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THE 7TH ANNUAL BASIC SCIENCE INTERNATIONAL CONFERENCE

7-8 March 2017
Ijen Suites Resort and Convention
Malang, Indonesia

Basic Science for Improving Survival & Quality of Life

Sub Topics:
Energy
Molecular and Health Science
Science and Technology Education

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BaSIC 2017

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ABOUT BASIC

The Annual Basic Science International Conference is a scientific meeting aimed to promote mutual exchange between scientists and also experts, to discuss innovative ideas in scientific research, and to tackle contemporary problems through the application of knowledge that rise from sciences. The scope of this conference is fundamental and applied research in chemistry, biology, physics, and mathematics. The origin of this conference was initiated in year 2000, by the Faculty of Mathematics and Natural Sciences of Brawijaya University, under the name of Seminar Nasional Kemipaan (National Sciences Conference). Since then, the conference has been organized regularly on annual basis. In 2004, the conference changed its name to Basic Sciences Seminar (BSS) and started to invite international speakers and participants. The conference then expands its scope to international in 2011 and formally adopting the current name. The previous Basic Sciences International Conference was held at Atria Hotel Malang in 2016 with participants from many countries including Australia, Malaysia, Thailand, Japan, UK and Germany.

WELCOME MESSAGE

On behalf of the organizing committee, I would like to welcome you to the 7th Annual Basic Science International Conference.

Firstly, I would like to thank all participants who have spent their time to come and join us for the conference. I believe that we will not be able to hold this conference successfully without participation from all of you. Secondly, I would like to thank the dean of faculty of Mathematics and Natural Sciences, Brawijaya University, because the faculty has provided us supports and facilities. I am thankful to our great keynote and invited speakers for their willingness to join the conference and share their scientific knowledge to all of us. Thanks to our reviewers who have made assessments and suggestions related to the abstracts. I also want to thank the sponsors which have made their contributions to this conference. Finally, I want to thank all members of the committee for their hard work to make this conference successful.

The Basic Science International Conference is held every year since 2010, and always organized by the Faculty of Mathematics and Natural Sciences, Brawijaya University. This conference is a forum that enables us to share our ideas among us. The participants are expected also to take their time and opportunities to know each other during the conference, in order to strengthen their networks and collaborations. In this conference, we have more than 300 participants from counties such as Indonesia, Japan, Australia, Germany, Switzerland, and Thailand. In the conference, we have plenary lectures and sessions for parallel oral presentations as well as poster presentations.

We hope that all participants enjoy all activities during the conference and this proceedings book will be useful for all of us.

Thank you very much.

Best regards,

Hari Arief Dharmawan, Ph.D.

Chairman of BaSIC 2017

WELCOME MESSAGE

On behalf of the Dean of Faculty of Mathematics and Natural Sciences, Brawijaya University, I would like to extend my warmest welcome to all delegates from all over the world. Welcome to Malang, where Malang is one of the educational city in Indonesia. Malang, which is about more than 400 meters above sea level, has many tourist destinations. Malang is like a bowl, surrounded by some volcanoes in the east (Semeru and Bromo), west (Kawi and Kelud) and north (Arjuna and Welirang Complex), and in the south are coastal areas, where we have many beautiful new opening beaches.

We are very pleased to welcome you in the proceedings book of the seventh Annual Basic Science International Conference 2017. I would like to express my gratitude to all of the participants, keynote and invited speakers as well. Many thanks also go to the reviewers and the editorial team for their big effort in supporting this book of abstracts. Last but not least my big appreciation to the steering and organizing committees, in realizing this proceedings book.

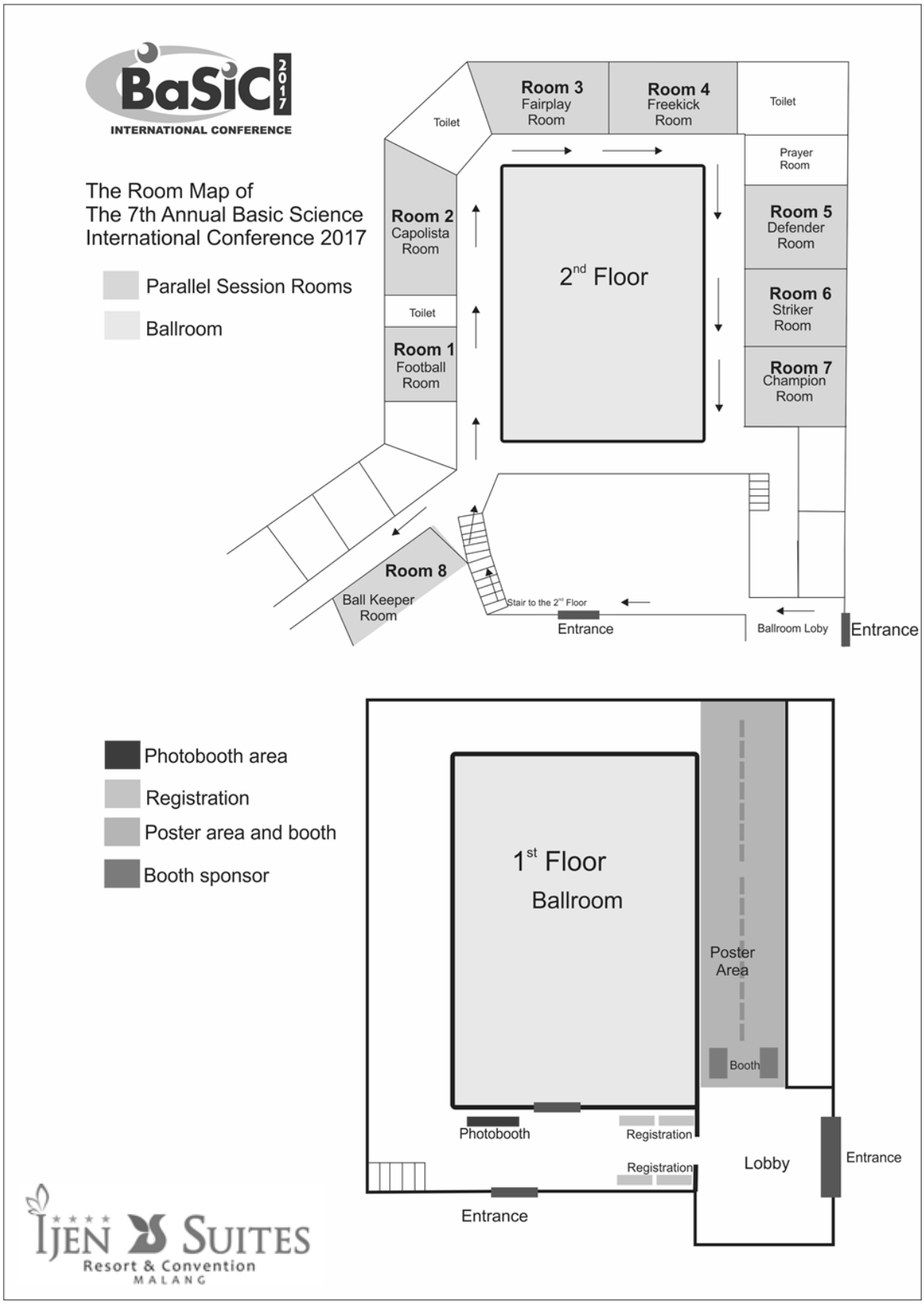
Thank you.

Faculty of Mathematics and Natural Sciences,

Dean,

Adi Susilo, Ph.D.

CONFERENCE VENUE



CONFERENCE PROGRAM

Day One: March 7th, 2017

07.30 – 08.30	Registration
08.30 – 09.00	Opening Ceremony
09.00 – 09.45	**Plenary Lecture 1**: *CRISPR/Cas9: Basics and Applications in "gene surgery".* Prof. Dr. Wolfgang Nellen, Institut fur biology, Germany
09.45 – 10.00	Coffee Break
10.00 – 10.45	**Plenary Lecture 2**: *Use of Wavelet Analyses with Potential Field Data in Exploration and Monitoring Studies* Dr. Guillaume Mauri, Neuchatel University, Switzerland
10.50 – 11.35	**Plenary Lecture 3**: *Mathematics for Solving 5G Massive Wireless IoT Networks Problems* Dr. Eng. Khoirul Anwar, S. T., M. Eng., Telkom University
11.35 – 12.30	Lunch
12.30 – 15.00	**Parallel Session 1**
15.00 – 15.30	Poster Session & Coffee Break
15.30 – 17.30	**Parallel Session 2**
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19.00 – 21.00	Gala Dinner

Day Two: March 8th, 2017

07.30 – 08.10	Registration
08.10 – 08.55	**Plenary Lecture 4**: *The Roles of Metal Ions in Diabetes – Metal Drugs and Supplements* Prof. Peter Andrew Lay, Sydney University, Australia
09.00 – 09.45	**Plenary Lecture 5**: *Functionalization of Stainless Steels Via Low Temperature Plasma Nitriding* Prof. Tatsuhiko Aizawa, Shibaura Institute of Technology (SIT) , Japan
09.45 – 10.00	Coffee Break
10.00 – 12.00	**Parallel Session 3**
12.00 – 13.00	Lunch
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BaSIC 2017

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Plenary Lectures

CRISPR/Cas9 – the new gene surgery

Wolfgang Nellen

Kassel University, Germany and Brawijaya University, Malang, Indonesia

Abstract – The new gene editing tool CRISPR/Cas9 allows for easy and efficient targeted changes in the genomes of microbes, plants and animals (including humans). CRISPR/Cas is a prokaryotic immune system that "memorizes" infections by phages and plasmids. The molecular biology and biochemistry of the machinery is very well understood and it has been engineered in different ways to serve specific needs in biotechnology. I will briefly present the origins and biochemistry of bacterial CRISPR/Cas systems, the technical use in gene technology and some applications that have already been achieved and others that are in the pipe-line.

1. SUMMARY

In 2012, a revolutionary paper appeared in the journal Science: Jennifer Doudna and Emmanuelle Charpentier and co-workers published the application of the bacterial CRISPR immune system to modify, delete and insert genes in essentially every living cell.

In bacteria and archaea, CRISPR represents an array of short DNA pieces that were captured from infecting phages or plasmids. Using enzymes encoded by the adjacent Cas locus, the microorganisms can fight subsequent infections. Transcripts of the short DNA pieces (crRNA) serve as guides to direct a nuclease encoded in the Cas locus to the invader and destroy it.

The Cas-nucleases can thus be programmed to cleave any specific sequence in any genome. Out of a plethora of different Cas-nucleases from different bacteria and archaea, the Cas9 enzyme from *Streptococcus pyogenes* proved to be the most convenient enzyme from the family for technical applications. In eukaryotes, the cut in the DNA is reversed by the cellular repair machinery. However, relegation of the cut ends usually results in sequence mistakes by short deletions or short insertions. Consequently, the gene targeted by a synthetic crRNA becomes functionless.

An additional piece of DNA with (partial) homology to the targeted sequence can be introduced to the cell. This will be used as a template and can replace the original sequence around the cut – including large insertions and deletions as well as single nucleotide changes.

CRISPR/Cas can thus be used in any organism to delete, insert or modify genes at a defined target site. The applications are endless and only a few examples will be presented here.

1. Compatibility in bone marrow transplantation: For BMT in leukaemia patients, compatible donors have to be found which have the same surface antigens as the recipient. With CRISPR/Cas9, genes for surface antigens of the donor can be adjusted to the recipient. If the genetic defect in the patient is exactly known, bone marrow cells can be removed, repaired in vitro and reintroduced into the patient.
2. Resistance of crop plants to fungal or viral diseases: In many cases natural resistances of plants against viral and fungal infections were accidentally lost during breeding and selection over the last few hundred years. Resistance genes are still present in some old variants or the wild forms of modern crops. Backcrossing these traits into the modern strains would take decades while introducing the resistance by CRISPR/Cas9 could be done in approximately one year.
3. Animal diseases: The same approach can be used to deal with diseases of farm animals like chicken flu or swine fever, where resistance genes exist in related species and can be transferred to livestock by CRISPR/Cas9
4. Nutritional value: Malnutrition is a substantial problem, though sufficient calories are supplied, in many regions of the world insufficient amounts of vitamins and micro-nutrients are contained in the common staple crops. Multiple genes to construct biochemical pathways for the synthesis e.g. vitamin A can be introduced into plants easily by CRISPR/Cas9.
5. Gene drive: CRISPR/Cas9 can be used for a copying (recombination) mechanism that generates homozygous individuals from heterozygous ones. This is currently being used for spreading infertility genes in disease vectors like Anopheles and Aedes. A realistic aim is to reduce the population of mosquitos by 80 to 90% and thus reduce infection by Malaria, Dengue, Yellow Fever and others.

6. Antibiotic resistant bacteria: it may be feasible to target resistance genes in pathogenic bacteria by CRISPR/Cas9 and thus solve the increasing problem of multi-resistant infections.

The advantages of CRISPR/Cas9 are that it can be universally applied, that the method is quite easy to perform and that it is inexpensive. These advantages could lead to a "democratisation of gene technology", meaning that the method is not only available to large, financially strong companies but also to small companies and even local breeders.

These advantages may also create the risk of intentional or unintentional misuse since any well trained molecular biologist may carry out CRISPR/Cas9 constructions with quite simple and inexpensive equipment.

There are also concerns on ethical issues, e.g. the interference with ecosystems or the genetic modification of human life. Though especially the latter issue requires serious and rational discussion, we are still very far from "designer babies" with specific (complex) traits and behaviour and it is questionable if this can be achieved at all. Rules and regulations on gene technology in general and CRISPR/Cas9 in particular have to be made wisely. In Europe, especially in Germany, legislation has strangled progress in genetic engineering, companies have moved out of the country and innovation is significantly slowing down. Even worse, western political parties and NGOs try to force their anti-science opinion onto developing countries, preventing progress that is so much needed. One should always consider that using a technology requires responsibility. However, prohibiting a technology also requires responsibility. Indonesia should find its own way to make best and responsible use of this new break-through technology. There are many challenges in agriculture, animal breeding, nutrition and human health that can be approached by CRISPR/Cas9. Unfortunately, after more than five years, no university or research institution has yet picked the technology to pursue serious projects.

For further details: there is a huge number of excellent review papers, cartoons, videos, graphs, podcasts and discussions in various languages available on the internet. A reference list would be endless or a very subjective, arbitrary choice. Readers interested in CRISPR/Cas will have no problem to find further material on every level of knowledge.

Use of Wavelet Analyses with Potential Field Data in Exploration and Monitoring

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1. INTRODUCTION

Developed in the 1980's, first for seismic analyses [1,2,3,4,5], the wavelet method is used since the 1990's to analyses potential field data [6,7,8,9]. The aim of this work is to present some of the benefits when using wavelet analyses on potential field data (e.g., gravity, self-potential) when conducting geothermal exploration and volcano monitoring.

2. METHOD

Wavelets are mathematical equations that allow for analyzing either time series or spatial data set, which are used in a wide variety of domains, which include but not limited to seismic/acoustics [1], image or signal processing [9,10], fluid mechanics (turbulence) [12], archeology [13], volcano monitoring [14], or biology [15].

The wavelets analyses are organized into different categories, such as orthogonal wavelet [16], discrete wavelet analyses [20] and continuous wavelet analyses [5,8,10]. Many wavelet exists and are organized into families [8,9,10,12]. Here, we present the use of the Poisson wavelet family [8,9,13]. Wavelet analysis is a multi-scale analyses method, which is used to determine power and frequency spectrum, and distribution (space, time) of a processed signal [1,9,13,16,17].

3. RESULTS AND DISCUSSION

In 2010, we developed the multi-scale wavelet tomography approach, which use multiple wavelets to better constrain depth of source generating observed potential field anomaly [18]. Later on, we published a wavelet code using Poisson family for potential field data, named MWTmat, based on matlab platform [19]. Since, we have applied MWTmat on several projects that cover volcano monitoring [20,21] and geophysical study for geothermal exploration [18,20,22].

Our results show the usefulness of the MWTmat method that brings information on depths, location and structural shape of the source. We present examples that include but not limited to Kawah Ijen aquifer monitoring (Indonesia) [21], geothermal fluids circulation on Waita volcano (Japan) [18]. Fig. 1 presents an example of MWT method applied on gravity data to better constrain the geological structures affected by increase of fracture density due to fault movement and rock dissolution through karstification processes (study case of Jura range, Switzerland) [22].

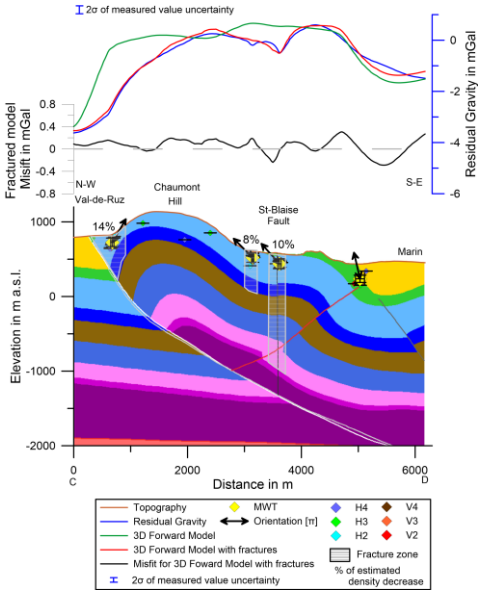

Fig. 1. Example of MWT analyses using Poisson wavelet on gravity data to locate rock density decrease associated to fault and karstification on geothermal exploration [22].

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Mathematics for Solving 5G Massive Wireless IoT Networks Problems

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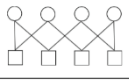
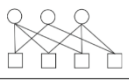
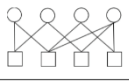
1. INTRODUCTION

Requirements of the fifth telecommunication generation (5G) aspects expected in 2020, according to the definition of international telecommunications union (ITU), are: (1) data rate beyond 10 Gbps, (2) massive machine-type communications, and (3) latency below 1 milisecond. This talk considers solution to massive machine-type communications, which is expected to serve about 40-50 billion devices connected to the internet, called the internet of things (IoT). Based on the contention-based access mechanism, IoT technologies are divided into four categories: (i) pure ALOHA, (ii) slotted ALOHA, (iii) non-slotted carrier sense multiple access with collision avoidance (CSMA/CA), and (iv) slotted CSMA/CA.

TABLE I
THE SIX MOST IMPORTANT STOPPING SETS.

Set	Graph	$P_o(\cdot)$	$\mathcal{X}(\cdot)$
$\mathcal{S}_1$		$\frac{M!}{(M-2)!}\frac{\Lambda_2^2}{2!}\frac{2}{(N-1)N}$	$\binom{M}{2}\Lambda_2^2$
$\mathcal{S}_2$		$\frac{M!}{(M-3)!}\frac{\Lambda_2^3}{3!}\frac{8(N-2)}{(N-1)^2N^2}$	$\binom{M}{3}\Lambda_2^3$
$\mathcal{S}_3$		$\frac{M!}{(M-4)!}\frac{\Lambda_2^4}{4!}\frac{288(N-3)(N-2)}{3(N-1)^3N^3}$	$\binom{M}{4}\Lambda_2^4$
$\mathcal{S}_4$		$\frac{M!}{(M-3)!}\frac{\Lambda_2^2\Lambda_3}{2!}\frac{24}{(N-1)^2N^2}$	$\binom{M}{3}\frac{\Lambda_2^2\Lambda_3}{2}$
$\mathcal{S}_5$		$\frac{M!}{(M-3)!}\frac{\Lambda_2\Lambda_3^2}{2!}\frac{36(N-3)}{(N-2)(N-1)^2N^2}$	$\binom{M}{3}\frac{\Lambda_2\Lambda_3^2}{2}$
$\mathcal{S}_6$		$\frac{M!}{(M-4)!}\frac{\Lambda_2^3\Lambda_3}{3!}\frac{288(N-3)}{(N-1)^3N^3}$	$\binom{M}{4}\frac{\Lambda_2^3\Lambda_3}{6}$

In this talk, we propose new categories of IoT [1]-[6] to provide better probability of success in detection, where collision is even beneficial [1]-[2]. Using the basic concept of mathematics [3]-[4], especially on probability, binomial and exponential distribution (combined with some findings in information theory), we provide better IoT technologies [5]-[6] in terms of: (a) higher throughput (serve more devices), (b) lower packet loss rate , (c) optimizable networks (using extrinsic information (EXIT) chart analysis), and the most important finding, i.e., (d) the theoretical IoT limits given multiple user/devices detection capability per time slot.

2. METHOD, RESULTS AND DISCUSSION

We use EXIT chart analysis to design the rate of each IoT devices. To make it applicable in practice, the time-slot can not be set very large. As a consequence, the stopping set happens causing performance limitation. We derive stopping sets from multinomial distribution. We found 6 stopping sets shown in Table I [4] for the case of no multiuser detection. We found that the probability is accurate enough to predict the performance of massive IoT wireless networks.

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The roles of metal ions in diabetes – metal drugs and supplements

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1. INTRODUCTION

Metal ions have diverse roles in controlling diabetes, with Cu, Zn and Mn deficiencies known to contribute to the disease through loss of control of oxidative stress, and there is debate about whether vanadium may also be an essential trace element that is involved in both insulin mimetic and insulin enhancing roles [1]. Both vanadium and chromium supplements are consumed widely for glucose metabolism and control of diabetes, and there has been considerable interest in developing anti-diabetic drugs based on these metals and also Mo and W [1]. Recntly, we have shown that it is important to consider speciation of such species in cell media and other biological fluids [2], and that supposedly safe Cr(III) supplements can be oxidized to carcinogenic Cr(VI) and that there are natural pathways to prevent Cr toxicity [3,4]. This and other evidence points to neither a natural role for Cr in diabetes nor safety in its long-term consumption [1-4]. Thus, most evidence is currently focused on vanadium and to a lesser extent Mo and W. These aspects will be discussed in this talk.

2. METHOD

The speciation of metal ions within cells and tissues were investigated with X-ray absorption spectroscopy, EPR spectroscopy and UV/Vis spectroscopy [2], whereas elemental distribution in cells and tissues were measured by X-ray fluorescence microscopy [3]. Various biochemical assays including vibrational spectroscopy and imaging of glucose metabolism in cells, capillary immunoassays of cell signaling and phosphatase inhibition have been studied.

3. RESULTS AND DISCUSSION

The results of the experiments described above throw considerable doubt on the efficacy and safety of widely consumed Cr dietary supplements for the control of glucose metabolism. On the other hand, evidence has been obtained for both a potential essential role for vanadium and insights into the reasons for its efficacy as a drug, which will be discussed.

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Functionalization of Stainless Steels via Low Temperature Plasma Nitriding

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Abstract – AISI420 type martensitic stainless steels were utilized to experimentally describe the low temperature plasma nitriding behavior with respect to the inner nitriding process in matrix, the lattice expansion and straining process, the strain-induced refinement process and the phase transformation from α'- to γ-phases. The physical modeling was built from these experimental results, which was difficult to explain by the classical nitriding models. This model took account of the inner nitriding process with consideration on the occupation of nitrogen solute with high contents into the vacancy sites in the a'-lattice. The strain induced phase transformation and microstructure refinement was investigated to describe the material model in Fe(Cr) – N system.

1. INTRODUCTION

The plasma-based nitrogen processes have been high-lighted; e.g. the plasma nitriding, the ion implantation, and the plasma immersion ion implantation [1]. In particular, the DC and DC-pulsed plasma nitriding processes have been utilized in industries with the name of ion-nitriding and radical-nitriding [2]. Those conventional plasma nitriding processes are characterized by higher holding temperature than 773 K and longer processing time than 20 ks. The hardening process is also driven by the precipitation strengthening where the synthesized chromium nitride (CrN) precipitates with nano-meter size and large volume fraction in the stainless steel substrates. The inner nitriding process is mainly governed by the nitrogen diffusion; e.g., the nitrided layer thickness is proportional to the square root of nitriding time [2-4]. As pointed out by [5], the high temperature plasma nitriding process for most of stainless steels and tool steels abides by this diffusion-oriented mechanism; the nitrogen solute content makes exponential decrease from the maximum content at the surface to the depth. Besides for the bound nitrogen in content in the form of CrN or Fe_4N, the surface maximum nitrogen solute content is limited by 0.1 mass %, which is equivalent to the maximum nitrogen solubility limit in the phase diagram. In the low temperature plasma nitriding below 673 K, the diffusing nitrogen interstitial atoms never react with the chromium and iron to form CrN or Fe_4N but occupy the vacancy sites in the fcc- and bcc-structured supercells in the stainless steel [6]. Owing to this occupation of vacancies by nitrogen solute, the original fcc- and bcc-structured lattices expand in their c-axis and contact in their a- and b-axes, respectively [7, 8]. In those previous studies, the nitrogen solute content [N] was limited at most by 20 at%; e.g., [N] = 10 at% in case of the ion implantation [9], [N] = 12 at% in case of the glow discharge plasma nitriding [10], and, [N] = 18 at% when using the low temperature plasma nitriding [11].

Authors have developed the high density plasma nitriding process to be working in low temperature [12-15]. In this low temperature plasma nitriding of martensitic stainless steels type AISI420 and austenitic stainless steels type AISI304, the nitrogen interstitials were infiltrated into the stainless steel substrates with significantly high content. Under this condition, the nitrogen interstitials occupy the vacancy sites in the α'-phase bcc structured lattices of martensitic stainless steels, and in the γ-phase fcc structured lattices of austenitic stainless steels, respectively. Due to this occupation of nitrogen solutes in the lattices with high concentration, each constituent grain is refined in the nitrided layer together with high straining by the lattice expansion with and without phase transformation. Hence, the nitrogen solute diffusion process still plays an important role in this low temperature nitriding; the occupation process must be taken into account for description of this process. In the present study, new physical modeling is proposed to describe the inner nitriding process without formation of nitrides as well as the strain-induced phase transformation from α'- to γ-phases. In addition, the strain-induced refinement in microstructure is also discussed to develop the heterogeneous structuring in stainless steels.

2. EXPERIMENTAL PROCEDURE

2.1 High density plasma nitriding

The high density nitriding system has no mechanical matching box with slow response time of 1 s to 10 s to adjust the applied power. Since both the input and output powers are automatically matched by frequency

adjustment around 2 MHz, the matching response time is only limited to 1 ms at most. This prompt power control provides to make full use of mesoscopic plasma pressure range over 50 Pa. Figure 1 a) illustrates the standard RF/DC plasma nitriding system. Different from the conventional processes, the vacuum chamber is electrically neutral so that RF-power and DC-bias should be controlled independently from each other. A dipole electrode is utilized to generate RF-plasma; DC bias is directly applied to the specimens. Heating unit is located under this DC-biased cathode plate. In the following nitriding experiments, the specimens are located in the inside of the hollow as shown in Fig. 1 b) before evacuation down to the base pressure of 0.1 Pa. This hollow cathode device is effective to intensify the density of activated nitrogen atoms and NH-radicals as well as electrons toward its outlet of hollow.

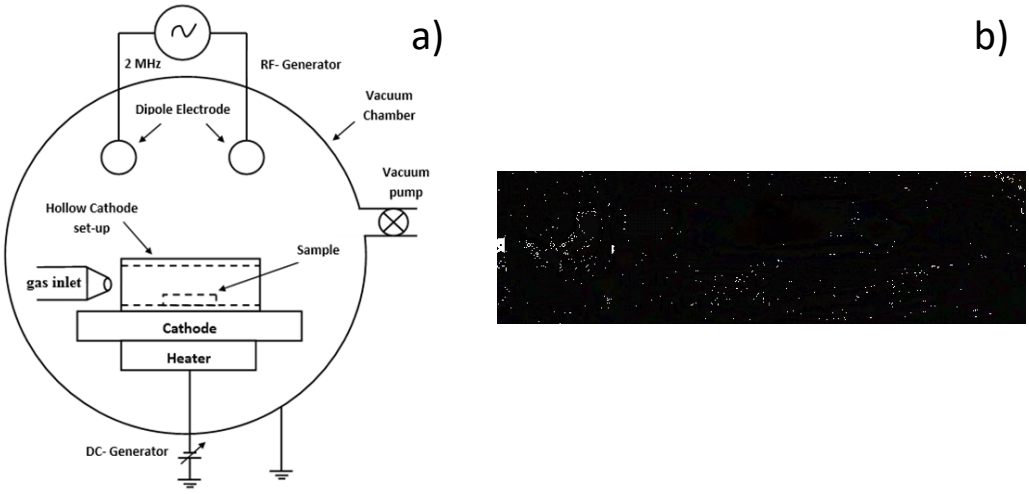

Figure. 1 High density plasma nitriding set-up. a) Standard RF/DC plasma nitriding system, and, b) Hollow cathode device to intensify the plasma density.

2.2 Measurement and observation

The microstructure of the nitrided layer was analyzed by scanning electron microscope (SEM). Energy dispersive spectroscopy (EDS) and electron backscattering diffraction (EBSD; HITACHI SU-70) were employed to make a precise analysis. The phase analysis was also performed by the X-ray diffraction.

3. RESULTS AND DISCUSSION

Figure 2 depicts the SEM image and nitrogen mapping on the cross-section of the nitrided AISI420 substrate at 673 K for 14.4 ks by 70 Pa. The original coarse grains were seen below the nitriding front end in Fig. 2 a); little grain boundaries can be detected even with the trace level in the nitrided layer. This implies that grain size should be significantly reduced during this low temperature plasma nitriding. Highly concentrated nitrogen atoms are present from 31 at% to 10 at% at the vicinity of surface down to 20 □m in depth. The nitrogen contents detected are much higher than the solubility limit of 0.08 at%; no chromium nitride (CrN) precipitates are detected. The high nitrogen concentration at the surface is responsible for driving the nitrogen diffusion process down to the nitriding front end at 80 □m in depth.

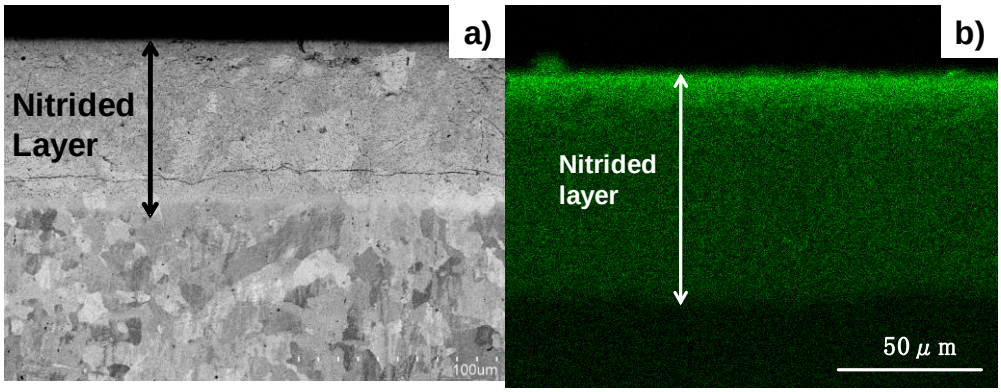

Figure. 2 Cross-sectional view of the plasma nitrided AISI420 specimen at 673 K for 14.4 ks. a) SEM image of cross-section, and, b) Nitrogen mapping by EDX.

Figure 3 depicts the nitrogen content depth profile from the surface to the nitriding front end in correspondence to Fig. 2 b). In the present plasma nitriding, the nitrogen content decreases form the surface to the nitriding front end; however, the plateau of nitrogen content is observed to have almost 9 at% from 20 μm to 80 μm in depth.

In the normal nitrogen diffusion process, the nitrogen content decreases from its maximum solubility limit of 0.1 at% to the nitriding front end exponentially. This essential difference in the nitrogen depth profiles is never explained by the normal diffusion model. The evolution of the nitrided layer thickness (E) is described by the classical Wagner's law where E^2 is proportional to the nitriding duration time (τ). This E^2 of the nitrided AISI420 specimen at the higher temperature than 723 K was proportional to τ; in case of this low temperature nitriding, no proportionality held on between E^2 and τ.

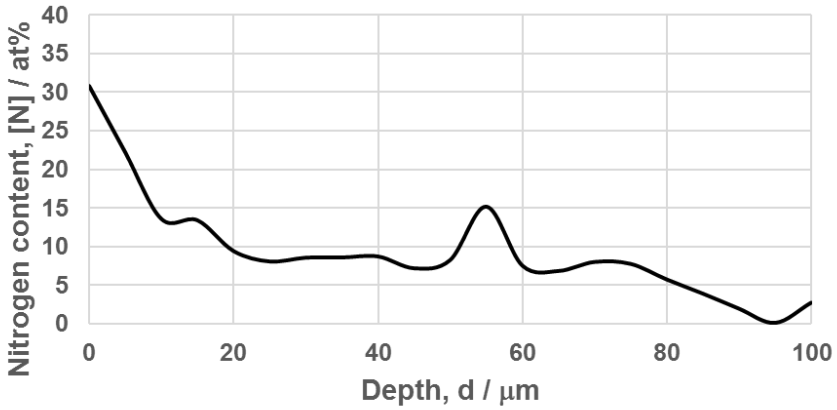

Fig. 3 Comparison of the nitrogen diffusion process between the high temperature and low temperature plasma nitriding treatments.

This nitrogen diffusion process in the low temperature nitriding accompanies with occupation process of nitrogen solute atoms into octahedral vacancy sites in the α'-bcc martensitic lattice structure. This occupation process is detected as an α'-lattice expansion by XRD analysis. As shown in Fig. 4, the original peak for α' (110) shifted itself to α'_N (100) in the lower 2θ direction; this large peak shift reveals that α'-lattice expands by occupation of nitrogen solute into lattice.

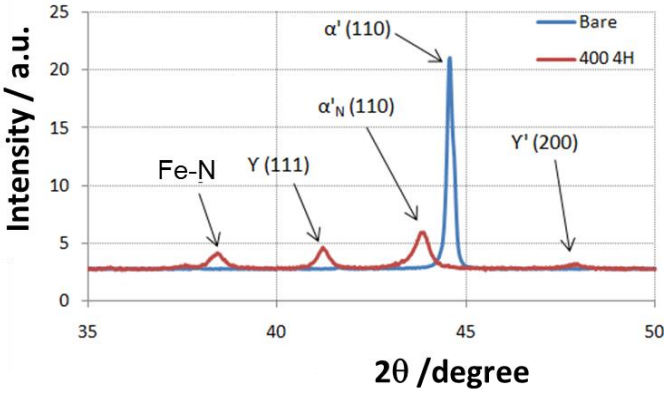

Figure. 4 Comparison of the XRD diagrams before and after nitriding at 673 K for 14.4 ks.

After [16], the original diffusion partial-differential equation is modified by considering this occupation process. The chemical reaction between the vacancies and the diffusing nitrogen atoms is taken into account in formulation; the occupation process is theoretically modeled by the single-step reaction kinetics. For simplicity, the nitrogen content is normalized to be represented by u (t, x) for $0 < u < 1$. Then, this new diffusion model is expressed by

$$\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} + \left(au - bu^2\right), \quad a, b > 0 \qquad (1)$$

where the second term in its right hand side denotes for the occupation process, and, a and b are material constants. If a and b are assumed to be unity, Eq, (1) reduces to the Fisher – Kolmogorov equation. Hence, this solution of Eq. (1) predicts that the nitriding front end advances in the depth with a wave velocity.

Beside for the α'-lattice expansion, the γ-phase peaks were seen in Fig. 4. The unknown peak around $2\theta = 38^o$ was identified to correspond to the Fe – N bonding state; more precise analysis is necessary in future. The physical analysis by EBSD works to describe this phase transformation from α'- to γ-phases during the plasma

nitriding. Figure 5 shows the relationship among the crystalline structure, the staining and the phase mapping. As shown in Fig. 5 b), the highly strained network is formed into the matrix by α'-lattice expansion in each grain. Corresponding to this network, the phase transformation to γ-phase is induced in the network structure in Fig. 5 c). In parallel with this high straining, the grain size is significantly refined to be invisible by EPSD-analysis at the vicinity of the surface in Fig. 5 a).

4. CONCLUSIONS

The low temperature plasma nitriding provides a method to describe the structure of α'- or γ-lattices in the stainless steels in the wider range of nitrogen contents up to 30 at%. Two phase structure such as (α', γ) is induced by high straining during this nitriding. Furthermore, the original coarse grain structure is refined by this high straining to have ultra-fine grain sizes. This low temperature nitriding also becomes a tool to make heterogeneous structuring for functionalization of stainless steels.

5. ACKNOWLEDGEMENTS

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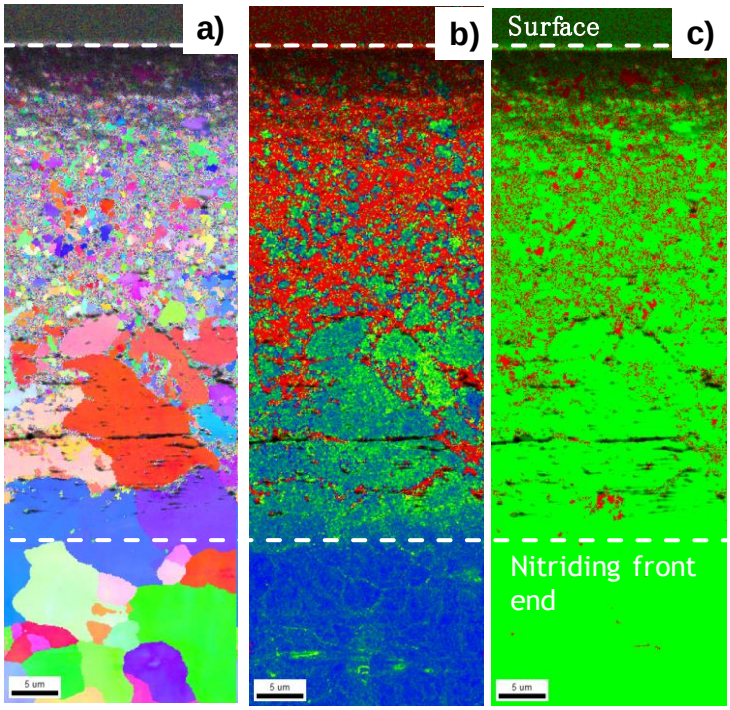

Fig. 5 EBSD analysis. a) IPF-mapping, b) KAM distribution, and c) Phase-mapping.

Volume 3

BaSIC 2017

The 7th Basic Science International Conference

Basics Science for Improving Survival and Quality of Life

Invited Papers

Complexity and Nano Science Approach in Life Sciences: The way to overcome our partial understanding on living system

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Abstract – Universe is organizational system built from complex interactions and interconnections of components where biological system is complex structure with specific function dedicated to perform normal ordered organizational system. Developing Complexity Science and Nano Biological perspective giving the ideas of interfacing between modern physical and biological sciences into more comprehensive understanding of life system. The ideas initiated for more than 7 decades by physicist Erwin Schrodinger in a 1944 book What is Life? The argument that life feeds on negative entropy or negentropy was asserted and may initiate the way to more comprehensive and better understanding life. We can then develop understanding biological behavior on nano size biological materials and its higher order using modern physics. While other physicist namely Firtjof Capra give another stimulating definition of biological system that enable to apply thermodynamic law. He defined that life is the ceaseless flow of energy and material through complex chemical interaction namely metabolism to perform self organization, perpetuation, regeneration, reparation as well as movement. These ideas are interesting while most people are not even conscious that biologists when they coming into nano size molecular biological discussion, they remain using what is called Newtonian principles respecting the mechanical view of reality when they think and talk. This Newtonian based principles, in order to fulfil the scientific methodologies, many biological scientists including who works in applied sciences such as edical Sciences, they practiced reductionism, reducing things into their parts and examining the parts to understand what made them tick. They reduced life to cells, molecules while the concepts is remain within Newtonian. In the early 20th Century, the certainty of Newton's mechanics was undermined by quantum mechanics and the Uncertainty Principle developed by Werner Heisenberg. This is the new challenges to the old Newtonian view of reality. So some biological scientists began abandoning the Newtonian worldview, while most of them and the ordinary people held on to it.

Surface Modification for Quartz Crystal Microbalance using Polystyrene as a Basis for Biosensor

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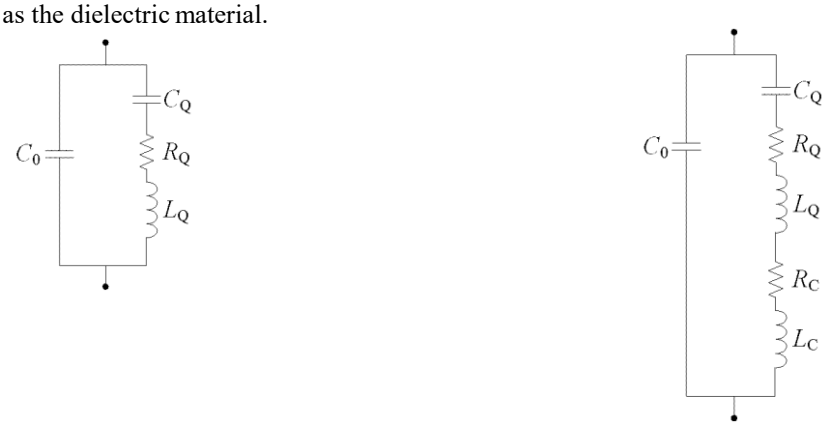
Abstract – Surface modification of the Quartz Crystal Microbalance is one importance factor in the application of the sensor as a biosensor. The property of the surface physically must not affect the performance of the sensor. It is indicated by a minimal damping of the sensor, while biochemically the sensor surface must be able to bind or adsorb the biomolecules being immobilized as a sensitive layer of the biosensor. Polystyrene is one from many materials which can be used as a sensor coating for the basis of the biosensor as the polystyrene can be used as a matrix for immobilizing antigen or antibody. Polystyrene coating on top of the QCM sensor can be done by using spin coating, airbrush spray coating, and ultrasonic spray coating. Those different methods result in a different surface structure. Good coating using glassy material will not increase the electrical impedance of the sensor. Therefore it is suitable to be used as a mass sensitive biosensor. In addition, modification of the polystyrene surface can be done to alter the surface hydrophobicity. UV radiation and plasma treatment can effectively alter the polystyrene surface hydrophobicity without affecting the electrical impedance of the QCM sensor which can be influenced by the acoustic impedance characteristic of the coating layer. Surface hydrophobicity plays an important role in the used of the QCM biosensor which detects the viscosity and density of the target molecule in liquid. Polystyrene surface with hydrophilic surface decreases the resonance frequency of the QCM biosensor in contact with liquid more than the decreasing resonance frequency on a hydrophobic surface.

1. INTRODUCTION

Since the first Quartz Crystal Microbalance has been used, the application of the quartz crystal microbalance can be found in many areas. The interesting aspect of the sensor is its ability to works in gas and liquid. The sensor responds to physical, chemical and biological quantity results in a frequency change or impedance change. The wide use of the QCM sensor for chemical and biological sensors are mostly based on the sensor responds to mass change on its surface, and it responds to viscosity and or density of contacting material on the sensor surface 1. The first respond is described by Sauerbrey equation 2 and the second respond by Kanazawa and Gordon 3.

The electrical behavior of the QCM sensor and its coating and loading can be modeled as an electrical circuit model (RLC model) 4,5. The model is well known as a Butterworth van Dyke (BvD) model. The electrical model is presented in Figure 1a. The right part of the model is the electromechanical equivalent model of the piezoelectric property of the sensor, while the capacitance in the left is the capacitance of the sensor as a parallel plate capacitor with the quartz as the dielectric material.



(a) (b)

Figure 1. Electrical equivalent model of the QCM sensor without coating (a) and with coating (b)

The coating layer and liquid behaviour is also modelled as a resistive and inductive elements in the extended BVD model 6. The model is depicted in Figure 1b. The load contribution of the coating layer can also be expressed in an RLC model. The resistive part of the model shows a dissipative behavior of the sensor and the coating material. A glassy coating material does not contribute to the resistance equivalence value, and those the value of the resistive element is close to zero. The coating material behaves only as a rigid mass on top of the sensor which can be expressed only by an inductor element. In the impedance spectrum of the sensor, the glassy coating material causes the series resonance frequency of the sensor goes down, and the minimum impedance of the sensor at the series resonance remains. In contrast, a rubbery coating material lowers the series resonance frequency of the sensor together with an effect of increasing minimum impedance of the series resonance sensor and decreasing maximum impedance at the parallel resonance. It was studied that the glassy and rubbery property of the coating material is determined by the shear moduli of the coating material. Also, the thickness of the coating material affects the property of the material. A very thin coating most likely behaves as a glassy material at high frequency, for example at 10MHz.

The rubbery coating material is not preferred to be used for QCM sensor because it contributes an additional damping to the sensor indicates by the increasing impedance of the coated sensor at series resonance. In the worst condition, the additional damping causes the sensor to stop oscillating. Therefore it is importance to maintain the low damping caused by the coating materials. The low damping is indicated by a non-significant or zero increase of the sensor impedance at series resonance.

Polystyrene is one from many polymers which can be used as a coating material for QCM biosensor 7–9 The polystyrene up to view micrometer thickness still behaves as a glassy material. Therefore polystyrene coating does not contribute additional damping to the sensor. The surface property of the polystyrene can be modified by physical and chemical modification. Method to deposit the polystyrene on top of the QCM sensor affects the surface roughness of the sensor. UV radiation to the polystyrene coating alters the hydrophobicity of the polystyrene surface. Plasma treatment can also change the hydrophobicity.

2. COATING FOR MASS SENSITIVE BIOSENSOR

Those indicated that the polystyrene coating is a good matrix layer to immobilized sensitive biomolecule for QCM biosensor. The high shear moduli of the polystyrene make the polystyrene coating behaves as a glassy coating material. Those it is expected that a polystyrene coating on QCM sensor does not add damping to the sensor. The sensor surface needs to be modified to make the sensor responds only to a specific quantity target. Surface modification of the QCM sensor using polystyrene can be done easily and targeted for many difference applications. The polystyrene coating on the sensor can be used as immobilization matrix for a biomolecule to be used as an immunosensor for various target molecules such as insulin, human serum albumin, a specific protein of cow milk, matrix metalloproteinase-3 (MMP-3) antibody and many others.

The simple method of coating development using polystyrene on top of the QCM sensor is done by spin coating, air pressure spray coating, and ultrasonic spray coating. Proper selection of the coating method results in a homogeny coating thickness with no damping contribution to the QCM sensor. However, come condition of the inhomogeneous coating exists caused by preparations and coating process. Inhomogeneous coating thickness raises the minimum impedance at series resonance of the QCM sensor.

Spin coating is the simplest method to make a polystyrene coating on top of the QCM sensor with HC-49U form. Rotation speed, concentration, and solvent selection can be done to achieve the desired film thickness and surface roughness 10. The surface roughness of the polystyrene which was deposited using spin coating method is affected by the solvent. Polystyrene coating which was deposited using spray coating (airbrush) resulted in more roughness. However, optimization of some parameter of the coating such as solvent, pressure, and the distance between the nozzle and the sensor surface need to be optimized to get a homogeneous surface. The same action is also required when the deposition is done using an ultrasonic deposition method.

The surface roughness of the coating, however, does not contribute any additional damping to the sensor. Inhomogeneity of the coating deposition can add damping to the sensor. By measuring the electrical impedance of the sensor, one can identify whether the coating layer is electromechanically good or bad. A good coating layer will not add additional impedance in the measured impedance spectrum, while a bad coating results in an increasing minimum impedance of the sensor at series resonance.

Figure 2 shows impedance curves of a QCM sensor before and after coating. Both were coated using polystyrene. In the left figure, the minimum impedance of the sensor at series resonance after coating dows not increase. Before and after coating the minimum impedance is around 10Ω. On the right side, the impedance of the sensor with the coating is around 38Ω. The frequency change of both sensors caused by the coating layer is around 27KHz. The increasing minimum impedance of the sensor in the right figure is caused by inhomogeneity of the coating.

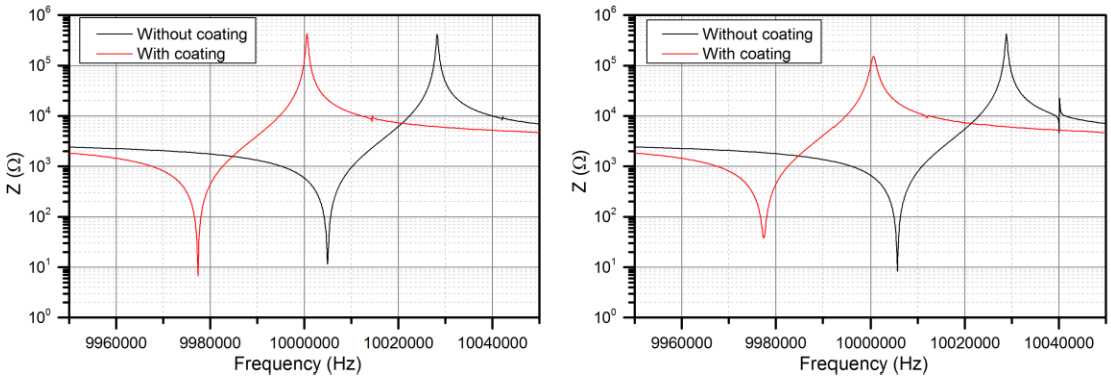


Figure 2. Impedance spectrum of QCM sensor with coating with no additional damping (left) and with additional damping (right)

Immobilized The surface roughness of the coating, however, does not contribute any additional damping to the sensor. Inhomogeneity of the coating deposition can add damping to the sensor. By measuring the electrical impedance of the sensor, one can identify whether the coating layer is electromechanically good or bad. A good coating layer will not add additional impedance in the measured impedance spectrum, while a bad coating results in an increasing minimum impedance of the sensor at series resonance.

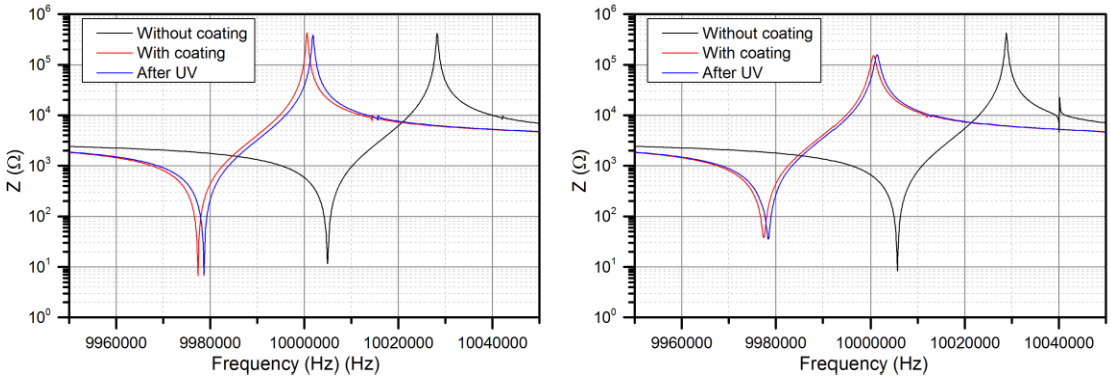
3. COATING FOR LIQUID VISCOSITY AND DENSITY CHANGE

Hydrophobicity of the polystyrene surface affects the loading properties of the liquid to the sensor response. This result in a different frequency change of the sensor at the same viscosity caused by different hydrophobicity. Biological sensor by detecting viscosity change of the sample liquid caused by interaction between the substance in the liquid and target molecule was demonstrated for endotoxin detection using QCM sensor 11. In those works, the change in the hydrophobicity of the surface resulted in a significant effect on the sensor response. A higher signal was obtained using a hydrophilic surface.

For the application where the detected target sample will result in viscosity and density change of the liquid on top of the QCM sensor, the surface property of the coating layer needs to be modified to effectively couples the liquid and the sensor. The good coupling is required to transfer the energy of the vibrating quartz to the liquid. The surface property of the sensor should be modified to minimize the slip effect of the liquid 12,13. The mechanical coupling between the surface and the liquid affects the frequency change of the sensor caused by the liquid viscosity and density change as described by Kanazawa and Gordon.

Different surface hydrophobicity can be achieved using many different approaches. One can use different materials or by modifying the surface property of the material by chemical or physical treatment. Polystyrene is one material where its surface hydrophobicity can be changed physically and chemically. The surface hydrophobicity of the polystyrene can be change to be more hydrophilic by UV radiation or by using plasma treatment. Both modifications do not change the bulk property of the polystyrene coating. Therefore the modulus elasticity of the polystyrene coating remains. Figure 3 shows an example of impedance curve of a QCM sensor with polystyrene coating before and after UV radiation. It can be seen that the minimum impedance of the sensor remains.

Figure 3. Impedance spectrum of QCM sensor with coating with no additional damping (left) and with



additional damping (right) before and after UV radiation

A significant change of the impedance curve can be observed when the sensor surface is in contact with water. The minimum impedance of the sensor in contact with water increases significantly after the Polystyrene coating treated with UV radiation and become a hydrophilic surface (Figure 4). When the polystyrene surface become hydrophilic, the shift of the resonance frequency is higher, and the minimum impedance increases. Therefore for

a biosensor which uses a liquid viscosity and density change of the water-based solution needs a hydrophilic surface to get a higher signal change.

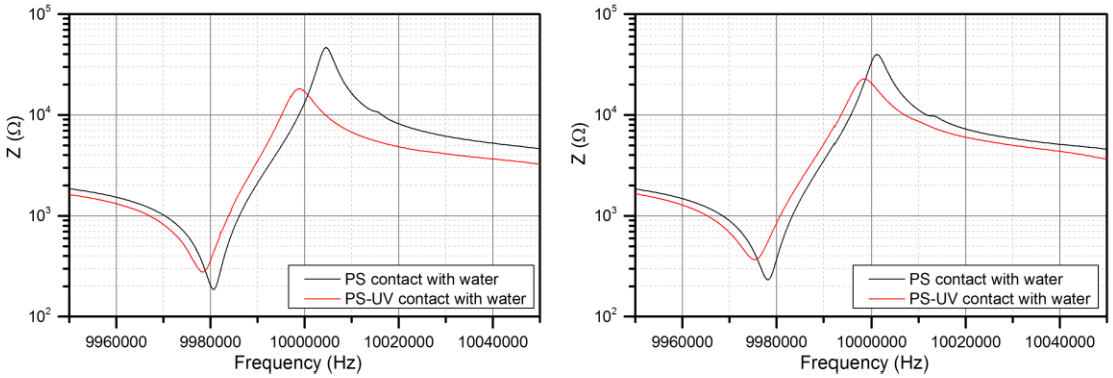


Figure 4. Impedance spectrum of QCM sensor in contact with water

4. CONCLUSIONS

Polystyrene can be used as a coating material for QCM sensor. The mechanical property of the polystyrene as a glassy material gives an advantage to the polystyrene coating which does not add additional damping to the sensor. Meanwhile, the surface property of the polystyrene can be modified to make the surface as a good matrix for biomolecule immobilization and to make a better coupling between the sensor and water solution containing target molecule.

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Structure and Dynamics of Water: An Insight from Molecular Simulation

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Abstract – While being termed as the "matrix of life", in which the majority of important biochemical reactions took place, water is the most puzzling substance and does not share many properties with other liquids [1]. Upon cooling and/or pressurized, water has at least 14 crystalline polymorphs in pure state [2]. This number increases with the presence of small hydrophobic molecules that can be encaged in a network of water molecules that are connected by hydrogen bonds [3,4]. A deep understanding on the structure and dynamics of water is of paramount importance in life science, cryobiology, climate prediction, and material design. Molecular simulation methods provide an access to experimentally difficult conditions such as extreme temperature and pressure or metastable state; thus provides possibilities to discover novel phenomena and peculiar character of a chemical system [5–7]. The methods enable a systematic investigation at nanoscale level with a great numerical detail. In the absence of technical difficulty in preparing certain material, molecular simulation methods facilitate creative minds to tweak the system with more freedom, and the results are useful to interpret the nature and to design innovative materials. Here we will introduce a number of our molecular simulation works in attempt to unravel the structures and dynamics of a water dominated system.

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Electrochemical Sensor for Industry and Medical

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Abstract – Electrochemical sensor had been developed for many purposes. This type sensor mostly can be easily adopted to solve the problem in industry and medical with a high degree of accuracy, precision, sensitivity and selectivity. This study we review a highlight recent in the using of electrochemical sensor for industry and medical. We developed a spicy sensor based gold nanoparticle for industry, which can be used for spicy analysis of any kind food. Both of anodic and cathodic can be used for spicy measurement with limit of detection of the sensor at about 0.500 μM. The sensitivity at anodic and cathodic is 0.102 and -0.268 $\mu A.\mu M^{-1}.mm^{-2}$ respectively. The spicy sensor has been applied to detect the quantity of capsaicin at the seed and fruit of red pepper. We also fabricate and utilize the extract of Maja (*Aegle marmelos*) leaves and pulp to modify silver electrode for medical biosensor. The maja leaf extract modified silver electrode gives a good performance as a urea biosensor at pH 13 with LOD and sensitivity are 2.299 μM and 19.781 $\mu A.\mu M^{-1}.mm^{-2}$ (for anodic) and 5.165 μM and 38.177 $\mu A.\mu M^{-1}.mm^{-2}$ (for cathodic) respectively. Whereas, the maja pulp extract modified silver electrode can be used as glucose biosensor. The biosensor has a good LOD and sensitivity for glucose detection, i.e., 1.920 μM and 1.222 $\mu A.\mu M^{-1}.mm^{-2}$ (for anodic) and 1.701 μM and 1.522 $\mu A.\mu M^{-1}.mm^{-2}$ (for cathodic) respectively. The urea and glucose biosensors were also proven have good selectivity.

1. INTRODUCTION

Jaroslav Heyrovsky, Czech scientist discovered voltammetry which is a form of electrochemistry in 1920s. This technique can analyze a samples by measuring current as a function of the applied potential [1]. In recent years, the sensor technology was developed using electrochemical technique. This sensor commonly used because it was relatively fast, simple and low-cost. Therefore, the electrochemical sensor have been widely applied in industry and medical [2–6].

Improvement of selectivity and sensitivity of the sensor can be conducted by modify the electrode surface using definite material. Most of the electrodes that modified are platinum, gold and silver. The modification on the electrode surface give general result in the:

1. Transfer of physicochemical properties of the modifier to the electrode
2. Enhanced electrocatalytic activity due to the use of materials with large surface area which in turn allows better sensitivity
3. Selectivity towards analyte due to immobilized functional groups and dopents
4. Fast diffusion kinetics in case of some materials
5. Extraction and accumulation of an analyte at the electrode surface [7].

Based on the above explanation, the scientist already modify many electodes to increase the sensor performance [8–12]. In this article, we reviewed our electrode modification as a sensor for industry and medical purpose. Our sensor was proven have a good limit of detection (LOD), selectivity and sensitivity. For industry purpose, we develop a gold nanoparticles for spicy sensor. We also utilize the Indonesian original plant (i.e., Maja (*Aegle marmelos*)) to modify silver electrode for urea and glucose biosensor. The performance of our modified electrode will be compared with another works.

2. APPLICATION ELECTROCHEMICAL SENSOR FOR INDUSTRY

2.1 SPICY SENSOR

Chili is one of important commodity in the world, include Indonesia. In the period 2000-2014, Indonesia is able to export an average of 8.38% per year for fresh chili and 31.74% per year for processed chili such as sauce. Determination of the pungency level of chili is needed, especially in the export-import industry. The pungency level of chili is related to the presence of capsaicinoid compounds [10,13–16]. Monitoring of this compound is

important because excessive intake of capsaicinoid may be harmful to health [15,17]. In fact, the Council of Europe has recommended a certain limit to the total capsaicinoid content permitted in a few categories, for instance, 5 ppm as the general limit for food and beverages, 10 ppm for spicy food and beverages, 20 ppm for hot sauces, and 50 ppm for tobacco, harissa, pimento hot oil, and so forth [15].

Conventional methods used to determine the pungency level or capsaicin concentration are using tongue by a panel of tasters (Scouville Organoleptic test method). This test is leveled in Scouville, which is the spiciness measurement of 30 chilies or its derivatives. Pure capsaicin itself has 16 million Scouville. Currently, the industrial Scouville test measurement becomes the standard of spicy flavor, but it is less accurate due to its subjectivity [18]. Quantitative methods such as spectrophotometry and high performance liquid chromatography (HPLC) has been used for the detection of capsaicinoid. HPLC results are reported in spicy ASTA units (15 multiple converted into Scouville) and claimed more reproducible than the conventional method. Unfortunately, this technique requires high cost and complex preparation. Therefore, a simple and accurate technique is still needed to detect and determine capsaicinoid.

Electrochemical method have been reported for fast, simpler and accurate technique. The first claimed of this method for capsaicin analysis is reported on the patent number WO 2009/115840A1. The active material used is double walled carbon nanotubes. Fabrication double walled carbon nanotube is relatively difficult since it requires relatively expensive. We provides another alternative active material that work in the similar system but using simpler process, i.e., composite polyamide 11 and gold nanoparticles. Synthesis of gold nanoparticles was done using wet method, which is simpler than the making of double walled nanocarbon active materials.

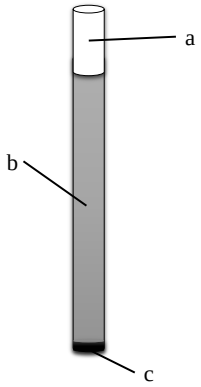

Fig 1. Polyamide composite electrode-gold nanoparticle.

In general, the composition of our modified electrode can be seen at Fig. 1. The surface of gold electrodes was coated by gold nanoparticles (Fig. 1a). The edge of the gold electrodes was isolated (Fig. 1b) and make the bottoms were opened. Deposition of polyamide 11 on the gold electrode surface is carried out using LbL (Layer by Layer) technique. Solution of polyamide 11 appeared by dissolving 0.2 grams powdered polyamide 11 with 7 mL of concentrated H2SO4. Gold electrodes immersed for 5 seconds in a solution of polyamide 11 and was directly included in the distilled water to rinse. The electrode was dipped in the suspension of gold nanoparticles for 24 hours (Fig. 1c), then rinsed again with distilled water. The electrodes are ready to analyze, after dried. The characterization of electrode surface by microscope optic was presented at Fig. 2.

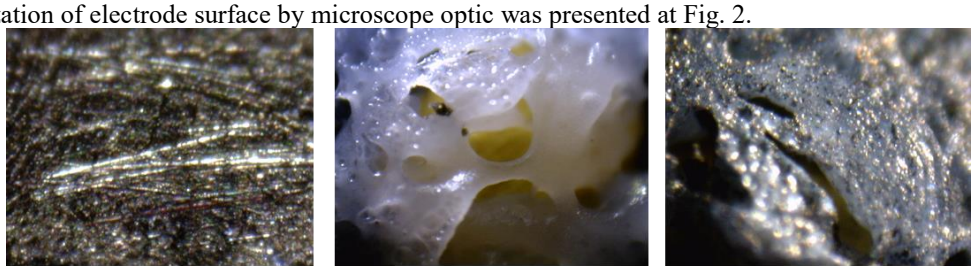

Fig 2. Gold electrode surface before (a) and after deposited by polyamide 11 (b), and the surface of modified gold electrode with polyamide 11-gold nanoparticles.

The combination of polyamide and gold nanoparticles are able to oxidize capsaicin, which cannot be done by gold electrodes. The peak of oxidation and reduction are observed at 0.439 V and 0 V, respectively. The detection limit (LOD) of electrode polyamide 11 gold nanoparticles at 0.439 V reaches up to 0.5 μM. Linear concentration range that can be used as a calibration curve is in the region of concentration 220 μM. In linear regression equation of the calibration curve obtained y = 6.75 + 0.08 x with a value of R of 0.99468, so the sensitivity of electrodes

polyamide/gold nanoparticles was 0.102 μM^{-1} $\mu A.mm^{-2}$. A calibration curve in this concentration range can be seen in Fig. 3. Besides the oxidation current, the reduction current can also be used to determine the concentration of capsaicin. This regularity is observed at a potential of 0 V. Detection limit of reduction current on the electrode polyamide 11 gold nanoparticles is 0.5 µM. The reduction current detection limit has the same value as oxidation currents. The range of linear concentration was 230 µM. The calibration curve in the concentration range of 230 µM can be seen in Fig. 4. From linear regression, calibration curve equation y = -16.69 - 0.21x the value of R for -0.97535 can be obtained. Based on the linear equation, it can be seen that the sensitivity of the electrode is equal to -0.268 mA $\mu M^{-1}.mm^{-2}$, where the negative value indicates reduction current, while the value exhibits positive oxidation current.

Comparison with the patent number WO 2009/115840A1 indicates that the polyamide11-gold nanoparticles modified gold electrode has nearly the same value of LOD (Table 1). Thus, the polyamide11-gold nanoparticles modified gold electrode is demonstrated as an alternative spicy sensor for industrial purposese

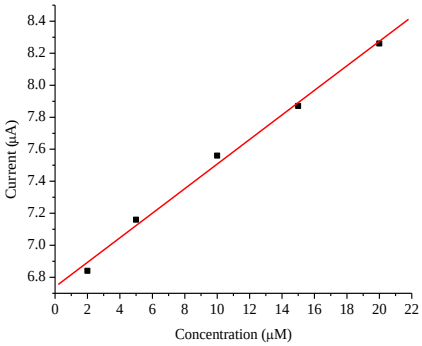

Fig 3. Calibration curve of anodic..

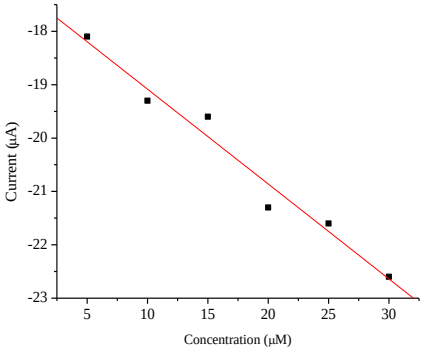

Fig 4. Calibration curve of cathodic

Table 1. Performance comparison of spicy sensor.

Electrode	Linear range (µm)	LOD (µM)	Sensitivity ($\mu A.\mu M^{-1}.mm^{-2}$)	Reference
Modified MWCNTs	0.5-35	0.45	-	[19]
Modified gold	2-20 (anodic) and 5-30 (cathodic)	0.500 (for anodic and cathodic)	0.102 (anodic) and -0.268 (cathodic)	This work

2.2 APPLICATION ELECTROCHEMICAL SENSOR FOR MEDICAL

Since medical analysis in laboratory are expensive and time-consuming process, more measurements of analytes are performaed in various locations, including hospital point-of-care setting, by caregivers in non-hospital settings and by patients at home. Today one of main challenges is the development of methods to perform these rapid 'in situ' analysis. These methods must be sensitive and accurate, and able to determine various substances with different properties in 'real-life' samples. Electrochemical sensors for the measurement of analytes of interest in medical analysis are ideally suited for these new application, due to their high sensitivity and selectivity, portable field-based size, rapid response time and low-cost. We already develop and use the extract of leaves and pulp of Maja (*Aegle marmelos*), Indonesian original plant, to modify the silver electrode and used it as urea and glucose biosensors. Similar with spicy sensor, we also provide a simpler process to urea and glucose detection.

2.2.1 UREA SENSOR

Urea is widely distributed in nature and its analysis is of considerable interest in clinical and agricultural chemistry. It is known to be an important marker for evaluating uremic toxin levels. The normal level of urea in serum is from 15 to 40 mg/dl (2.5–7.5 mM/l). In patients suffering from renal insufficiency, urea concentrations in serum vary from 180 to 480 mg/dl and, at elevated levels above 180 mg/dl, hemodialysis is required [8,12]. Hence, urea detection is important. In our study, we presented another alternative method for urea detection, that is electrochemical non-enzymatic biosensor using the extract of Maja leaf [5].

The composition of the electrode was reported in our previous paper [5]. The response of electrode was presented at Fig 5. It shows that the Maja leaf extract-modified silver electrode can detect urea without any interference from glucose. This result prove that the electrode have a good selectivity. The cathodic and anodic current signals are appears at 0.4 V and 0.625 V, respectively. The performance of the electrode also proven with LOD and sensitivity. According to Table 2, our Maja leaf extract-modified silver electrode is better that the other studies.

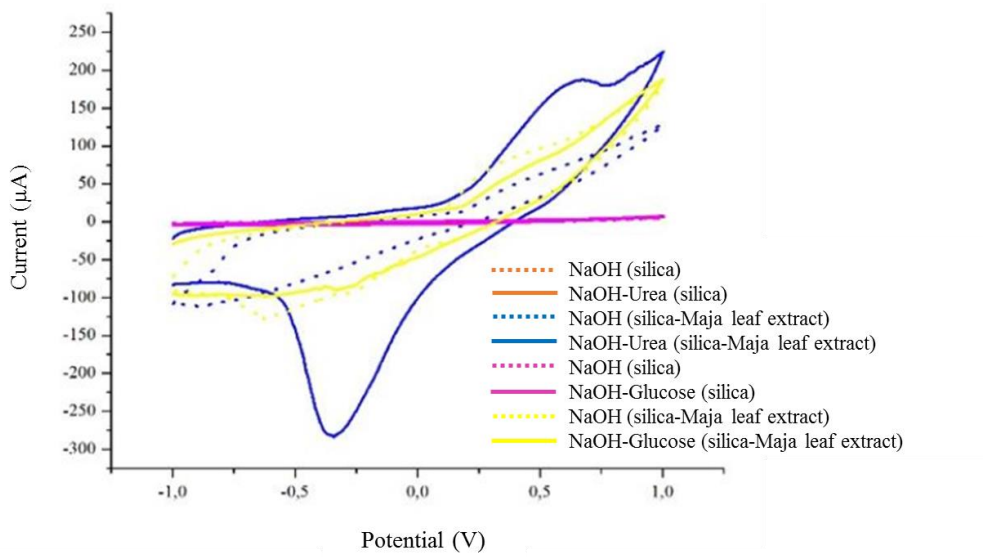

Fig 5. Response comparison between silica gel and silica gel/Maja leaf extract to glucose solution and urea at pH 13 condition.

Table 2. Comparison of the non-enzymatic urea biosensors.

Electrode	Linear range (μM)	LOD (μM)	Sensitivity ($\mu A.\mu M^{-1}.mm^{-2}$)	Reference
Modified Pt	80-1440	40	1.11	[8]
NiO/cellulose/CNT	10-1400	7	371	[12]
Ni-MOF/MWCNT	10-1120	3	685	[20]
Modified silver	1-6 (anodic) and 2-7 (cathodic)	2.299 (anodic) and 5.165 (cathodic)	19.781 (anodic) and 38.177 (cathodic)	This work

2.2.2 GLUCOSE SENSOR

Development of simple, reliable and fast methods for glucose detection is important, especially for medical diagnostics. The methods usefull to determination of glucose in the blood for diabetes screening and treatment. Many researcher reported and develop the electrochemical glucose sensors and biosensors both in enzymatic or non-enzymatic form. Enzymatic sensors normally have a short life due to the properties of the enzyme, which is very easy to decompose. Therefore, we used Maja pulp extract to modify silver electrode for non-enzymatic glucose biosensor [4].

The composition of the electrode was reported in our previous paper [4]. The response of electrode was presented at Fig 6. It shows that the Maja pulp extract-modified silver electrode can detect glucose. The signals of cathodic and anodic current discovered at 0.38 V and -0.25 V, respectively. There is no interference signal from urea and ascorbic acid. The performance of our Maja pulp extract-modified silver electrode was compared with other studies, which summarized at Table 3. It justifice that our electrode show a better performance than other research.

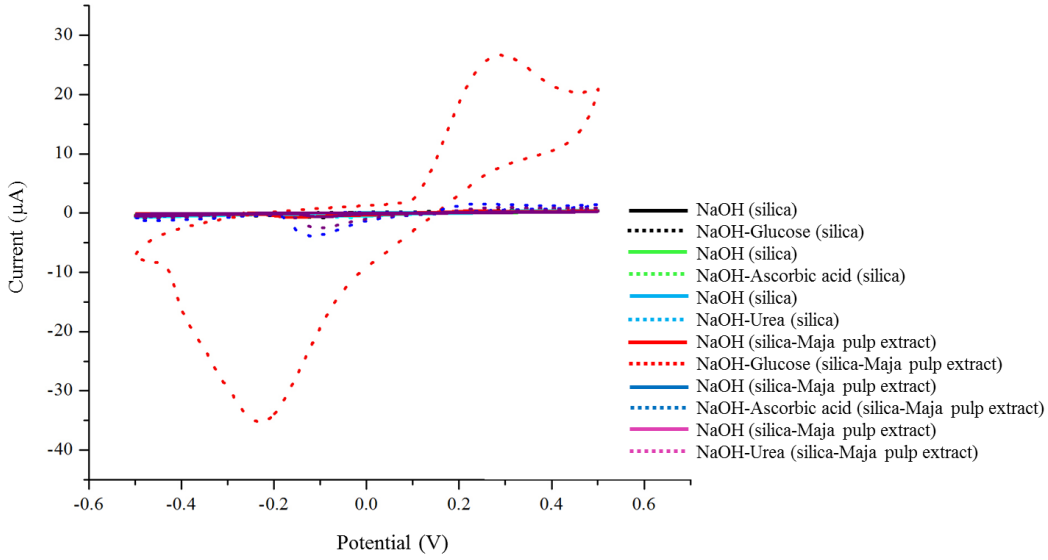

Fig 6. Response comparison between silica gel and silica gel/Maja pulp extract to glucose solution and urea at pH 13 condition.

Table 3. Performance comparison of the non-enzymatic glucose biosensors.

Electrode	Linear range (μM)	LOD (μM)	Sensitivity	Reference
Nanoporous Au	1000-18000	3	0.002 $\mu A.\mu M^{-1}.mm^{-2}$	[21]
ZnO/MWCNT/GCE	1000-10000	820	0.0064 $\mu A.\mu M^{-1}.mm^{-2}$	[22]
Micro/nano hybrid structured	55.6-13890	9	0.0749 $\mu A.\mu M^{-1}.cm^{-2}$	[23]
Modified silver	0-4 (anodic and cathodic)	1.920 (anodic) and 1.701 (cathodic)	1.222 (anodic) and 1.522 $\mu A.\mu M^{-1}.mm^{-2}$ (cathodic)	This work

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Polyaniline-Modified Zeolite NaY: A New Sorbent for Dispersive Solid Phase Extraction of Multiclass Pesticides

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1. INTRODUCTION

Pesticide residues are highly concerned as one of contaminants in environment, foods and agricultural products since their applications are continually expanding and their consumptions are ever increasing. Trace analyses of these substances require analytical techniques for the detection of the greatest number of compounds possible, with the fewest number of extraction and clean-up steps. The analytical techniques for these trace residues usually consist of sample preparation and quantification using analytical instruments. Our work is intended to developed new materials for preconcentration of chemical residues as well as to propose a sample preparation procedure [1]. A novel polyaniline (PANI)-modified zeolite NaY was proposed as sorbent for the dispersive solid-phase extraction (DSPE) of diverse pesticide residues [2].

2. METHOD

The PANI-coated zeolite NaY sorbent was created via oxidative polymerization of aniline onto the surface of the zeolite. The sorbent was applied for DSPE of commonly used pesticides belonging to five different chemical groups, including carbamate, organophosphate, sulfonylurea, pyrethroid and neonicotinoid. To perform the DSPE, 150 mg sorbent was added to 125 mL of sample. A suspension was then shaken for 4 min to promote the sorption of the analytes onto the sorbent before transferring to the SPE eluting column. The column was washed with 10 mL water before eluting with an appropriate eluent. The final extract was analyzed by HPLC-PDA.

3. RESULTS AND DISCUSSION

The applicability of synthesized PANI-modified zeolite NaY as a sorbent for multiclass pesticides extraction was investigated. An average sorption capacity of 833 mg kg^{-1} sorbent was reached. The experimental conditions of the DSPE procedure were optimized. The coupling of DSPE and HPLC resulted in an efficient method for multiresidue analysis of pesticides. The preconcentration factor of up to 42 was observed. The LODs and LOQs of the proposed method were found in the ranges of 0.001–1.00 mg L^{-1} and 0.005–2.50 mg L^{-1}, respectively. The method was successfully applied to the determination of pesticide residues in environmental and food samples. The satisfactory recoveries were obtained.

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Mathematical Model of a Growing Tumor and Its Interaction with Immune System: The role of dendritic cell in controlling the immune system

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1. INTRODUCTION

Immune system such as natural killer (NK) cells and cytotoxic T cells are capable of recognizing and killing tumor cells. In recent years, it is found that dendritic cells (DCs) also have a potential role in controlling a tumor growth, besides their function as a regulator of immune system [1]. This talk relates to a mathematical model of a growing tumor and its interactions with the immune system that is emphasized in the role of dendritic cells in controlling immune system. First, we construct a mathematical model in the form of system of ordinary differential equation. Then, existence of the equilibrium point and their stability are analyzed. We also investigate which parameters that play important role in this model. Second, we develop a hybrid cellular automata model that can describe the system in more detail, including cell-cell interactions of every single cell in the system. Besides providing more detail of the immune system, this model also include the chemokine which function to activate dendritic cells. Furthermore, to include the effect of chemokine, we use a partial differential equation to describe the concentration of chemokine.

2. METHOD

First, we construct the model based on some assumptions stated in [2]. This model consists of tumor cells, NK cells, cytotoxic T cells, DCs and chemokine in the form of system of nonlinear ordinary differential equations. The stability analysis is done by linearizing the system near equilibrium points and Routh-Hurwitz creterion is then used. In the second model, we combine the numerical solution of partial differential equation model with the discrete cellular automata used to model individual cells [3]. Finally, some numerical simulations are conducted to illustrate the behavior of the solution of both models.

3. RESULTS AND DISCUSSION

Based on analysis result, the first model has two equilibrium points which exist and asymptotically stable under certain conditions. It is also obtained that the parameters such as tumor growth, NK cells tumor cell kill rate, death rate of NK cells and source term of NK cells play important role in the growth of tumor.

Hybrid cellular automata model can describe the evolution of the tumor cells both in space and in time. From simulation, increasing the concentration of DCs will decrease the concentration of tumor cells.

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Spatial Panel Dynamic Econometrics Model of Land Value, Land Use Externalities and Their Dynamic: Case Study of the Jakarta's Fringe

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Abstract – Land value is the product of past decision of its use leading to its value. It is also affected by the local characteristic and the observed surrounded land use (externalities) from the previous period. The effect of each factor on land value has dynamic and spatial virtues. An empirical land value model with a spatial panel dynamic setting is appropriate to capture them. The model will be useful to estimate the extent of land use externalities on land value in the short run as well as in the long run. It serves as a basis to formulate an effective urban growth management's policy. The objective of this paper is to derive the indirect effects – externalities on land value and their dynamic. The result will be applied to analyze the significance of land use externalities on land value in the fringe of Jakarta Metropolitan. There is some evidence about the significance of neighborhood urban activity – negative externalities, the previous land value and local accessibility on land value. The effects are accumulated dynamically over years, but they will fully affect the land value after 5 until 6 years.

How Data Science Shapes Personalized Medicine Revolution

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Abstract – Personalized Medicine coined firstly in 1969 is defined as tailoring of medical treatment to the individual characteristics. This field has made significant progress with key discoveries boosted by rapid development of new biotechnologies such as Microarray Chip and Next Generation Sequencing (NGS).

From the completion of Human Genome Project 2003 scientist in this field have produced billions of data points on properties, structure and functions of genes, proteins and other molecules are compiled in enormous databases and systematically studied. As the price is getting cheaper, the growth of the data on genomes, proteins and cells would be unstoppable by approximately 25 petabyte/year worldwide.

Dealing with this "big data" requires a different approach and poses challenges for data scientist in retrieving, managing, visualizing, analyzing, interpreting and presenting the data. Conventional statistical approaches can no longer be used. Numerous advanced statistical techniques have been proposed to understand and extract more insight and information of the biological data.

The objective of this article is to review and describe the role and increasing importance of data scientist in the research of personalized medicine, as well as discuss the challenges and some current topics.

Furthermore, this article would discuss the development of various advanced statistical techniques from simple to the latest implemented in Bioinformatics in shaping the revolution of personalized or precision medicine. Wide range approaches in classification and clustering techniques used in Microarray and NGS would be presented. Future development and research direction would be discussed as well.

In conclusion, data scientists not only have major role in all aspects of discovering precision medicine but also would be able to speed up the translation of all findings to the clinical practice.

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Energy

Two Dimensional Seismic Modeling for the Identification of Free Gas in Marine Sediments

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Abstract – Seismic modeling is basically a construction of geologic computer model, which simulating the seismic wave propagation responses of the earth. The synthetic seismic traces generated are then compared with the real seismic data. If the geological models are known with sufficient accuracy, such synthetic responses are used to validate the choice of acquisition and processing parameters, and to verify interpretation decisions. The physical properties of earth materials are not uniform because subsurface variations occur in lithology, porosity, mineralogy, density, permeability, and pore fluids. To understand wave propagation in these materials, simplified mathematical models are usually constructed.The goals of this study is to identify the presence of free gas by performing seismic modeling. This paper investigates the relative abundance of free gas associated with the BSR by modeling the reflection coefficient, or amplitude changes. The models are based on multichannel seismic profiles, and seismic velocity data.
Because the strength of the reflected signal is proportional to the change in elastic impedance (product of velocity and density), the top of free gas sediment produces a strong reflection amplitude (bright spot). Furthermore, the reflection coefficient for the top of free gas was estimated and evaluated for elastic isotropic situations. The presence of gas produces a significant reduction of seismic wave velocity in the reservoir rocks, relative to the water-saturated condition. The low velocity zone can be directly related to the derived interval velocity from RMS, and/or stacking velocity when layer boundary is flat and constant velocity is assumed based on Dix's equation, and thus the separated reflections signals can be used to image the top and the bottom of free-gas bearing zones.

1. INTRODUCTION

Reflection and transmission of plane wave boundary between two media are one of the most fundamental problems in wave propagation. The seismic amplitudes represent, primarily, contrasts in elastic properties between individual layers, lithology, porosity, pore-fluid type and saturation and pore pressure –, the information that cannot be gained from conventional seismic interpretation.

One such seismic model assumes only the propagation of compressional, or P-wave types, and is usually called the acoustic media model. However, when a P-wave strikes an interface between two solids, at an angle that is below the critical angle, it generates reflected and transmitted P- and S-waves. Similarly, an incident SV-wave also generates reflected and transmitted waves of both types. Such a process called mode conversion. Models that consider such effects are called elastic-media models and fully consider the propagation of S-waves and mode-converted waves, in addition to P-waves.

2. METHODS

Mathematically, the propagation of waves can be described by solving the wave equation, which is based on two fundamental laws of physics, i.e., Newton's second law of motion (F=ma), and Hooke's law of elasticity (related to the stress deformation). Properties of isotropic elastic medium are described by 3 spatial-varying parameters: compression-wave velocity of Vp(x_1, x_3), shear-wave velocity Vs(x_1, x_3), and density ρ (x_1, x_3). $\mu = \rho V_s^2$, which correspond to elastic constants $a_{13} = \lambda$, $a_{55} = \mu$.

Aki and Richards (1980) and Shuey (1985) give an approximation relation of how the reflection amplitudes vary with the rock properties. The approximation at a small reflection angle is given by Aki and Richards approximation. At normal incidence, the magnitude of the reflection coefficient from soft rock to hard rock is the same as from hard rock to soft rock, whereas at non-normal incidence, the magnitude of the reflection coefficient is generally different from soft rock to hard rock, and from hard rock to soft rock. Generally speaking, soft rock refers to sedimentary rocks, and hard rock refers to igneous and higher grade metamorphic rocks.

One of the issues in AVO analysis is observing the AVO response of gas-bearing reservoirs. In this synthetic calculation, the parameters of gas hydrate and free gas, as well as the density, are chosen from the paper of Ecker

et al (1998). The layer model and the parameters used in numerical simulation are shown in Figure 1 and Table 1 respectively.

As a reference calibration, the seafloor reflection was modelled. A Poisson's ratio of 0.5 for deep seafloor sediments is estimated from the data of Hamilton (1979), and the simple relation between Poisson's ratio and Vp of Castagna et al (1985) using seafloor velocity of 1,5 km/s. The selected velocitiy for sediments with gas hydrate is about 2.7 km/s and for gas-saturated sediment is 1.74 km/s. Figure 2 shows the corresponding synthetic seismogram at 6 km long (468 channels) receiver streamer with interval 12.5 m. The source depth is 8 m while the receiver depth is 9 m. The ricker wavelet is used with dominant frequency 20Hz. The total wave recorded length is 4s with sampling rate 2 ms.

3. RESULTS AND DISCUSSION

Because the strength of the reflected signal is proportional to the change in elastic impedance (product of velocity and density), the base of hydrate sediment produces a strong reflection amplitude. This characteristic seismic reflector, which runs parallel to the seafloor and with reversed polarity compared to the seafloor reflection has been termed as bottom simulating reflector (BSR).

Therefore, the BSR marks an interface across which there is a decrease in elastic impedance. This behavior was explained by a high P-wave velocity above the BSR and an anomalously low S-wave velocity above the BSR than that below the BSR [4]. Amplitude of the reflection from just below the base of the hydrate reflection is quite strong, indicating a sharp contrast in the velocity that may indicate the base of free gas layer.

Further, the reflection coefficient for BSR was estimated and evaluated for elastic isotropic situations. As the comparison, the reflection coefficient was also calculated based on Aki and Richards approximation, then the AVO curve for gas-bearing sediment is shown in Figure 3. It is observed that the absolute values of the gas-saturated sediment curve show an increase in amplitude. The variation of reflection amplitude versus offset or amplitude versus incidence angle can be an important indicator of free gas at an interface [9]. A downward transition to sediment containing free gas with low Poisson's ratio, from overlying sediment with or without hydrate having a more normal Poisson's ratio should give reflection (negative) amplitudes that become substantially larger with increasing incidence angle or offset [8]. The BSR reflection amplitude as a function of source-receiver offset depends on the compressional velocity (Vp), shear velocity (Vs) and the density structure. The AVO behavior also depends primarily on the contrast in Poisson's ratio.

Based on the pre-defined model, Vp for hydrate sediment is about 2.7 km/s and for gas saturated sediment is about 1.74 km/s. As the background lithology, the brine-saturated sediments was used which is above the hydrate sediment layer and below the gas saturated sediment layer with Vp is about 2.37 km/s [3]. Then by changing the Vp and Vs values separately, the variation of reflection coefficient is observed. First of all, the initial Vp is adding and/or reducing by 5%, 25% and 65%. The reflection coefficient is calculated based on Aki and Richard's approximation. By changing the Vp value, it can be observed that if Vp is increasing then the reflection coefficient become lower. On the contrary, when Vp is decreasing then the reflection coefficient value become higher (more negative) (c.f. Figure 4). Similar behavior also occurred in the second case (Figure 5) that both Vp and Vs are changed by adding and/or reducing with 5%, 25% and 65%. The lower reflection coefficient will be obtained if Vp and Vs are increasing, and if Vp and Vs are decreasing then the reflection coefficient would be higher (more negative). So, this kind of simulation is performed to observe the changing of Vp and/or Vs in affecting the reflection coefficient value.

Table 1. Model parameters (Ecker et al, 1998)

	P-wave Vp (km/s)	S-wave Vs (km/s)	Density ρ (g/cm³)	Poisson's ratio σ
Sea water	1.50	0.00	1.00	0.5
Brine-saturated sediments	2.37	1.10	2.17	0.36
Sediments with gas hydrate	2.70	1.11	2.11	0.39
Gas-saturated sediments	1.74	1.13	2.05	0.13
Brine-saturated sediments	2.37	1.10	2.17	0.36

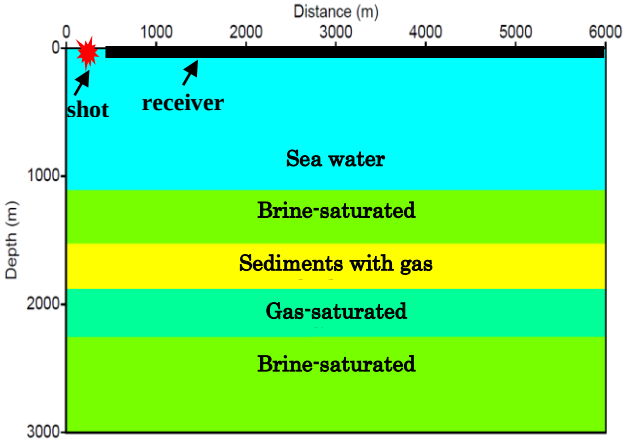

Figure 1. Comparison of different Ricker wavelets 10-100 Hz (left) and table of model parameters (Ecker et al, 1998)

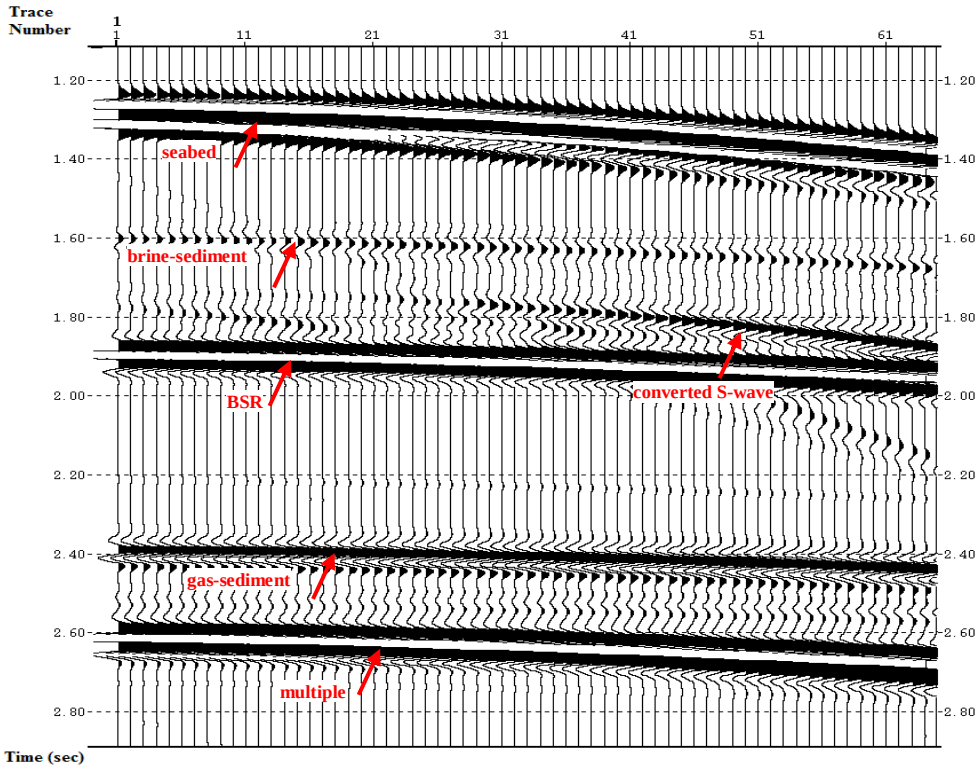

Figure 2. Synthetic seismograms for gas hydrate and free gas model

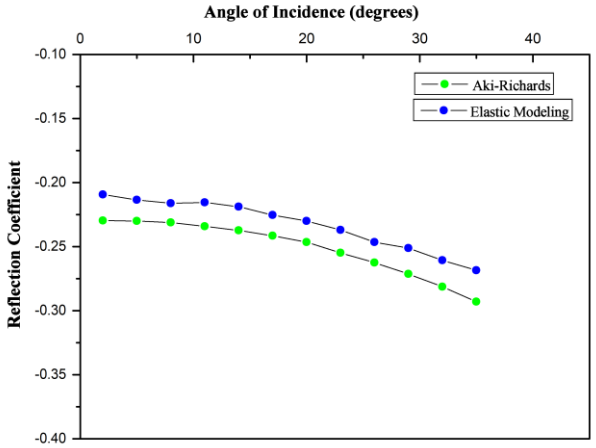

Figure 3. Reflection coefficients (RC) of BSR. The reflection coefficient shows the negative trend with increasing incidence angle. The difference in the estimated RC from layer-over-half-space model of Aki-Richards theory appears to be underestimated. The scaling factor is 0.02.

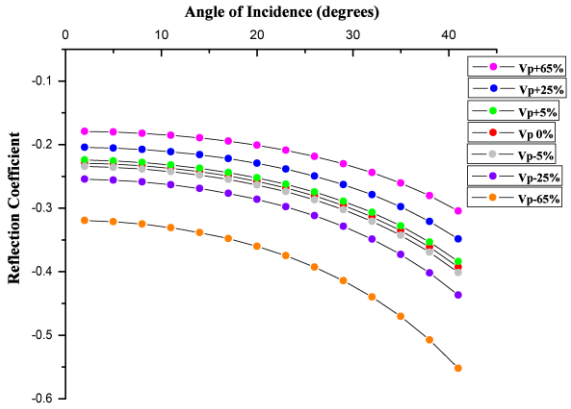

Figure 4. Variation of Reflection coefficient values corresponds to the perturbation of Vp changes. P-wave velocity varying from -65%, -25%, -5%, 0%, +5%, +25% and +65%. When Vp is increasing then the reflection coefficient become lower. On the contrary, when Vp is decreasing then the reflection coefficient value become higher (more negative)

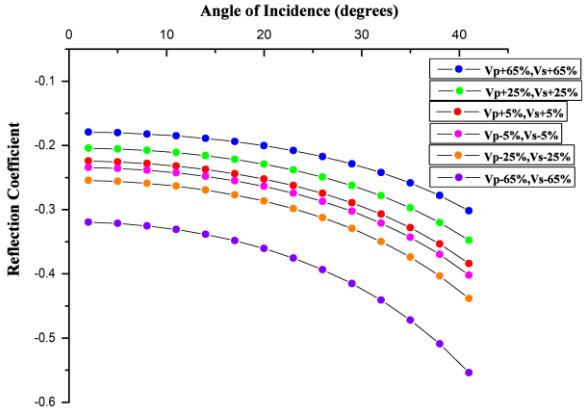

Figure 5. Reflection coefficient values with Vp and Vs changes. The lower reflection coefficient will be obtained if Vp and Vs are increasing, and if Vp and Vs are decreasing then the reflection coefficient would be higher (more negative)

4. CONCLUSIONS

Based on the observation, it can be stated that the absolute values of the gas-saturated sediment curve show an increase in amplitude. By changing the Vp value, it can be observed that if Vp is increasing then the reflection coefficient become lower. On the contrary, when Vp is decreasing then the reflection coefficient value become higher (more negative).

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Analysis on Electrical Energy from Cylindrical-Buoy-Type Sea Wave Power Plant Model

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Abstract – As **e**lectrical energy needs keep growing as technology and information advances, so it is necessary for the existence of renewable energy as a source of electrical energy in the future. One of them is sea wave energy. In previous studies, it has been discussed about cylindrical buoy type sea wave power plant with dimensions of length variation of the swing arm and the length of the buoy to the energy generated. Therefore, this research will discuss about the influence of different inertia due to the ballast mass-base swing arm distance variation and the generator wire diameter variation, on the laboratory scaled sea wave power generator, to the generated electrical energy. The system modeling and numerical simulation are used for getting the generated power results. The simulation parameter variations arethe ballast mass - base swing arm distance, 7 cm, 15 cmand 23 cm, and the the generator wire diameter, 0.2 mm, 0.3 mm and 0.4 mm. The ballast mass - base swing arm distancedoes not give the significant effect. Whereas the greater generator wire diameter gives the higher generated power. The greatest occurson the 0.4 mm wire diameter with value 0.0265 W. The system has 3% of efficiency. The system with the same mechanism and on the real sea scaled (1:37) will give 95.97 W generated power with the 0.4 mm wire diameter and 2.9 m average sea wave height.

1. INTRODUCTION

Sea wave is one of many kinds of renewable energy source. The previous researches inform that the sea energy has potential to provide the important contribution for future energy needs [1,2]. This kind of energy has been existed since decades ago, and provided great contribution recently [3,4]. There are three common ways how sea based energies can be converted to electrical energy, such as sea wave, tide, and flow. Sea wave energy generation have many kinds of form. One of them is with buoy. The bouy based application can use power transmission to rotary type permanent magnetic generator with translation to rotation motion gearing mechanism [6]. The previous researches also effort the maximum result by developing its generator [5,7]. Zulfikar *et al* estimated the potential sea wave energyon the Java Sea with 3 m wave height [8]. Made *et al* simulated cylindrical buoy type sea wave electric generator with buoy dimension and its arm lengthvariations, and concluded 25 Watt generated energy on real scale.

This research provides the results of influence of ballast mass addition to the bouy arm and rotary generator wire diameter not been researched before. The varied ballast mass-buoy arm base distances are 7 cm, 15 cm, and 23 cm. The generator wire diameters are 0.2 mm, 0.3 mm, and 0.4 mm. The objectives are getting model, simulating it, and analyzing the generated energy characteristic. This model can be the base for designing and building the real scaled object.

2. METHODS

The cylindrical buoy type sea wave electrical energy generator is simulated by using 3 main section model mechanisms, buoy with arm, base shaft, and variation of its ballast mass position-arm base distance; gearset with 1:4 velocity ratio and one way bearing addition; and DC generator with wire diameter variation. The modeling uses state-variable equation method, then it is applied to numerical software, solved with ODE4 type and time interval 0,001 sec, and gotten its electrical power output. The model of mechanicalis showing in Figure1.

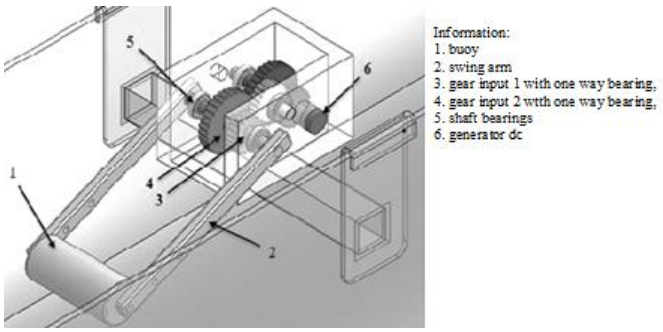

Figure 1 (The mechanical model schematic)

The simulation and modeling of sea wave power plant cylindrical buoy type is done by state-variable method. Where do the simulation process by several stages, the first stage begins with a mathematical equation - and the second stage is to make state-variable of system and after that, making the block-diagram of numerical simulation software, simulations performed by harmonic excitation by variation the parameters used, after which the power output in watt.

Inputs used in the simulation model is in the form of sea wave equation obtained from the reference [6] as follows:

$$F_w = \frac{\rho g^2 H^2 T^2}{64\pi\lambda}\left(\cos\frac{2\pi t}{T}\right)^2 \quad (1)$$

Where *Fw* is the wave force, gravitational acceleration *g* (9,8 m/s^2), density of water ρ (1000 kg/m^3), amplitude *A* (m), wave length λ in (m), and the wave period *T* (second).

While modeling cylindrical buoy and ballast mass-base swing arm distance variation on the dynamic condition is as follows.

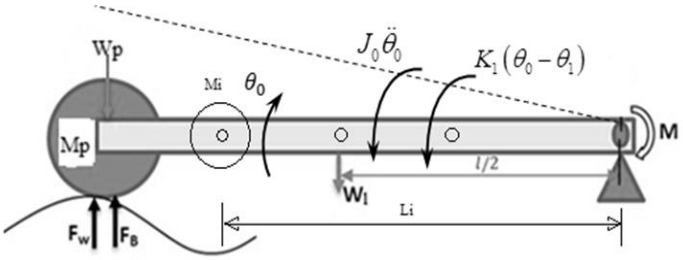

Figure 2 (swing arm mechanism with a ballast mass and buoy)

where the equation is

$$\ddot{\theta_0} = \frac{1}{M_P L^2 + \frac{1}{3}M_i L^2 + M_i L_i^2}\left[\frac{\rho g^2 H^2 T^2}{64\pi\lambda}\left(cos\left(\frac{2\pi t}{T}\right)\right)^2 L + \frac{d_2}{d_1}\frac{d_7}{d_6}k_1\theta_8 - k_1\theta_0\right] \quad (2)$$

Where θ_0 same as ω_0 is the movement of the swing arm (rad/s), weight of the buoy *Wp* (kg), wave force *Fw* (N), bouyancy force *Fb* (N), weight of the swing arm *Wl* (kg), weight of the ballast mass *Mi* (kg), ballast mass distance *Li* (m), inertia of swing arm (kg.m^2), rigidity the shaft K_1 (Nm/rad).

From figure 2 is shown the model structure of mechanism of sea wave power plant cylindrical buoy type, where *Mp* is mass of buoy (kg), wave force *Fw* (N), bouyancy force *Fb* (N), ballast mass *Mi* (kg), swing arm mass *Ml* (kg), inertia of the swing arm and buoy J_0 (kg.m^2), inertia equivalent of gear box J_{Eq} (kg.m^2), as much as 7 gears. The equation is :

$$\ddot{\theta_8} = \frac{1}{\left[J_{Eq}\left\{\frac{d_7 d_2}{d_6 d_1}k_1\theta_0 - \frac{d_7^2 d_2^2}{d_6^2 d_1^2}k_1\theta_8 - 2NBLri(t)\right\}\right]} \quad (3)$$

Where $\ddot{\theta_8}$ is motion of the armature, diameter of gears *d* (m), whereas the equation of motion the DC generator model is :

$$\frac{d_i}{d_t} = \frac{1}{L}\left(2NBLr\theta_8 - R_{load}\,\dot{\imath}\,\frac{4\rho_{wire}L_{wire}}{\pi d_{wire}^2}\,i\right) \quad (4)$$

With R_{load} is resistance of gauge (Ω), electrical inductance *L* (H), number of coil *N*, magnetic flux *B* (T), length of coil *l* (m), radius of coil *r* (m), length of wire L_{wire} (m), the copper resistivity ρ_{wire} (Ωm), wire diameter *d* (m).

The equation is entered into the simulation is the equation 2, 3 and 4.

3. RESULTS AND DISCUSSION

The simulation on cylindrical-buoy-type sea wave power plant model employed some variations i.e. the augmentation of ballast mass on the distance variations and the alteration of wire diameter on the DC rotary generator. The variations on the distance between the ballast mass and swing arm were 7.15 cm and 23 cm while those on the wire diameter of generator were 0.2 mm, 0.3 mm, and 0.4 mm. The another parameters for used in the simulation is shown on table 1.

Table 1 (The parameters of sea wave simulation)

Symbol	Predicate	Value	Unit
H*wave*	High sea wave	8.04	cm
T*wave*	Wave period	1	s
F*wave*	Wave frequency	1	Hz
A*wave*	Wave amplitude	4.02	cm
P*water*	Density	1000	kg/m^3
g	acceleration of gravity	9.81	m/s^2
λ*wave*	Wave length	0.97	cm

The parameter is used at the numerical simulation by using available equation can be got the result as following :

Then the power that is resulted by the ocean wave simulation is showed at the figure 3.

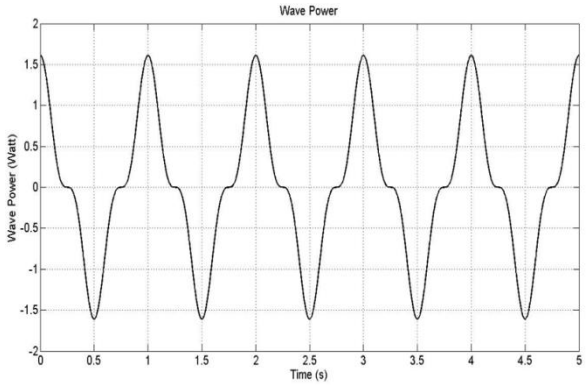

Figure 3 (Sea wave power chart)

The RMS power that is resulted by sea wave simulation at the laboratory scale is used 0.9018 watt.

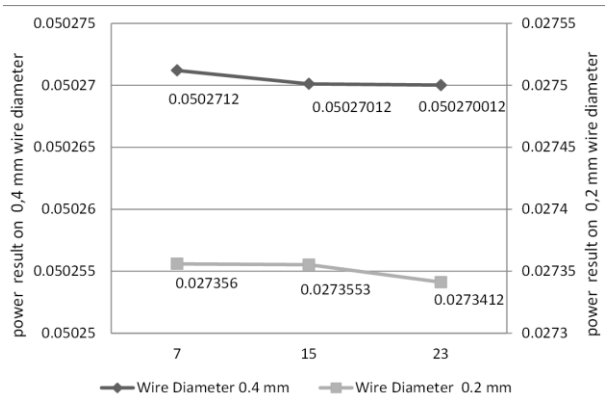

Figure 4 (The chart of distance variations of ballast mass in swing arm on the generator with wire diameter 0,2 and 0,4)

Figure 4 is showed about the result of variation of voltage toward the distance between ballast mass on the swing arm. The graphic is shown about decline trend. At the distance 7 cm has the biggest result affected the system and the lowest result is showed at 23 cm. The phenomenon is caused by the mass increased at the swing arm that is not affected the insetia system too much, so the energy resulted has not different. But the inertia become bigger when the ballast mass is increased. When the position of the ballast move at near the shaft center can make the power resulting become increased.

The simulation result is showed the distance of ballast mass is not too large, which may affect the system, that is caused by the ballast mass is small and the distance between the ballast mass is short.

Meanwhile, the result of variation of wire diameter of generator is showed at the figure 5.

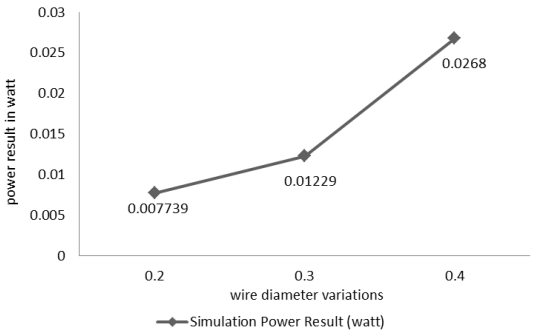

Figure 5 (The RMS power resulted on wire diameter variations chart)

From the figure 5 is showed about the result of simulation with different wire diameter that is used at the generator DC. The lowest resulted at the wire diameter 0,2 mm by 0.0077 watt, and the wire diameter 0.3 mm by 0.01229 watt. The biggest result at the wire diameter about 0.4 mm by 0.0268 watt. The result of simulation is very small because the scale that is used on the laboratory scale.

The scale of the real dimension refer to the research before [8] that is used at 1:37. The ratio is used by refer to the high of real sea wave. And also the volume of mechanism become increased reach the real dimension. The real dimension of sea wave electrical generator on cylindrical buoy can be resulted the power about 95.97 watt at the average high of sea wave at 2.9 m

4. CONCLUSIONS

According to the result obtained from analysis and simulation in this research, then can be concluded.

1. From the ballast mass variations on swing arm, the lowest resulted at the distance 23 cm at RMS power is 0.007738 watt, and the better resulted at the distance 7 cm at RMS power 0.007739 watt.
2. From the wire diameter variations that is used at the generator DC. The lowest resulted at the wire diameter 0,2 mm by 0.0077 watt, and the biggest result at the wire diameter 0,4 mm by 0.0268 watt. The result obtained by the laboratory scale.

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Poly (Eugenol Sulfonate) - Sulfonated Polyetherimide - Titanium Dioxide (TiO_2) New Blends Membrane Promising For Direct Methanol Fuel Cell (DMFC)

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Abstract – Proton conducting new blend membrane has been prepare with ternary mixtures comprising poly (eugenol sulfonate) (PES), sulfonated polyetherimide (SPEI) and titanium dioxide (TiO_2) for direct methanol fuel cell (DMFC). The membrane was characterized by Fourier transform infrared spectroscopy (FTIR), and scanning electronic microscopy (SEM). Ion exchange capacity (IEC), proton conductivity, methanol barrier, water uptake, and water contact angle of the membrane, were also being determined. The new PES-SPEI-TiO_2 membrane with 3 wt.% PES, 20 wt.% SPEI, 5wt.% TiO_2, show higher IEC, water uptake, proton conductivity and methanol barrier properties as compared to Nafion 117 membrane. As a conclusion, the results indicate that the PES-SPEI-TiO_2 membrane has potential to be used as PEM for DMFC application.

1. INTRODUCTION

Proton exchange membrane (PEM) has been studied as one of key components in direct methanol fuel cells (DMFC), which can directly convert chemical energy into electrical energy, in a wide range of applications, from vehicles to portable utilities [1]. Nafion is a commercialized membrane for DMFC because it has high proton conductivity, low dimensional change in water, and excellent cell performance in various environments. However, Nafion membranes are expensive, have high methanol permeability, and lose their proton conductivity and durable properties at high temperature (>80^0C)[2]. Therefore, many researchers have endeavored to develop inexpensive membranes using a variety of aromatic polymers. Polyetherimide, an aromatic hydrocarbon based ionomer, has been reviewed as one of PEM materials to alternate Nafion membrane over the past decade because of its high thermal resistance, good mechanical strength, and low methanol permeability [3]. Above all, it has an inexpensive production process for commercialization, and helps to reduce the total cost of PEMFC application. [3]. The cost of polyetherimide (255.4US$/kg) is much lower than Nafion 117 solution (363.7US$/100ml)[3].

Blending is a combine material to improve the comprehensive properties of a matrix [4]. Rajagopalan *et al.* and Guhathakurta *et al*., had successfully synthesized the PEM from SPEI polymer. The conductivity values for the SPEI copolymers at an ion exchange capacity of around 0,553 meq/g were 0.0014 S/cm at 25 °C, indicating that they are promising candidates for PEM materials [3,5]. Also, polymer blending is an effective method to improve the mechanical and chemical stabilities via addition of another more stable components as filler.

In this study, as an effort to realize this goal, we develop a new blend membranes material from sulfonated polyetherimide and poly(eugenol sulfonate), with TiO_2 as filler. Poly(eugenol sufonate) have been aromatic phenol units provide high performance properties such as considerable mechanical strength, thermal stability and chemical resistance, while the flexible sulfonate linkages provide good processability [6]. Titanium dioxide (TiO_2) is a good candidate for the hydrophilic filler for the Nafion and other polymers because it provides suitable hydration of the membrane under fuel cell operation conditions. Studies on Nafion or polymers with TiO_2 composite membranes have attracted the attention of many research groups [7]. Their results show that the morphological properties of the filler play a key role in the performance of the composite membranes at a high operating temperature [7].

2. METHODS

2.1 Chemicals

The polyetherimide and methyl-2-pyrrolidone (NMP) were supplied by Sigma Aldrich. The Eugenol was supplied by Merck, and the Nafion® 117 membrane was purchased from DuPont Co. Sulfuric acid, lead (II) sulfate (PbSO4), dichloroethane, chlorosulfonic acid, dimethylacetamide, isopropanol, sodium chloride, sodium hydroxide were purchased from Sigma Aldrich.

2.2 Procedures

Synthesis of poly (eugenol sulfonate) (PES) [6]

Poly (eugenol sulfonate) was synthesized from eugenol as per our previous study. The poly (eugenol sulfonate) (PES) was synthesized from a mixture of H2SO4, eugenol and PbSO4 in a steam bath at 90 °C. The resulted polymer was analysed using infrared spectrophotometer.

Sulfonation of polyetherimide [3, 5, 8]

The synthesis of SPEI was carried out based on procedure from previous study. First, 10 g of PEI was dissolved in dichloroethane (50 ml) at 60 °C for 5 h. The solution was stirred at room temperature for 1 h. a mixture of chlorosulfonic acid (2.06 ml) and dichloroethane (35 ml) was added drop by drop into the polymer solution under vigorous stirring for 45 min. After 2 h, the final solution was dissolved in DMAc and precipitated in isopropanol. The product was filtered and washed with isopropanol three times to remove excessive acid, and dried in an oven at 50 °C for 48 h. The resulted polymer was characterized using infrared spectrophotometer.

Preparation of PES-SPEI-TiO_2 membrane

Three polymer solutions of PES-SPEI-TiO_2 with different PES-SPEI-TiO_2 mass ratio (15%:3%, 20%:3%, 25%:3%) were prepared for this study. The SPEI and PES polymers were simultaneously dissolved in NMP to form a 15 wt.% solution. The solution was stirred at 60 °C for 12 h. After that, the solution was cooled down to room temperature before being casted on glass plates. The resulted membrane was dried in room temperature for 12 h before being immersed in sulfuric acid for 12 h. The resulted membrane was characterized water uptake, methanol uptake, swelling ratio, ion exchange capacity (IEC), contact angle, proton conductivity and methanol permeability.

3. RESULTS AND DISCUSSION

Table 1 Membrane Properties

Blend Membrane	water uptake (%wt)	methanol uptake (%wt)	swelling ratio (%)	IEC (mmol/g)	Contact Angle (°)
20% PEI	6.1	5	4	0.4	89
20% SPEI	23.5	18	14.7	1.24	78
20% SPEI – 3% TiO_2	49.3	30	12	1.9	68.97
20% SPEI – 5% TiO_2	**47.2**	**29**	**10**	**2.1**	**66.36**
20% SPEI – 7% TiO_2	39	28	9	2.0	66.23
20% SPEI- 3% PES – optimum (5%) TiO_2	**50.6**	**31**	**13**	**2.7**	**58.31**
20% PEI- 3% PES – optimum (5%) TiO_2	24.5	19	8	2.2	72.05
Nafion 117	19.3	41	16.4	0.98	80

Table 2 Methanol permeability and proton conductivity of membranes

Membrane	Proton Conductivity ($S.cm^{-1}$)	Methanol Permeability ($\times10^{-7}cm^2.s^{-1}$)
SPEI 20%	0.0078	10
20% SPEI – 3% TiO_2	0.00017801	11
20% SPEI – 5% TiO_2	0.000180203	17
20% SPEI – 7% TiO_2	0.000181783	19
20% SPEI- 3% PES – optimum (5%) TiO_2	0.002038357	7
20% PEI- 3% PES – optimum (5%) TiO_2	0.000243088	8
Nafion® 117	0.090	25

Based on the result in table 1 Membrane properties and table 2 Methanol permeability and proton conductivity of membrane, the SPEI-PES-TiO_2 membrane with 20 wt.% SPEI loading blend with 3% PES and 5% TiO_2 is the

best composition to be use as PEM for DMFC application. Water uptake, methanol uptake, swelling ratio, IEC, contact angle, proton conductivity and methanol permeability are the important PEM characterizations that need to be calculated for DMFC application. Water uptake and methanol uptake are parameter for ion and methanol exchange membrane. Swelling ratio is stability of membrane in aqueous solutions [9]. Ionic exchange capacity (IEC) is defined as the fixed milli-equivalents of exchangeable group per gram of polymer, usually correspond to the number of sites for proton transfer [4]. Contact angle is degree for hydrophilic or hydrophobic materials. Proton conductivity is one of the most important factor for proton-conducting membranes used in fuel cell. In general, the proton transport in hydrated polymeric matrices is described on the basis of either two principal mechanisms: "proton hopping" or "Grotthus mechanism" and "diffusion mechanism" which water as a vehicle or "vehicular mechanism"[10]. Methanol permeability measures the ability of the membrane to prevent methanol crossover through its structure [11]. They are all parameter interrelated for good performance in DMFC application.

The proton conductivity of the good performance membrane generally increases with high water uptake, low methanol uptake and methanol permeability, high ionic exchange capacity (IEC), low swelling ratio and also reduce degree in contact angle (more hydrophilic material). The uptake of more water improves the formation of the hydrophilic domain that conducts protons [12]. The water uptake of the polymer can be increased by an increase in the ionic group content in the polymer chain, but most often, the uptake of more water increases the swelling of a membrane, leading to the loss of the mechanical stability of membranes and high methanol permeability [12]. IEC value depends on the number of sulfonic acid group in the polymeric membrane chemical structure and can impact water uptake and proton conductivity properties of the polymeric membrane [11]. The IEC value of membrane decreased as the loading of filler increased because agglomerations problem [10]. Therefore, all parameter must be optimized for successful operation in fuel cells.

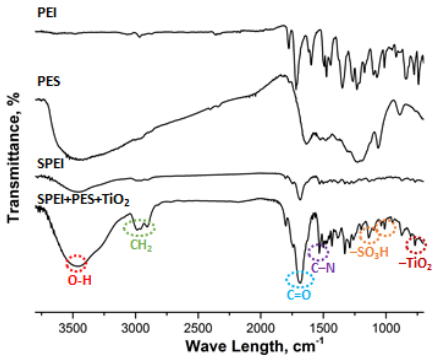

Figure 1. FT-IR spectra of PEI, PES, SPEI and SPEI+PES+TiO_2 membranes

FT IR spectra of PEI, PES, SPEI, and SPEI+PES+TiO_2 membrane are shown in Figure 1. Based on the Figure 1, the bands at 708 cm ^{1}, 1071 cm ^{1}, 1279 cm ^{1} shows that the stretching vibration of SO_3H groups [3, 11]. The broad band around 3600 cm ^{1} was assigned to O H vibration associated with the interaction between sulfonic acid groups and water molecules [3, 11]. The FT IR results confirmed that the sulfonic acid group was successfully introduced to the resulting polymer. Presence of sulfonic acid group is significance for PES/SPEI membrane to allow proton transfer through its structure. The bands for the stretching vibration of the C-N groups of SPEI can be observed at 1467 cm ^{1}, and the stretching vibration of the C=O groups of SPEI and PES can be seen at 1650 cm ^{1}. The bands at 700 cm ^{1} presence of TiO_2.

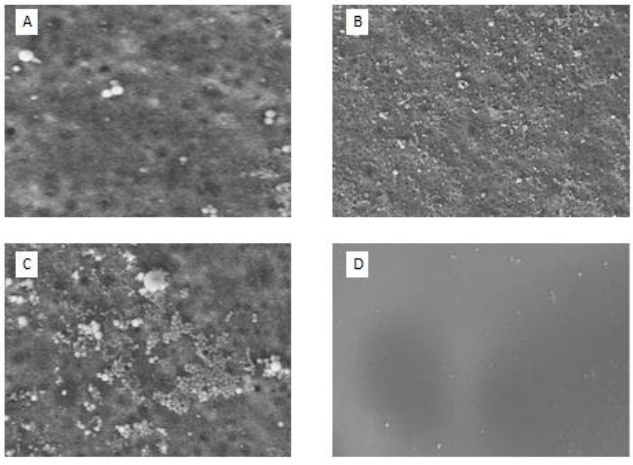

Figure 2 Sem image of (A) 20% SPEI 3% TiO_2, (B) 20% SPEI 5% TiO_2, (C) 20% SPEI 7% TiO_2, (D) 20% SPEI 3% PES 5% TiO_2

Based on Figure 2 (A), the surface of 20% SPEI 3%TiO_2 membrane was less smooth due imperfect casting technique and the TiO_2 filler not homogen yet The Figure 2 (B) shows that the TiO_2 filler was a good dispersion and homogenously blended with the SPEI polymer than figure 2 (A) and 1 (C). Meanwhile, severe agglomeration on the surface of 20% SPEI 3%TiO_2 was observed as shown in figure 2 (C). The SEM images proved that the PES and form a smooth surface SPEI-PES-TiO_2 membrane shows in figure 2 (D).

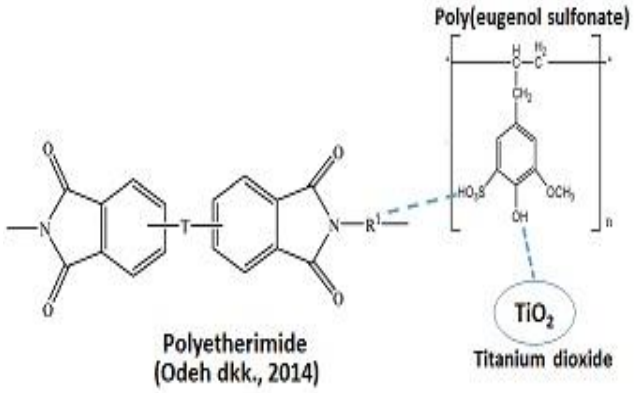

Figure 3 Proton transport mechanism between PES,SPEI and TiO_2

Figure 3 shows that the probably proton transport mechanism between PES, SPEI and TiO_2 in the membrane.

4. CONCLUSIONS

The PES-SPEI-TiO_2 membrane was successfully prepared using solution casting method. SEM image shows that the PES polymer was successfully blended with the SPEI polymer to form a homogenous PES SPEI TiO_2 membrane. The water uptake for PES SPEI TiO_2 membrane increase as the TiO_2 loading increase. The PES SPEI-TiO_2 membrane shows higher proton conductivity and lower methanol permeability values as compared to the Nafion® 117 membrane. Finally, this study can be concluded that the PES-SPEI-TiO_2 membrane has promising as PEM for DMFC applications.

5. ACKNOWLEDGEMENT

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Geothermal Energy: Case Study Identification Based on Analysis of Ion Balance and Reservoir Characteristic

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Abstract – Some samples of data from the world's geothermal system consisting of: (1) geothermal systems Padang Cermin (a. Padang Cermin 1, b. Padang Cermin 2, c. Margodadi, d. Wadok, (2) Kawah Ijen (East Java) , (3) Wairakei (New Zealand), (4) Sea water, and (5) Te Aroha (New Zealand). Analysis of ion balance is performed on the fifth geothermal system data stretcher. Analysis triangle ternary diagram (Cl-HCO3-SO4) performed on all the data from the five samples. Ion balance analysis results showed that: (1) showed good hydrothermal system (ion balance value between 0.1 up to 2.19%), (2) shows that the hydrothermal system is not good (the value of the ion balance 47%), (3) showed good hydrothermal system (ion balance value of 1.2%), (4) shows the hydrothermal system that is not good (the value of the ion balance 64%), and (5) indicate that the hydrothermal system is not so good (the value of the ion balance between 8.5%), (Cl-HCO3-SO4) triangle ternary diagram analysis results showed that: (1) produce a type of chloride water reservoir, (2) produces chloride water reservoir types, (3) produces chloride water reservoir types, (4) produce a type of chloride water reservoir, and (5) to produce the type of carbonate reservoir water.

Keyword: geothermal system, Ion balance analysis, (Cl-HCO3-SO4) triangle ternary diagram analysis.

1. INTRODUCTION

The unifying scheme for classifying geothermal system was very simplified classification on hydrologic style [1]. Geothermal fluids have diverse chemical compositions. Many of these chemical differences depend on the sources of recharge waters and the contribution of volatiles from metamorphic or magmatic sources[2]. Fluid composition change caused by the effect the degree of boiling of mixing. Large-scale fluid hydraulic factors, further determine whether a system undergoes fluid convection in the stagnant reservoir. While general trends in fluid chemistry exist for various geothermal environments. It is our task as geochemists to learn the processes which govern fluid compositions and thereby use this information to understand the individual geothermal system.

2. METHODS

The method in this research using: (1) analysis of ion balance, (2) Cl-SO4-HCO_3 and (3) Na-K-Mg chart analysis. Ion balance is the method of checking how good the chemical composition of the geothermal system. In most solutions are the dominant ions Na^+, K^+, Ca^{+2}, Mg^+, Cl^-, HCO_3^-, and SO_4^{-2}. To calculate the value of the formula used Ion Balance:

□Change% =
{(□cations+□anions)/|□cations+□anions|}x100
Calculation Cl-SO_4-HCO_3 using the formula:
S = cCl + cSO_4 + $cHCO_3$;
% Cl = (100 cCl)/S and % HO_3 = (100 $cHCO_3$)/S.

Calculation Na-K-Mg using the formula:
S = cNa/1000 + cK/100 + $cMg^{1/2}$
% Na = (cNa)/ 10S and % Mg = (100 c $cMg^{1/2}$)/S.
[3]; [4].

3. RESULT

The results of this study are calculation of ion balance and calculation of Cl, HCO3, SO4, Na, K, and Mg concentration in Table 1. While the results of ternary diagrams analysis (Cl-HCO3-SO4) and (Na-K-Mg) are illustrated in Between Figures 1 and 6b.

4. DISCUSSIONS

From Figure 1a. Cl-SO4-HCO3 and NA-K-Mg chart analysis of the geothermal system from spring Padang Cermin 1 (Pc1), indicated that the Padang Cermin 1 geothermal system (Pc1) is chloride water within partial equilibrium. Figure 1b. Cl-SO4-HCO3 and Na-K-Mg chart analysis of the geothermal system from spring Padang Cermin 2 (Pc2), indicated chloride-water system within the immature liquid condition. Figure 1c. Cl-SO_4-HCO_3

and Na-K-Mg chart analysis of the geothermal system from spring Margodadi Padang Cermin (Mg), indicated carbonated water reservoir condition within partial equilibrium condition. Figure 1d. Cl-SO_4-HCO_3 and Na-K-Mg chart analysis of the geothermal system from spring Wadok Padang Cermin (Wadok), indicated chloride water reservoir condition situated between partial equilibrium and immature liquid condition. The Padang Cermin geothermal reservoir should be a hight temperature geothermal system if we compare to Wairakei geothermal system New Zealand). The Wairakei Na-K-Mg analysis shows in Figure 1.

Figure 2. Cl-SO4-HCO3 and Na-K-Mg chart analysis of the geothermal system from spring of Kawah Ijin East Java (IjenS), indicated chloride-water system within the immature liquid condition. Figure 3a. Cl-SO4-HCO3 chart analysis of Wairakei geothermal system well (WrkW), and Na-K-Mg chart analysis of Wairakei geothermal system well of (WrkW) and Wairakei geothermal system spring (WrkS), and Figure 3b. Cl-SO4-HCO3 chart analysis of Wairakei system spring (WrkS). indicated the reservoir of Wairakei is chloride water. The liquid condition is a partial equilibrium in the spring, and full equilibrium in the well and the measures temperatures were 99o C in spring and 240^{o}C in well. The temperature determination using Na-K-Mg chart analysis was consistent with well-measured temperature. Figure 4. Cl-SO4-HCO3 chart analysis of Sea Water (SW), indicated that sea water is rich of chloride altho has much SO4 and HCO3. So that chloride mineral still dominant in sea water. Figure 5. Cl-SO4-HCO3 and Na-K-Mg chart analysis of the geothermal system from Salton Sea well USA (SsUsW), indicated the chloride water and liquid condition of full equilibrium. The liquid condition in full equilibrium so consistent temperature analysis chart of Na-K-Mg with well-measured temperature. For example, the temperature measured the Salton Sea well USA was 330^{o}C and the Na-K-Mg chart analysis more than 300o C (see Figure 5 and Tabel 1). The other example, the temperature measured the Mirabilis was 245o C and the Na-K-Mg chart analysis closed to well temperature measured (see Figure 6a and Table 1). Figure 6b. Cl-SO4-HCO3 and Na-K-Mg chart analysis of the geothermal system Mirabilis spring Cero Pro Mexico (MvCrS), indicated the chloride water reservoir and liquid condition in partial equilibrium.

Table 1. Data and results of geochemical analysis of sea water and geothermal system of Padang Cermin, Ijen Crater, Wairakei, Salton Sea and Miraviles.

No	Station	C	Na	K	Ca	Mg	Li	B	Cl	SO4	HCO3	%Cl	%Hco	%Na	%Mg	HCO/Cl	Ion Balance
1a	Pc1		1243	93	117	7	15	3	1972	113	306	82,5	49,1	25,8	54,9	0,155	**-0,1**
1b	Pc2		326	72	51	13	8	13	510	15	250	65,8	56,8	7,0	77,5	0,490	**2,2**
1c	Mgd		1545	100	142	13	13	2	2588	52	104	94,3	26,6	25,1	58,6	0,040	**1,4**
1d	Wadok		1166	170	124	7	15	1	2037	68	100	92,4	31,7	21,2	48,0	0,049	**1,1**
2	IjenS	60	1030	1020	3150	680			1675	30	4	98,0	11,8	2,8	69,9	0,002	**71,1**
3a	WrkW	240	1170	167	20	0,01	10,7	26	1970	35	5	98,0	0,8	39,8	3,4	0,003	**-0,2**
3b	WrkS	99	1220	140	30	4,5	14,5	43	2100	30	30	97,2	7,9	25,7	44,7	0,014	**-1,5**
4	SW	4	10760	390	410	1290			19340	2710	140	87,2	4,9	21,3	71,0	0,007	**0,03**
5	SsUsW	330	38400	13400	22010	10			118400	4	140	99,9	97,2	21,9	1,8	0,001	**-3,6**
6a	MvCrW	245	1970	238	73	0,02	5,7	54	3300	36	40	84,0	6,0	43,9	3,1	0,012	**-5,3**
6b	MvCrS	73	1970	79	22	6,5	3,4	48	2600	120	910	71,8	79,7	37,1	48,0	0,350	**-0,7**

5. CONCLUSION

The fluid compositions should be informed to understand the individual geothermal system, is it chloride water, or acid water or carbonated water reservoirs. We concluded that Na-K-Mg chart analysis with full equilibrium fluid illustrated the consistent temperature with rail temperature well measure. The Padang Cermin geothermal field Pesawaran Lampung Indonesia should be the hight temperature geothermal system refer to Wairakei geothermal system New Zealand.

6. AKNOLEDGMENT

I would like to thanks to Simmons that have product the lecturer book for aNalysis reference and some data that using to be analyzed

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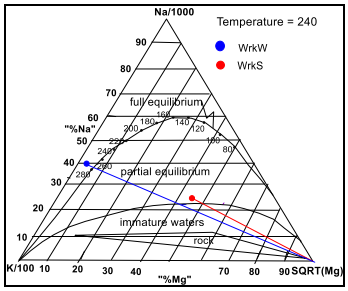

Figure 1. Interpretation from Na-K-Mg chart analysis does determine the temperature of the reservoir. Comparison between the Wairakei spring data analysis (WrkS), and Wairakei well data analysis (WrkW) compare to the good temperature.

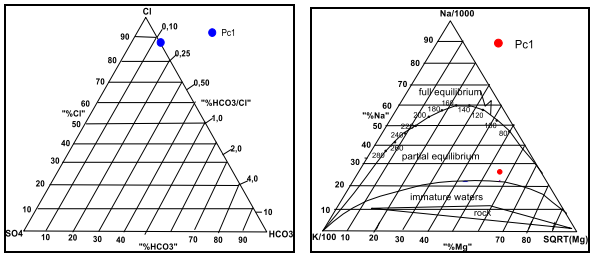

Figure 1a. Cl-SO$_4$-HCO$_3$ and Na-K-Mg chart analysis of the geothermal system from spring Padang Cermin 1 (Pc1)

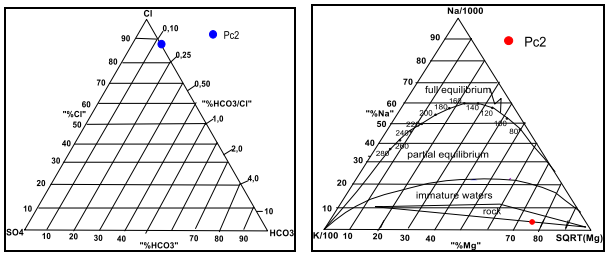

Figure 1b. Cl-SO$_4$-HCO$_3$ and Na-K-Mg chart analysis of the geothermal system from spring Padang Cermin 2 (Pc2)

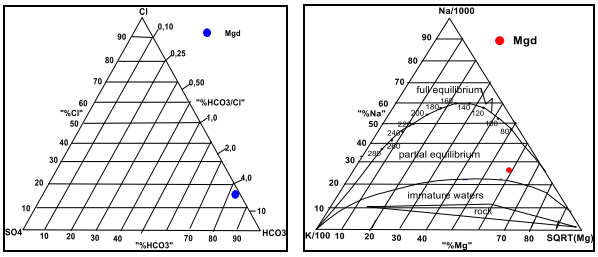

Figure 1c. Cl-SO$_4$-HCO$_3$ and Na-K-Mg chart analysis of the geothermal system from spring Margodadi Padang Cermin (Mgd)

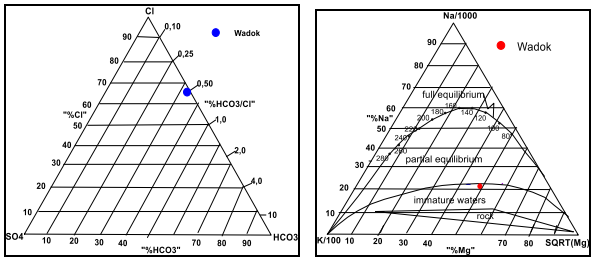

Figure 1d. Cl-SO$_4$-HCO$_3$ and Na-K-Mg chart analysis of the geothermal system from spring Wadok Padang Cermin (Wadok)

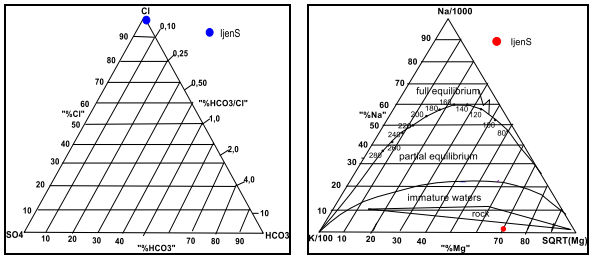

Figure 2. Cl-SO$_4$-HCO$_3$ and Na-K-Mg chart analysis of the geothermal system from spring of Kawah Ijin East Java (IjenS)

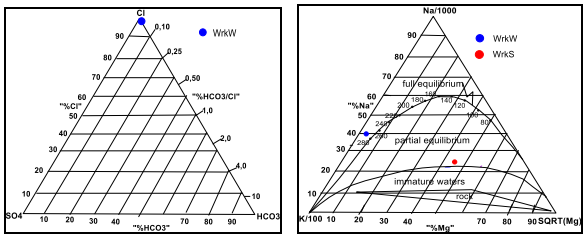

Figure 3a. Cl-SO$_4$-HCO$_3$ chart analysis of Wairakei geothermal system well (WrkW), and Na-K-Mg chart analysis of Wairakei geothermal system well of (WrkW) and Wairakei geothermal system spring (WrkS),

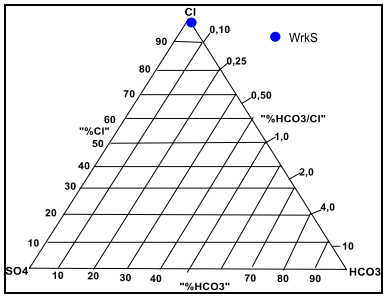

Figure 3b. Cl-SO$_4$-HCO$_3$ chart analysis of Wairakei geothermal

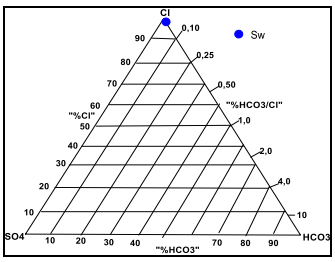

Figure 4. Cl-SO_4-HCO_3 chart analysis of Sea Water (SW),Waairkei system spring (WrkS).

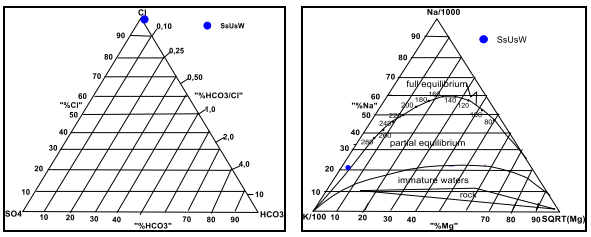

Figure 5. Cl-SO_4-HCO_3 and Na-K-Mg chart analysis of the geothermal system from Salton Sea well USA (SsUsW)

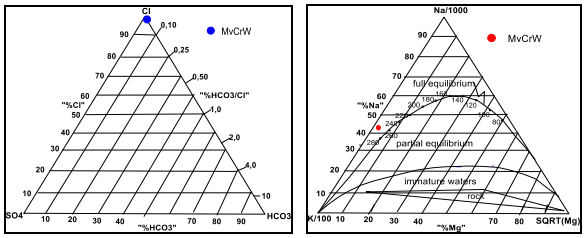

Figure 6a. Cl-SO_4-HCO_3 and Na-K-Mg chart analysis of the geothermal system Miraviles well (SsUsW)

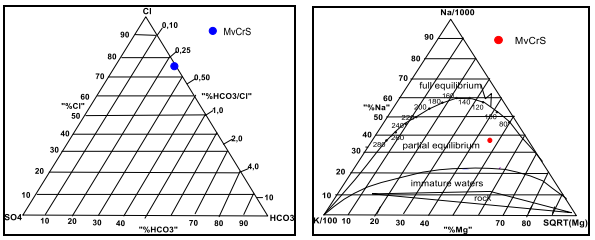

Figure 6b. Cl-SO_4-HCO_3 and Na-K-Mg chart analysis of the geothermal system Miraviles spring (SsUsS)

Energy Efficiency of Zinc-Carbon and Standart Accumulator

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Abstract – Energy Efficiency of Zinc-Carbon accumulator and Standart Accumulator Model 6N4-2A-4 have been studied. The accumulator has been made using Zn-C as an electrode which composes of three cells with sulfuric acid electrolyte. Two setups were used to assembly the cell namely series setup and parallel setup. Three types accumulator will be tested for charging and discharging characteristics to know the energy efficiency of Zinc-Carbon Accumulator with series configuration, Zinc-Carbon Accumulator with parallel setup and Standart Accumulator Model 6N4-2A-4. Operational voltage 1.39 – 7.96 V, 1.06 – 2.69 V and 1.73 – 7.55 V was applied to the charge and discharge process. Charging and discharging performances were measured and analyzed using three cycles for 36 hours. The results showed that Standart Accumulator Model 6N4-2A-4 is better than both the accumulators regarding the average energy efficiency. The average energy efficiency for Standart Accumulator Model 6N4-2A-4 is 67.9 % whereas Zinc-Carbon Accumulator with series configuration and Zinc-Carbon Accumulator with parallel configuration resulted in 35.3 % and 63.3 %, respectively.

Keywords: *Accumulator, Electrode, Energy Efficiency*.

1. INTRODUCTION

Electrodes Zinc - Carbon is a constituent material of alkaline batteries that have the nature of non-rechargeable batteries or primary cell battery, it is designed to be fully discharged only once, and then discarded [1]. People usually use and throw it carelessly so that it gives a very bad impact on the environment, it is because the content of the spent primary cell batteries generate specific residues such as mercury, zinc, manganese and other heavy metals [2], which is very susceptible to damage the environment and threaten public health [3]. Increased environmental awareness and consumption of raw materials led to tightened regulations on primary batteries worldwide. These rules and various things of issues - environmental issues pushed to collect the spent primary battery aimed at recovery of further use of metal [4]. One is in Turkey; the regulations on the Control of Spent Battery and Accumulators was published on August 2004 [5]. In Indonesia, regulations on environmental pollution by dry cell batteries have been published by the decision of environmental state minister and provincial regulation in Yogyakarta No. 2 of 2012 on the management of hazardous wastes and toxic [6,7].

Based on the regulation on environment ministers, Zinc is one of the hazardous heavy metals that pollute the environment [6]. So in this study, zinc metal developed to be active material as a concept of energy storage technique that is shaped like an accumulator. In the previous studies have been developed as an active material of a secondary battery design [8,9]. Likewise with carbon electrodes are also used previous studies as an inert material that has an influence as the good collector current behavior in the lead acid battery system [10,11]. Electrode system design uses a sandwich models, as is done on the research [12,13].

2. METHODS

In this study, using Zinc as the negative electrode made with levels of 72% with dimensions of 7 x 4.5 cm^2 total of 24 plates, while the carbon material as a positive electrode with a 94% level with dimensions of 7 x 4.5 cm^2 up to 12 plates. Connecting process at zinc plate bonded with a tin trunk that has dimensions of 28 x 4.5 cm^2 as well as carbon plate with the same connecting treatment. Both electrodes are stacked into one like a sandwich model and between the two sides were given a separator made of insulator material so that the two are not in contact with each other as is done in research [12,13]. This pile is composed of three layers with the configuration Zn | C | Zn. This electrode layer configuration was put into a cell with dimensions of 6 x 5 x 32 cm^3 in which there is a solution of sulfuric acid.

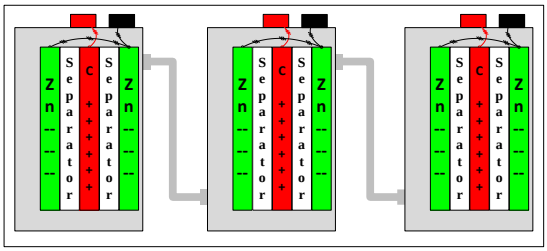


Figure 1. The Design Of Cell System.

Figure 1 shows an accumulator cell system block diagram[14]. Three cell system in which each one cell system connected in series with the pipe.

3. RESULTS AND DISCUSSION

In this study consists of two electrodes that is Zink electrode and inert electrode. Zink is as the anode electrode and inert electrode is carbon as the cathode. While the electrolyte used is sulfuric acid (H_2SO_4) at concentration of 0.1 M. The discharge and charge reaction on both electrodes is as follows [16,17]:

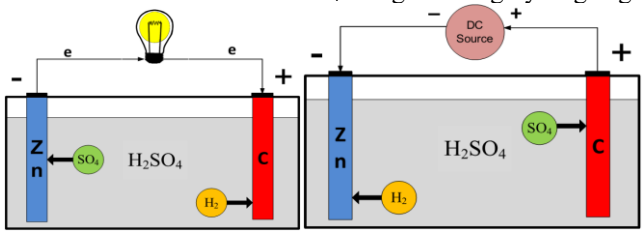
Cathode : $O_2\,(g) + 4H^+\,(aq) + 4e \rightarrow 2H_2O\,(l)$ $E^o = +1{,}23$ V
Anode : $2Zn\,(s) \rightarrow 2Zn^{2+} + 4e$ $E^o = -0{,}76$ V

Discharge Reaction Cells : $2Zn(s) + O_2\,(g) + 4H^+(aq) \rightarrow 2Zn^{2+} + 2H_2O\,(l)$ $E^o_{Cell} = +1{,}99$ V

Cathode : $2Zn^{2+} + 4e \rightarrow 2Zn\,(s)$
Anode : $2H_2O\,(l) \rightarrow O_2\,(g) + 4H^+\,(aq) + 4e$

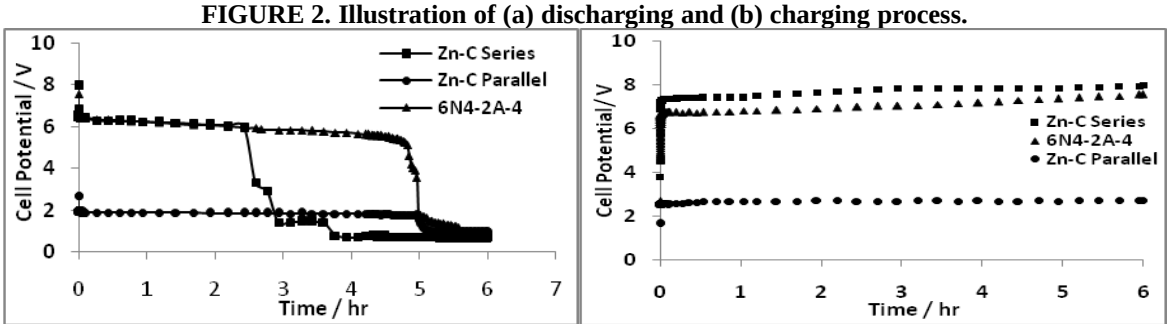
Charge Reaction Cells : $2Zn^{2+} + 2H_2O\,(l) \rightarrow 2Zn\,(s) + O_2\,(g) + 4H^+\,(aq)$

The discharging and charging process illustrate in figure 2, the chemical reaction at the discharge process is indicated by the formation of hydrogen bubbles (H_2 gas) at positive pole and oxygen gas in the anode when charging.On the negative pole Zink metal at room temperature have a solid form and has a negative standard potential (E ° = - 0.76 V), it means that zinc metal is easily oxidized by releasing two electrons forming Zn^{2+} ions. Zn^{2+} ions will react with sulfuric acid to form $ZnSO_4$ and generating hydrogen gas [15,17].



(a) (b)

FIGURE 2. Illustration of (a) discharging and (b) charging process.



(a) (b)

FIGURE 3. Constant current 0,5 A (a) discharge and (b) charge characteristics.

Figure 3 shows that the cell energy generated Standart Accumulator Model 6N4-2A-4 is greater than the Accumulator Zn-C series, but the time span discharge process between Standart accumulator 6N4-2A Model-4 and Accumulator Zn-C parallel has span time is almost the same. Based on the curves in Figure 3, the operational voltage Standart Accumulator Model 6N4-2A 4th is 1.73 Volt to 7.55 Volt. Accumulator Zn-C series is 1.39 volts to 7.96 volt, accumulator parallel is 1.06 Volt to 2.69 Volt. The chemical process that happens is the

opposite of the process of discharging. At the anode occurs oxidation reaction, the water will form oxygen gas. Furthermore, the zinc cathode $ZnSO_4$ is reduced forming solids Zinc. This happens because the carbon electrode is an inert material, which means it will not dissolve in acidic or alkaline solution so that there is no reaction. Because the electrolyte used is a sulfuric acid electrolyte that has negative ion SO_4^{2-} then water reacts at the anode. While on the cathode metal ions Zn^{2+} has a smaller potential than water reducible form a solid metal [16]. In Figure 3b shows the charging energy consumption in the Zn - C Accumulator series is bigger than all the graphs voltage charging performance accumulators Zn - C parallel and Standart Accumulator Model 6N4-2A-4.

Test of charge/ discharge cycle is done to look at the performance of each type of Accumulator. Based on the reference to the technical specifications data of Standart Model 6N4-2A-4 states that discharging duration required for 0.5 Ampere [19]. The data can be used as a reference for comparison with Zn-C Accumulator. The cell parallel circuit configuration can add the sectional area of the metal electrode so that Zink can reproduce Zink metal oxidation reaction and increase the amount of formation of the Zn^{2+} ion to react with sulfuric acid to form $ZnSO_4$.

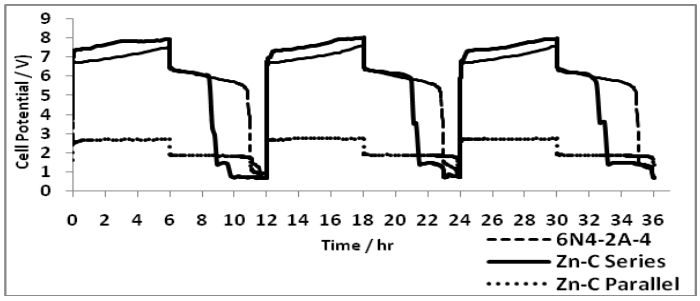


FIGURE. 4. Cell voltage vs. time response to three charge/discharge cycles at constant current 0.5 A.

In Figure 4, shows the test result of charge/discharge cycles in each accumulator of three cycles for 36 hours. Zn - C Accumulator parallel has the best performance. The longer carried out the process of charge - discharging for 3 cycles, the curve of surface area during discharge is getting bigger. Based on testing 3 cycles of charging-discharging by using equations energy efficiency[18], the value of the average energy efficiency of each accumulator is 35.3% (Zn - C series), 63.3% (Zn - C parallel), and 67.9% (Standart Model 6N4-2A -4), respectively.

4. CONCLUSIONS

Operational voltage for each accumulator were 1.39–7.96 V (Zinc–Carbon series configuration), 1.06–2.69 V (Zinc –Carbon parallel configuration) and 1.73–7.55 V (Standart Accumulator 6N4-2A-4). The process of charge - discharging is performed 3 times over 36 hours on each type of accumulator. As a result, Standart accumulator Model 6N4-2A-4 has the best performance. The average energy efficiency for Standart Accumulator Model 6N4-2A-4 is 67.9 % whereas Zinc-Carbon Accumulator with series configuration and Zinc-Carbon Accumulator with parallel configuration resulted in 35.3 % and 63.3 %, respectively.

5. ACKNOWLEDGMENTS

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Time Management of an Anaerobic Solid Waste Digester With Leachate As A Starter To Obtain The Continuous Biogas Production

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Abstract – The limitation resources of fossil energy requires an exploration of another energy resources which could be renewable energy. One proper energy sources that considerable as the biogas is generated from solid waste. Anaerobic digester technology could be applied to process solid waste into renewable energy sources such as biogas, yet the widespread application of these technologies can solve the problem of environmental pollution which caused by waste piles. In this study, the anaerobic digester technology is applied to obtain biogas from organic solid waste using the leachate as a starter. By using 4 pieces of digesters, it takes cultivation process of sewage which carried out within an interval of 14 days between one digester to another one. The production of the gas is controlled every day, and the quality of the gas is measured in every 10 days. The result of the measurement for all digesters show that the production of the gas from the day 20th to the day 30th produce the good quality of the gas which contains methane gas for more than 40 %. Therefore, as the biogas production that fulfills the requirement as the fuel which takes time of 10 days, so in order to keep the continuation of the gas production it is advisable to take the cultivation process of sewage for each digester is 10 days.

Keywords : renewable energy, solid waste, leachate.

1. INTRODUCTION

The human needs of energy are increasing from time to time. Nowadays the fulfillment of needs for energy majority comes from the fossil energy such as oil, coal, and gasses. On the contrary, the availability fossil energy gets more decreased so many efforts has been made to get more new energy and renewable. One of noticeable energy source is biogas which comes from the anaerobic degradation process of organic composition.

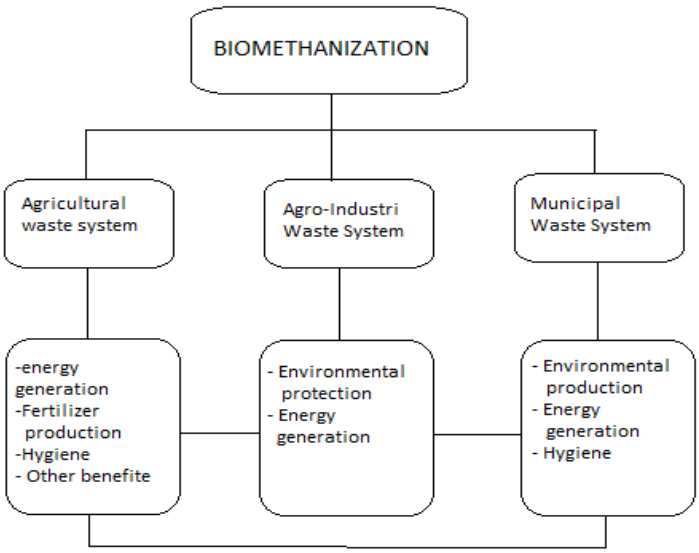

Fig 1. The benefit of biogas technology applications [sherwin]

Biogas technology is worth to applied for changing organic waste which comes from : Agriculture, farming, industrial and many more human activities to be an energy source. The application of energy source that comes

from wastes could make the better environment, health improvement and socio-economic improvement of the society as shown in Figure 1.

The potential of biogas as the energy source is enormous as it could solving the problem of waste that comes from industry, agriculture and domestic [1][2][3][4]. The application of biogas for an electric generator is possible to be done with some preparation on the specific gas turbine which will be used to solve the impurity of the gas fuel [5].

The handling technic of municipal waste to produce biogas and also to avoid the air pollution and bad odor is using landfill method. From the application of landfill, a method is still found the liquid waste which known as leachate that contains high toxic, so it is not allowed to be freely thrown to the environment. The usage of leachate that recirculated to the landfill bioreactor shows the improvement of quality and quantity of the biogas significantly [6].

This research has done the experiments of organic waste processing with leachate using as a starter on anaerobic biodigester with a hope that produces high quality of biogas which contains the methane more than 40%.

2. EXPERIMENTAL METHODS

The research is using leachate which earned from the final waste of Bandung , Cilegon City, which previously has using landfill method to process the pile of municipal waste. The result of a laboratory test to the leachate composition is shown in Table 1.

Table 1. Leachate composition

No.	Parameters	unit	values
1	COD	mg/l	3636
2	pH		9
3	Total Solid Suspension	mg/l	347
4	Fe	mg/l	6.5
5	Pb	mg/l	20

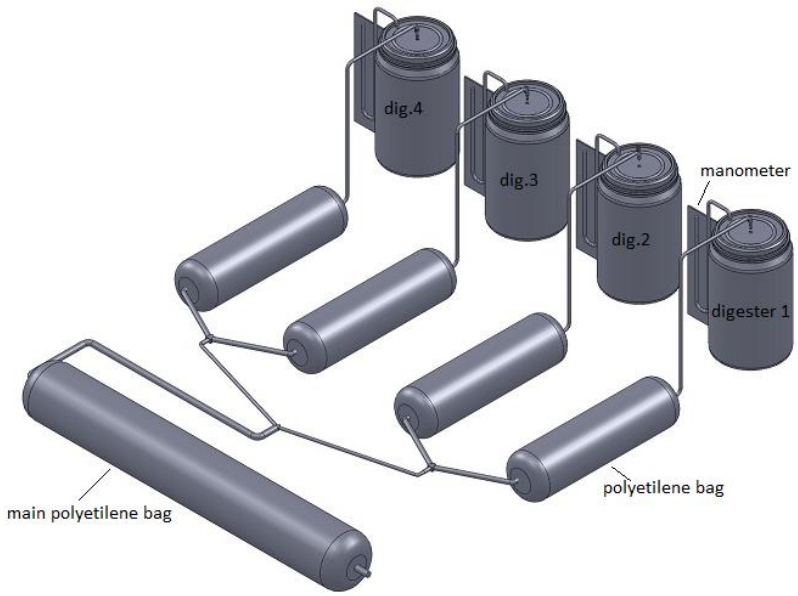

Figure 2. Digesters and gas storages.

Four digesters are used in this research with the cultivation of the composition has a duration of 14 days from one digester to another. Figure 2 shows the scheme of all digesters which haves tubes and valves that control the flow. From day one to the day fourteen only digester 1 that works and fill the polyethylene bag 1. When the contain of the methane on polyethylene bag 1 has reached 40 %, so the gas will be flown from the polyethylene bag 1 to the main polyethylene bag. Next, when the day 14th digester 2 start operated, biogas which produces on the polyethylene bag 2 will be measured by the quality and the quantity. If the contain of the methane has reached 40 % then the biogas will be flown to the main polyethylene bag. The same will be applied for the digester 3 and 4 which each of them will start to operate on the day 28th and 42th.

Specification of the tester

Digester drum capacity : 150 l
Gas storages : polyethylene bag
Composition : 50 l organic waste + 50 l leachate

3. RESULTS AND DISCUSSION

The results of the measurement of biogas production which produced from each digester are shown in Figure 3.

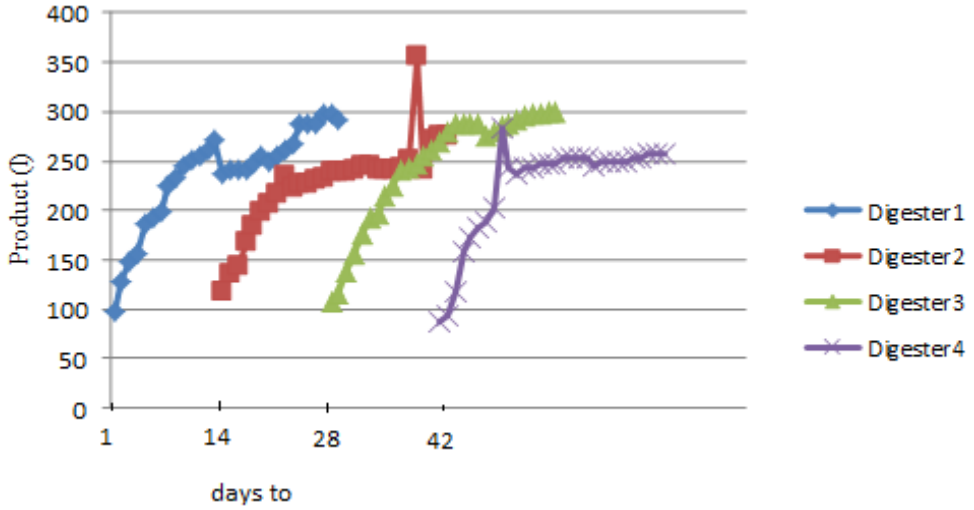

Figure 3. Biogas production (l) from each digester

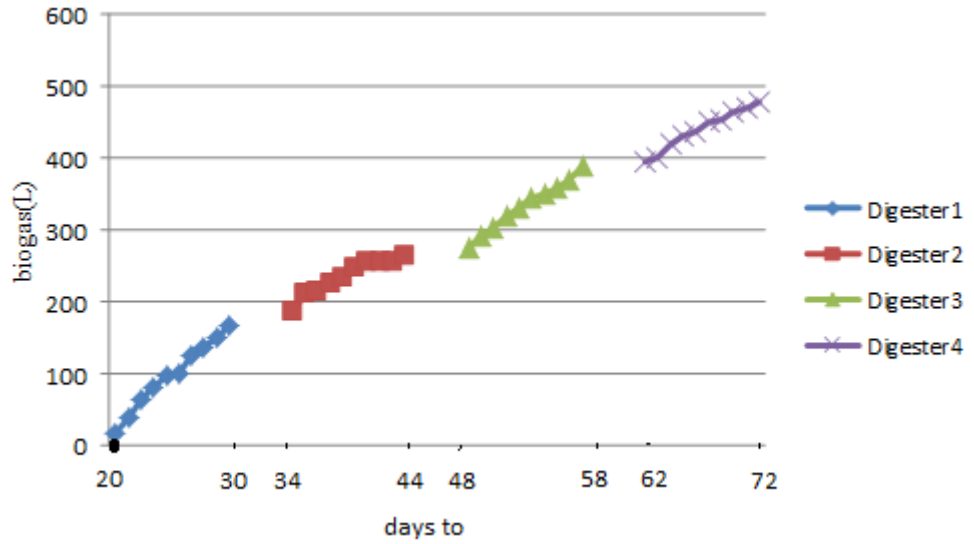

Figure 4. Volume of the biogas on the main polyethylene bag With the contain of methane > 40%

The production of biogas on the first 10 days generally increasing sharply, after that the rate of production get slower until the day 30 that shows nothing increased in the production.

The contain of methane on the polyethylene bag from each digester are scrutinized, and when the methane has reached 40 %, the biogas will be flown to the main polyethylene bag by opening the valve. The result on the polyethylene bag is shown in Figure 4.

The main polyethylene bag start to filled from the digester 1 on June 4th, 2016 to June 14th, 2016 (10 days duration). Next, on the 4 days break the biogas from a polyethylene bag of the digester 2 can be flown to the main polyethylene bag because the contain of the methane on the biogas has reached 40 %. The same is also done for the digester 3 and 4. In average the digesters are enabled to produce the biogas with the contain of the methane for more than 40 % on the days 20th – 30th. So to keep the continuity of the biogas production on the digester with leachate starter the duration of the cultivation of the composition to the digester tank should be done on 10 days.

4. CONCLUSIONS

The results of the experiment of forming of biogas from the organic solid waste using the method of the anaerobic digester with leachate as the starter show that the biogas with the contains of methane for more than 40 % happened on the duration of 10 days which is the day 20 to the day 30 for each digester. So in order to get the biogas continually the time of the early waste cultivation and leachate as the starter to the digesters should be taken the duration of 10 days from one digester to another one.

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Catalytic Hydrocracking to Biofuel of Kapok seed oil (*Ceiba pentandra*) using Zn-Mo/HZSM-5 Catalyst

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Abstract – Catalytic hydrocracking of Kapok seed oil (KSO) used to Zn(2.92%)-Mo(7.55%) supported HZSM-5 catalyst in a slurry pressure batch reactor at 350^0C (10 bars after flowing H_2) for 2 h was carried out to produce biofuel. A Zn-Mo/HZSM-5 catalyst was prepared by incipient wetness impregnation method and characterized using XRD. The volume of kapok seed oil in the reactor is 200 ml and catalysts used as much as 0.56% w/w. Liquid product obtained was analyzed using GC-MS. It displayed the highest compounds were 36.35 area% of palmitic acid and the aromatic hydrocarbon as much as 0.24 area% was found in biofuel and cycloparaffin, i.e., cyclo tetracosane as much of 1.36 area% was found through cyclization of olefin. Thus, the catalyst Zn-Mo/HZSM-5 can convert kapok seed oil through the catalytic hydrocracking process into biofuel.

Keywords: Catalytic hydrocracking, Kapok seed oil (*Ceiba pentandra*), Zn-Mo/HZSM-5, biofuel.

1. INTRODUCTION

Population growth resulted in an increase of energy needs, so it will affect the availability of petroleum fossil resources. The fossils fuel combustion caused a negative impact on the environment, one of which is increasing CO_2 emissions. Biofuel is an alternative measure to replace fossil fuels as renewable energy. In the last few years, developments experienced competition technological resource's food and energy plant selection, causing the expansion of biofuel production from non-food crops. Kapok (*Ceiba pentandra*) seed oil (KSO) is inedible vegetable oils. *Ceiba pentandra* locally known Kapuk Randu is grown in Indonesia, Pandaan district. This tree grows to 60-70 m tall and has a trunk large enough to reach a diameter of 3 m. The trees begin to bear fruit after the age of 3-4 years and will continue to generate up to 60 years [1]. Kapok seed oil (KSO) was extracted from seeds of *Ceiba pentandra*. So far, kapok seed oil has been used as an ingredient in cosmetics, oil additive lubricant, a mixture of fodder, etc. [2]. The use of kapok seed oil as a biofuel in accordance with the purpose than the production of second generation biofuels, namely the use of inedible feedstock to avoid conflicts with a source of food.

Fatty Acid Methyl Ester (FAMEs) has generated through kapok seed oil transesterification methanol with the homogeneous catalyst [3]. The high content of oxygen atoms in the form of carboxylic acid ester compounds and cause Fames less favorable for transportation machinery. The nature of FAMEs as poor heating value compared to diesel and oxidation instability in its use as a fuel for transport [4]. FAMEs also should blend with pure diesel because of FAMEs without blending does not affect the internal combustion engine. Until now, the hydrocracking is a way to reduce the content of oxygen atoms in the long chain of FAMEs and deciding on triglycerides with a bifunctional catalyst to produce hydrocarbon shorter molecules. Previous bifunctional catalysts such as zeolite impregnated with metals Ni and Mo are used in petroleum cracking process [5]. Based on this thinking, triglycerides with a C_{14}-C_{20} chain of atoms found almost the same as the structure of the oil so that it can crack with type zeolite catalyst to produce biofuels. Biofuel is expected to have a composition similar to petroleum diesel hydrocarbons, such as paraffin, cycloparaffin and aromatic.

Some research has applied about catalytic hydrocracking against with using vegetable oil-based catalysts Mo and HZSM-5 zeolite, either single promoted catalyst or double promoted catalyst. Using the catalyst, Mo-Zn/Al_2O_3 to produce biofuels, besides producing hydrocarbons but also oxygen compounds such as acids, alcohols, and esters [6]. In a different study, Ni-Mo/ZSM5 catalyst to convert soybean oil to obtain the increase in yield reached 50% it into hydrocarbon fuels with optimum conditions at a temperature of 360^0C and a pressure of 650 psi. The use of double promoted metal catalyst showed high activity and selectivity in the range of gasoline-diesel [7]. Therefore, it is interesting to investigate the Zinc (Zn)-Molybdenum (Mo)/HZSM-5 catalyst in catalytic hydrocracking kapok seed oil to produce biofuel at 350^0C.

2. METHODS

2.1 Chemicals

A commercial NH_4-ZSM-5 zeolite purchased from Zeolyst International was used as a catalyst support. A zinc nitrate salt of $Zn(NO_3)_2.6H_2O$ and ammonium heptamolybdate tetrahydrate of $(NH_4)_6Mo_7O_{24}.4H_2O$ purchased from Merck with 98% purity was used as metallic precursors.

2.2 Procedures

Catalysts HZSM-5 was prepared by calcined NH_4-ZSM5 at 550^0C for 5 h under at air atmosphere. Zn (2.92%)-Mo(7.55%) supported on HZSM-5 were prepared by incipient wetness impregnation method. A number of aqueous metal solutions were calculated by using the total pore volume of HZSM-5 as a support [8, 9]. Total pore volume can be investigated by BET analysis. It was obtained 0.2712 cm^3/gr. Catalyst characterization was performed using X-ray diffraction (XRD).

Catalytic hydrocracking process using a slurry pressurized batch reactor (Parr USA 4563) equipped with a stirrer. The operating conditions during the experiment carried out at a temperature of 350^0C for 2 (two) hours with a pressure of 10 bars after flowing H_2 for at least 1 (one) hour. The volume of *Ceiba pentrandra* oil is 200 ml and catalysts used as much as 0.56% w/w. Liquid product obtained was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS).

3. RESULTS AND DISCUSSION

3.1 Kapok (*Ceiba pentandra*) seed oil

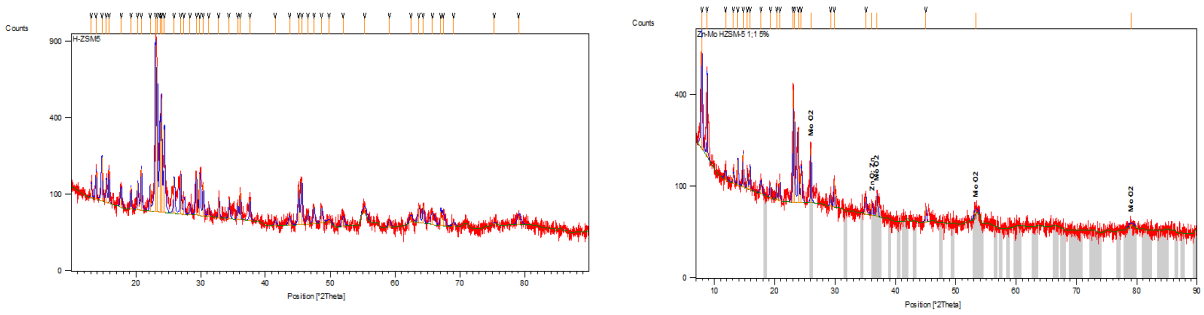
Kapok seed oil (KSO) was provided by Pandaan district, East Java, Indonesia. The beaches spool pieces are 26% kapok seeds, so every 100 kg pieces will produce 26 kg of kapok seed waste. The color of kapok seed oil is clear bright yellowish-colored liquid (**Figure 1c**). The analysis results of fatty acid KSO was analyzed by GC-MS. It indicated that the largest component contains linoleic acid (C18:2) 78.85% and palmitic acid (C16:0) 21.12%. So it can be grouped that KSO belonged to polyunsaturated fatty acids.

(a) (b) (c)

Figure 1 Kapok seed oil (a) fruit [1], (b) seeds [1] and (c) Kapok seed oil

3.2. Catalyst Characterization

Figure 2 shows the XRD patterns of (a) HZSM-5 and (b) Zn-Mo/HZSM-5 catalyst. The XRD patterns of catalyst showed small peak intensity at 2θ of 36.0899º for ZnO, while for MoO_2 detected on three main diffraction peak at 2θ i.e. 26.0182º, 36.9332º, and 53.3027º. Mo catalyst can facilitate the hydrodeoxygenation reaction [10]. Initial characterizations of metallic Zn and Mo were modified in HZSM-5 through impregnation given by analyses obtained BET surface area of 250.704 m^2/g.



(a) (b)

Figure 2 XRD pattern of (a) HZSM-5, (b) ZnMo/HZSM-5 catalyst

Making NH_4-ZSM5 can be converted being to the acid H-from simple calcination [8]. The HZSM-5 has a high surface area of 375.121 m^2/g, while the impregnation of bimetallic Zn and Mo into the HZSM-5 support causes the decrease of surface area and pore volume of ZnMo/HZSM-5 catalyst. Product HZSM-5 has a characterization, such as high surface area, large pore volumes and strong acidity [11]. These changes indicated that these metals are blocking some microporous and clogging the external mesoporous [12].

3.3. Biofuel Analysis

Figure 3 shows the GC-MS chromatogram of biofuel with ZnMo/HZSM-5 catalyst at a temperature of 350^0C. It was found that palmitic acid (molecular weight's of 256) and oleic acids (molecular weights of 264) were produced from catalytic hydrocracking of KSO in this study, as presented in **Figure 4a**. The palmitic acid of 36.35 area% was the most abundant compound in the liquid product. The presence of this carboxylic acid as an intermediate product showed that the triglycerides are broken down into free fatty acids through a mechanism that facilitated the process of hydrogenation. Previously, the saturation process of linoleic acid as the most abundant compound in kapok seed oil to occur first. It was expected that decarboxylation and/or decarbonylation of free fatty acids at a temperature above 350ºC could produce hydrocarbon, i.e. n-paraffin, isoparaffin, cycloparaffin and aromatic. The aromatic hydrocarbon, (e.g. ethylbenzene) of 0.24 area% was found in biofuel. In addition, the presence of olefin (e.g. 1-nonadecene) show that at temperatures of 350°C, dehydrogenation and/or hydrogenolysis of n-paraffin occurred and was followed by cyclization. As shown in **Figure 4b**, a small amount of cycloparaffin, i.e., cyclo tetracosane (molecular weights of 336) of 1.36 area% was found through cyclization of olefin. It indicated that at temperature of 350^0C some various reactions, such as aromatic compounds, hydrogenation, cyclization, and aromatization occurred during catalytic hydrocracking of KSO with Zn-Mo/HZSM-5. Although the hydrocarbon compounds produced are still in small quantities (less than 5% of total).

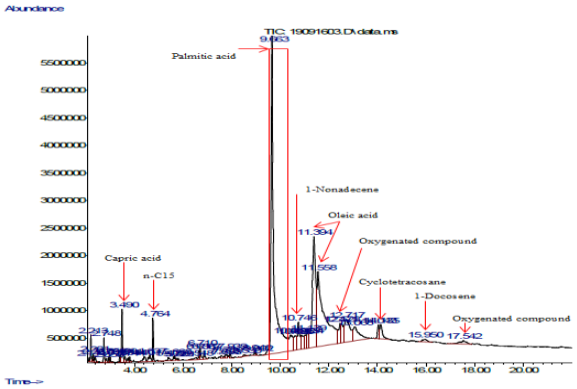


Figure 3 GC-MS spectra of biofuel at 350ºC with Zn-Mo/HZSM-5

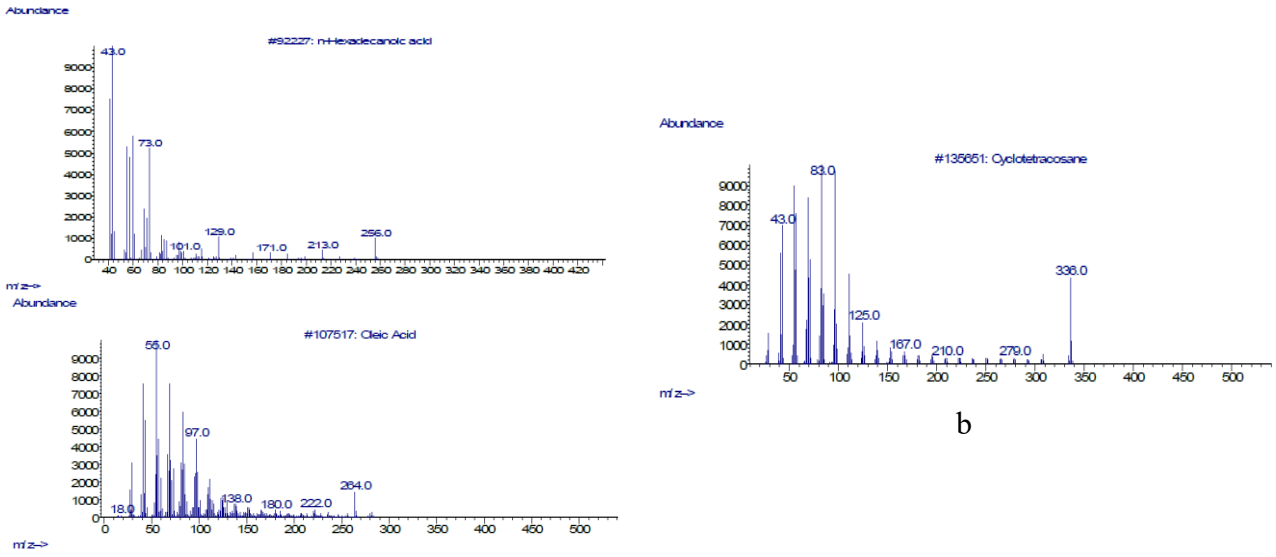


Figure 4. Mass spectra of (a) carboxylic acid, (b) cycloparaffin in biofuel produced from catalytic hydrocracking of KSO

4. CONCLUSIONS

In this study, Zn-Mo/HZSM-5 catalyst was prepared by incipient wetness impregnation. Zn-Mo/HZSM-5 used to produce biofuel through the catalytic hydrocracking process of cotton seed oil at a temperature of 350^0C in a slurry pressurized batch reactor at 10 bars. On the conversion of kapok seed oil into biofuel at a temperature of 350^0C occur a variety of reactions, for example, the reaction of aromatic compounds, hydrogenation, cyclization, and aromatization occurred. Palmitic acid, a fatty acid compound, are in the majority of liquid products as much as 36.35%area. The presence of the carboxylic acid on partially liquid products showed that chain triglycerides could be disconnected with mechanism hydrogenation reaction. The aromatic hydrocarbon as much as 0.24 area% was found in biofuel and a small amount of cycloparaffin, i.e., cyclo tetracosane as much of 1.36 area% was found through cyclization of olefin. This shows that through the process of catalytic hydrocarbon kapok seed oil by using Zn-Mo/HZSM-5 catalyst has been successfully producing biofuels. A number of hydrocarbon compounds produced are still in small quantities (less than 5% in total).

5. ACKNOWLEDGMENT

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Effect of Hierarchical Pore Structure on Performance of Co-Ni/HZSM-5 Catalyst in Hydrocracking of Sunan Candlenut Oil

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Abstract – This research is aimed to study the influence of hierarchical pore structure and Co–Ni supported on HZSM-5 on hydrocarbons in the liquid product produced from the hydrocracking of sun candlenut oil. The catalyst was characterized by SEM-EDAX and N_2 physisorption.The hydrocracking was carried out in a batch reactor under initial hydrogen pressure, the reaction temperature of 350 ^oC for 2 h, pressure in the range of 10-15 bar, and a catalyst/oil ratio of 0.005 g.catalyst/mL oil. The hydrocarbon compounds in the liquid product were determined qualitatively by gas chromatography-mass spectrometry. It was found that the Co(3.38)-Ni(4.32)/hHZSM-5 catalyst may support hydrogenation process on a poly-unsaturated chain into saturated chain fatty acid at a temperature of 350 ^oC. However, in liquid product hydrocarbon compounds such as paraffin, cycloparaffin and aromatic were still in a small amount (less than 7 area%). Therefore, for the future work the reaction temperature above 350 ^oC using the Co(3.38)-Ni(4.32)/hHZSM-5 catalyst was needed to be applied in hydrocracking of sun candlenut oil to produce a fuel range hydrocarbon..) All manuscripts must be accompanied by an abstract.

1. INTRODUCTION

So far, the potency of sun candlenut oil as non-edible vegetable oil has been optimized via transesterification technology to produce biodiesel [1]. The FAMEs produced already meet the characteristics of diesel. However, the detergent properties of FAMEs may cause damage the fuel system [2]. Therefore, to eliminate the oxygen content of FAMEs and/or vegetable oil to obtain hydrocarbon compounds is a hydrocracking process including deoxygenation (decarboxylation/decarbonylation), isomerization, cyclization and aromatization reactions.

According to Al-Muttaqii [3], aromatic hydrocarbons dominated the content of biofuel produced from hydrocracking of sunan candlenut oil with the Co-Ni/HZSM-5 catalyst at 350 ^oC. The cyclization reaction of paraffin compounds is due to the formation of aromatic. Saturated chain hydrocarbons around 0.5 area% were still in the relatively small area. It was different as reported by Malinda et al. [4], the hydrocracking of cerebral mangas oil with the Co(0.88%)-Ni(3.92%)/HZSM-5 catalyst at 350 ^oC produced a high amount of n-paraffins and became the dominant liquid product. The same result was also found that n-paraffins as the abundant component were found in biofuel derived from hydrocracking of cerebral mangas oil with a various metals ratio of Co-Ni/HZSM-5 catalyst [5]. According to the above results [3,4,5], the different metals ratio on Co-Ni/HZSM-5 catalysts influenced the properties of the catalyst. It was suggested that the porosity properties of catalyst and the content of polyunsaturated fatty acids in vegetable oil determined the product distribution of hydrocarbon compounds.

Hierarchical pore structure may improve diffusion of hydrated metal ions during impregnation because its size is larger than HZSM-5 pore. In addition, the presence of intercrystalline mesopores supports on mass transfer of reactant and product molecules from/to HZSM-5. The polymerase by-product or intermediates reaction covering active sites in micropore channel can be solved. According to Chen et al. when hierarchical NiMo/ZSM-5 treated with low NH_4^+ exchange amount was applied in hydroconversion of soybean oil at a temperature of 380 ^oC, and 3 Mpa under flowing hydrogen, gas oil-like hydrocarbons were produced. In addition, when this catalyst treated with an NH_4^+ concentration of higher than 0.2 M, aromatic hydrocarbons increased and became the most abundant compound [6].

Based on the result of previous works [3], it is interesting to study the influence of hierarchical pore structure and Co–Ni supported on HZSM-5 catalyst on distribution product of hydrocarbon compounds in the liquid product produced from hydrocracking of Sunan candlenut oil.

2. METHODS

2.1 Materials

NH4-ZSM-5 zeolite was purchased from Zeolyst International (CBV 8014, 400 m^2/g, Na_2O 0.05 wt.%). Furthermore, HZSM-5 zeolite was calcined at 550 °C for 5 h and had been characterized with the results as reported by Al-Muttaqii [3] dana measured Si/Al ratio of 24. The $Ni(NO_3)_2.6H_2O$ and $Co(NO_3)_2.6H_2O$ was purchased from Merck with 98 % purity. While, sun candlenut oil was obtained from pressing of seeds by screw press in Ballas, Malang, Indonesia. Sunan candlenut seed was collected from Bogor,West Java, Indonesia. Based on GCMS analysis, the oil contained a high amount of polyunsaturated fatty acid, such as linoleic acid.

2.2 Procedures

To observe the effect of hierarchical pore support, we use two support catalyst, namely HZSM-5 and hierarchical HZSM-5catalyst. The hierarchical HZSM-5 catalyst was prepared according to the literature [7]. To obtain Co and Ni impregnated on HZSM-5 catalyst, the $Ni(NO_3)_2.6H_2O$ solution of 2.08 M and Co $(NO_3)_2.6H_2O$ solution of 2.07 M were prepared. Impregnation procedure used in this work has been done by Malinda et al. [4], which was a combination of the incipient wetness impregnation and successive impregnation. By EDAX, the metals content was 3.86 wt.% of Co dan 2.95 wt.% of Ni of the total catalyst weight. A sample of the catalyst is named as the Co(3.86)-Ni(2.95)/HZSM-5 catalyst. Furthermore, by using the same impregnation procedure, Co and Ni were impregnated into the hierarchical HZSM-5 catalyst. Before be done, the $Ni(NO_3)_2.6H_2O$ solution of 0.4 M and Co $(NO_3)_2.6H_2O$ solution of 0.39 M were prepared. By EDAX, the metals content was 3.38 at.% of Co dan 4.32 at.% of Ni of the total catalyst weight. A sample of the catalyst is named as the Co(3.38)-Ni(4.32)/hHZSM-5 catalyst. Finally, the two type of catalysts wares characterized by SEM-EDAX and BET.

The hydrocracking reaction was conducted under initial hydrogen pressure in a batch reactor (Parr Instrument Company 4563) equipped with a mechanical stirrer. The reaction pressure was kept in the range of 10-15 bar. The reaction was carried out at a temperature of 350 °C for 2 h. Sunan candlenut oil of 200 ml was placed into the reactor, which was containing the catalyst of 1 g. The liquid product was analyzed by gas chromatography-mass spectrometry to identify hydrocarbon compounds qualitatively corresponding to the Wiley275 and NIST02 mass spectral library of data. The hydrocarbon components with a probability match equal to or higher than 80% were considered.

3. RESULTS AND DISCUSSION

Figure 1 shows a morphology of hierarchical Co-Ni/HZSM-5 catalyst. Desilication with alkaline solution caused the defects on the HZSM-5 surface which before desilication and impregnation, the typical MFI structure of HZSM-5 have hexagonal cubic morphology. Some previous study [8,9,10] reported that silicate anion and aluminum in zeolite framework are extracted and leave a non-uniform pore size. EDAX measurement shows that Si/Al ratio decreased from 24 to 11.97, as presented in Figure 1.

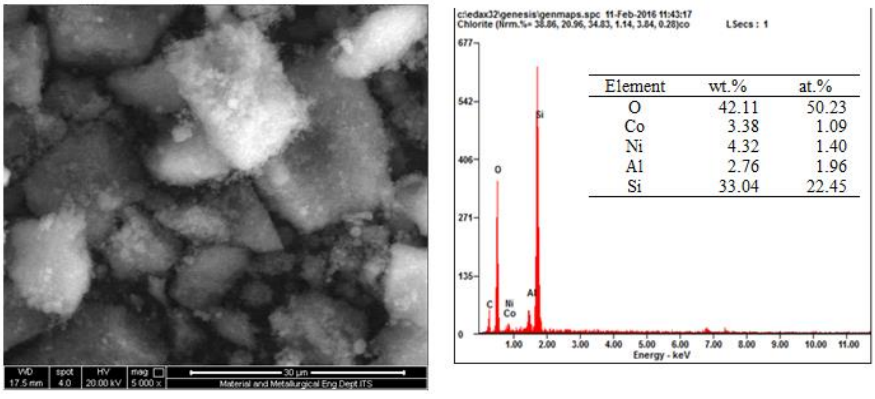

Figure 1 SEM-EDAX image of the Co(3.38)-Ni(4.32)/hHZSM-5 catalyst

The introduction of mesopores on HZSM-5 through desilication and followed impregnation of cobalt nickel gave BET surface area of the Co(3.38)-Ni(4.32)/hHZSM-5 catalyst from 267.045 m^2/g become 367.048 m^2/g, as shown in Table 1. It was interesting to be noted that pore volume and diameter increased from 0.22 to 0.83 cm^3/g and from 3.35 to 4.54 nm, respectively. According to previous result [11], after impregnation by nickel into HZSM-5, pore volume and diameter decreased because of blocking on micropores by nickel particle. After the introduction of hierarchical pore structure on HZSM-5, this problem can be solved so that the adding of nickel and cobalt into HZSM-5 pore may improve its porous properties. Overall, it was also found that the actual metal loading was approximately 6 wt.% with Co-Ni ratio = 1:1.

Table 1 The Porous properties of Co-Ni supported on HZSM-5 catalyst

Catalyst	Si/Al	S^a, m^2/g	V^b_{tot}, cm^3/g	D^c, nm
Co(3.86)-Ni(2.95)/HZSM-5	n.d	267.045	0.2238	3.3517
Co(3.38)-Ni(4.32)/hHZSM-5	11.45	367.048	0.8344	4.5463

n.d = not determined
a. BET surface area
b. Total pore volume
c. Average pore diameter

Table 2. Observation of hydrocarbon compounds according to GC-MS analysis

Compound	Retention Time (RT)	Abundance (qualitative, area%)	
		I	II
n paraffin			
Tridecane	3.02	0	0.13
Tetradecane	3.81	0	0.12
Pentadecane	4.77; 4.75	0.19	1.99
Hexadecane	5.79	0	0.11
Heptadecane	6.86;6.85	0.1	1.37
Nonadecane	8.91	0	0.15
Aromatic			
Benzene, pentyl	2.31	0.23	0.44
Benzene, hexyl	2.87	0.19	0.52
Benzene, heptyl	3.63	0.18	0.4
1 Methyl 2 n hexylbenzene	3.69	0	0.422
Benzene, octyl	4.55;	0.21	0.33
Benzene, nonyl	5.60; 5.59	0.27	0.34
Benzene, undecyl	7.78; 7.77	0.39	0.61
Benzene, decyl	6.69	0.28	0
Cycloparaffin			
Cyclopentadecanone	4.63	0.14	0
Olefin			
8 Heptadecene	6.86; 6.63	0.19	1.37
5 Tetradecene	3.73	0.08	0
Carboxylic acid			
Benzoic Acid	2.28	0	0
Octanoic Acid	2.23; 2.21	0.25	1.43
Nonanoic acid	2.75; 2.73	0.11	0.9
Undecanoic acid	4.37; 4.34	0..08	0.67
Dodecanoic acid	5.38; 5.35	0.09	0.45
n Hexadecanoic acid	9.74; 10.01;10.07;10.13; 10.32;10.60	22.09	23.46
n Decanoic acid	10.94; 3.45	0	1.34
Octadecanoic acid	11.7	0	27.20
Hexadecanoic acid, methyl ester	9.18	0.06	0
9 Octadecenoic acid	11.39;11.57;	30.23	0
18 Nonadecenoic acid		4.42	0
9,12 Octadecadienoic acid	12.45	7.02	0
Oxygenated compound			
2 Heptadecanone	8.97	0	0.19
Cyclopentadecanone, 2 hydroxy	8.97	0.09	0

I. The liquid product produced over Co Ni/HZSM 5, and II. Liquid product produced over the Co(3.38) Ni(4.32)/hHZSM 5 catalyst

Figure 2 shows GC-MS chromatogram of liquid product produced at a temperature of 350 °C. In the first step, triglycerides were converted into free fatty acids when the both of catalyst were used. The carboxylic acid peak area indicated that palmitic acid was the most abundant saturated fatty acid. When the Co(3.38)-Ni(4.32)/hHZSM-5 catalyst was used, poly-unsaturated fatty acid was not found again. It suggested that hierarchical pore structure supported hydrogenation process on a poly unsaturated chain into saturated chain fatty acid. In addition, some peaks appeared indicating the presence of hydrocarbons, such as paraffin and aromatics, as shown in Figure 2(c). When Figure 2 (a) and (c) are compared, it implied that retention time of n-C15 and n C17 in liquid product slightly shifted if be compared with the retention time of commercial petroleum diesel. Olefin was also detected in retention time of 6.7 However, hydrocarbon compounds were found in very small amounts in the liquid product so that the reaction temperature above 350 °C was needed. This phenomenon corresponded to the observed hydrocarbon compounds, as shown in Table 2. Based on Table 2, linoleic acid in Sunan candlenut oil may successfully convert into palmitic acid and stearic acid with the presence of the

hierarchical pore structure on the catalyst at a temperature of 350 ºC. It can be seen that short chain molecules of carboxylic acids were a dominant liquid product (see III in Table 2). The poly- and mono-unsaturated fatty acid were not found again in the liquid product. But, the various aromatic compounds were found, such as undecyl benzene (molecular weights of 232).

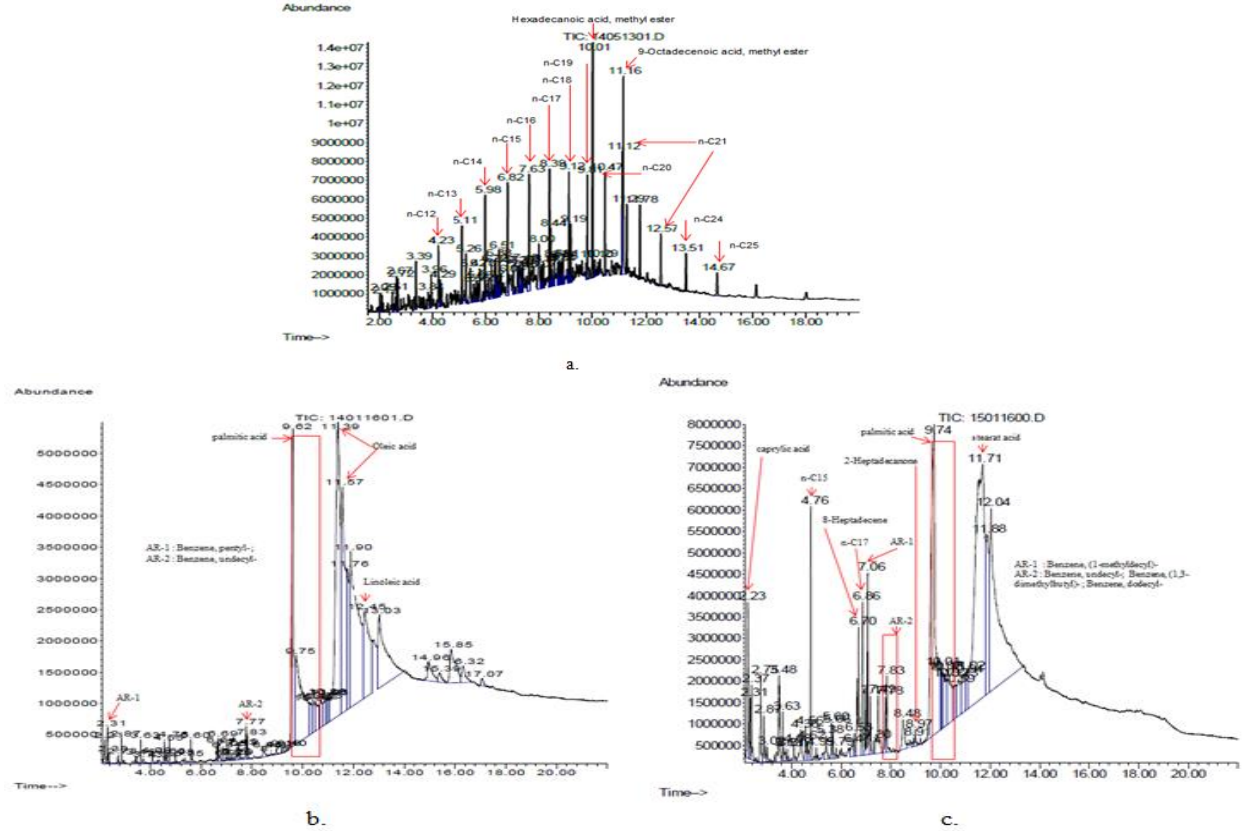

Figure 2 GC-MS Chromatogram of (a) commercial petroleum diesel [12] and liquid product produced at temperature of 350 ºC with (b) Co(3.86)-Ni(2.95)/HZSM-5 catalyst and (c) the Co(3.38)-Ni(4.32)/hHZSM-5 catalyst

4. CONCLUSIONS

The Co(3.38)-Ni(4.32)/hHZSM-5 catalyst with the mess–microporous character was studied. It was observed that hierarchical pore structure might support the hydrogenation process on a poly-unsaturated chain into saturated chain fatty acid at 350 ºC. In addition, the various aromatic compounds were found, such as undecyl benzene (molecular weights of 232). It proved that during hydrocracking, the various reactions including cracking, deoxygenation, aromatization and cyclization.

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Effect of Variable of Propeller Shaft Angle for Propulsion of Traditional Fishing Vessels – A Study Case in Kampung Wadas Bojonegara, Banten

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Abstract – Research on performance of traditional fishing vessel propulsion by a variable of propeller shaft in Kampung Wadas, Bojonegara was done. The fisherman is not attention to the propeller shaft angles and hydrodynamics of ships; they are only oriented to the payload capacity and quantity of catches. This research attempt to the improvement of propulsion performance of traditional fishing vessel by determining of propeller shaft angle. The study aimed to get the propeller shaft angle to generate the optimal characteristic propulsion such as Torque, Efficiency and Shaft Angle. The results show the propeller shaft angle of 22 ° was given an optimum speed of the fishing vessel and power of the engine.

Keywords: *traditional fishing vessel, open water test, propeller, propulsion, shaft angle*

1. INTRODUCTION

A traditional fishing vessel that used in coastal areas is built with specific techniques and skills of the craftsmen ship. The traditional vessel built with non-modern technology and has many weaknesses in term of resistance and propulsion analysis.

Generally, the fishing vessels operating in the Kampung Wadas is not considered to the propeller shaft angles and hydrodynamics aspect; they are only oriented to the payload capacity (Sukardono, 2010). Most of the fishermen only use they experienced when operating the fishing vessel. Actually, in the concept of ship propulsion, the angle of shaft propeller is one that can affect the efficiency and fuel consumption.

2. BASIC THEORY

2.1 Propulsion

The propulsion system is a system from engine to the propeller for ship operation. Ship Propulsion System consists of three main components, such as prime mover (main engine), transmission system and propeller.

2.2 Flow velocity

The measurement of the speed of water flow from any variation of different angles can be expressed:

$$V=L/T \tag{2.1}$$

where:

V: Flow velocity (m / s) L: The length of the test track (m) T: Time (s)

2.3 Velocity of flow average

Knowing how the flow velocity in sea water density 0.8985 m / s in the corners that have been determined.

$$\frac{\rho 1}{\rho 2}=\frac{v1}{v2} \qquad v2=\frac{v1\,.\rho 2}{\rho 1} \tag{2.2}$$

Where:

Vwater: velocity of water flow rate in the density of seawater
ρ1 ɤ: The density of sea water (1030 kg / m³). $v1$:The flow velocity in the density of sea water (m / s)
ρ2 ɤ: Density of fresh water (1000 kg / m³) $v2$: The flow velocity in the density of fresh water (m/s)

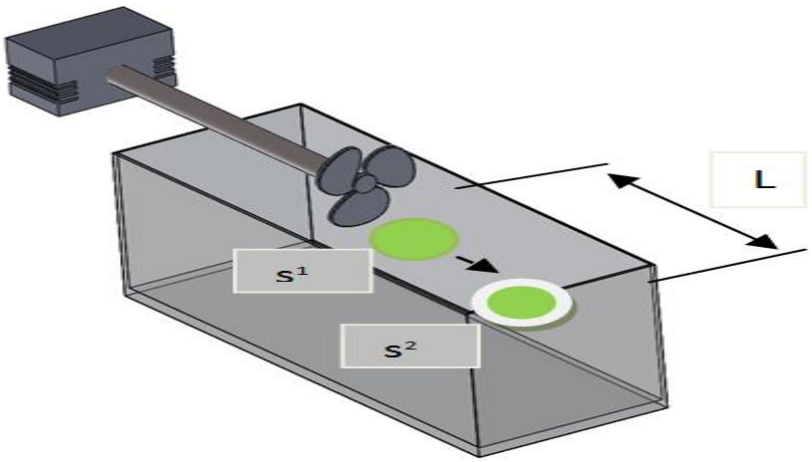

Figure 1 Test of water velocity

2.4 Power and Torque

The value of the power can be solve using the following equation:

$$PB = 2.\pi.Q.n \quad (2.5)$$

$$PS = PB.\eta GB \quad (2.6)$$

$$PD = PB.\eta S.\eta GB.\eta PD \quad (2.7)$$

$$PT = T.VA \quad (2.8)$$

$$Q = \frac{PB}{2.\pi.n} \quad (2.9)$$

where :

PB	= power engine (W)	ηGB	= Efficiency gear box
PS	= Power to the propeller shaft (W)	ηS	= Efficiency shaft
PD	= Power at the propeller (W)	ηPD	= Efficiency propeller
PT	= total power (W)	T	= Thrust vessel (N.m)
Q	= torque (N.m)	VA	= water flow rate of each variable angle (m/s)
n	= rpm		

2.5 Open water efficiency

Some equation for the find out the open water efficiency are:

$$KT = \frac{T}{\rho.n^2.D^4} \quad (2.10)$$

$$KQ = \frac{Q}{\rho.n^2.D^5} \quad (2.11)$$

$$J = \frac{Va}{n.D} \quad (2.12)$$

$$n_0 = \frac{J}{2.\pi} \cdot \frac{KT}{KQ} \quad (2.13)$$

$$T = \frac{SHP \times \eta_P \times \eta_{rr} \times 75}{ve} \quad (2.14)$$

where :

n	= 3768 rpm.	n_0	= efficiency*open water.*
J	= Advance number.	n_p	= efficiency propulsion.
KT	= Coefficient *thrust*.	n_{rr}	= efficiency Rotative.
KQ	= Coefficient torque.	P/D	= Ratio ranges from average leaf to its diameter.
Ae/Ao	= Blade area ratio		

3. METHODOLOGY

The experiment of measuring of flow rate was done with five test angle of propeller shaft (22 °, 24 °, 26 °, 28 °, 30 °). The sketch of experiment modeling used 3D *Software SolidWork 2010*. The experimental was set up in the Mechanical Engineering Laboratory of Faculty of Engineering UNTIRA campus Cilegon. Three blades propeller, cast iron material, and type B-Series propeller were examined.

4. RESULTS AND DISCUSSIONS

Based on the experimental results data, the results can be concluding that the best water flow velocity is at an angle of 22 ° with a value of 0.25 m/s the time gained by 1.26 seconds with a distance of 0.5 m, and minimum results found in a 30 ° angle to the value of the speed of 0.063 m/s time taken 4.9 seconds from a distance of 0.5 m ball release.

Tabel 1. Results of Data Experimental

No		Variation of Angle				
		22°	24°	26°	28°	30°
1	Q	2.053 W	2.053 W	2.053 W	2.053 W	2.053 W
2	T	6404.06 N.m	6404.06 N.m	6404.06 N.m	6404.06 N.m	6404.06 N.m
3	J	0.018	0.012	0.0102	0.006	0.0045
4	PD	470.2 W	470.2 W	470.2 W	470.2 W	470.2 W
5	Ps	729 W	729 W	729 W	729 W	729 W
6	PT	1601.01 W	1139.92 W	902.97 W	531.53 W	403.45 W
7	KT	0.69	0.69	0.69	0.69	0.69
8	KQ	0.39	0.39	0.39	0.39	0.39
9	Va	0.25 m/s	0.178 m/s	0.141 m/s	0.083 m/s	0.063 m/s
10	n_o	0.0016	0.0011	0.0009	0.00053	0.0004

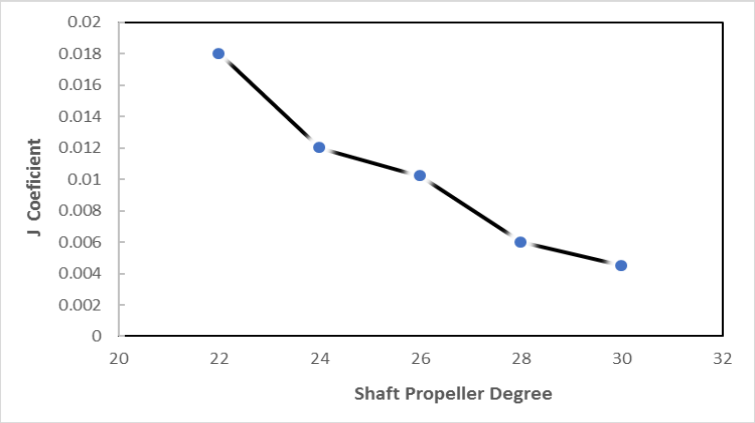

Figure 2 Graph of advance coefficient

Figure 2 shows the comparison chart between angle and advance coefficient. The angle of 22 ° give the highest advance coefficient.

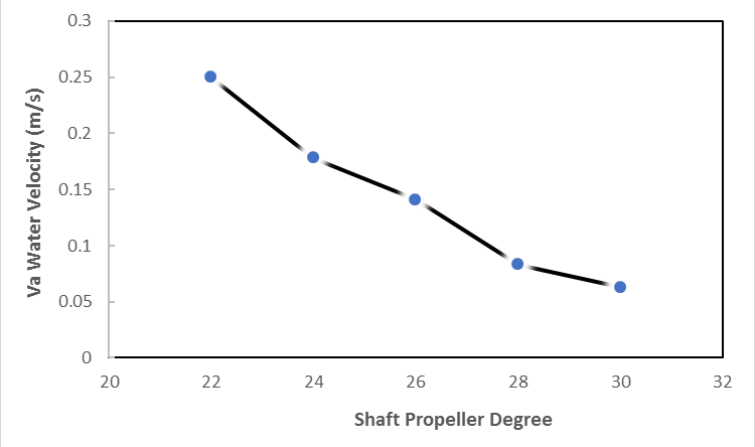

Figure 3 Graph of flow velocity

Figure 3 shows the graph of water flow velocity behind the propeller. The results show that the angle of 22 ° gives the greater of velocity water, it's around 0:25 m/s.

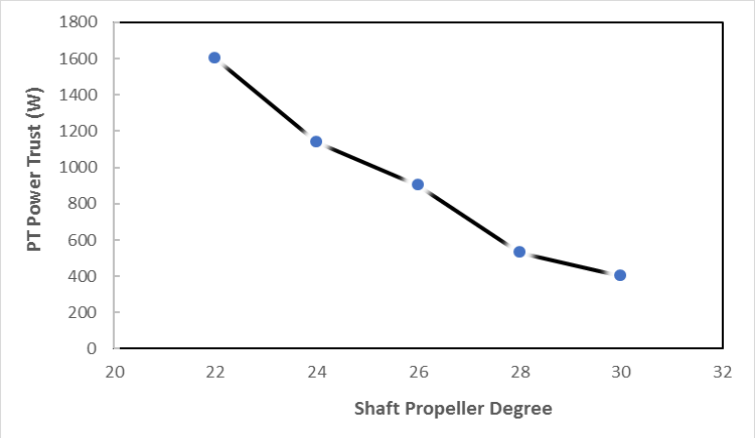

Figure 4 Graph of Power Trust

The result of power trust is shown in figure 4. The power maximum is given by the angle of 22, this phenomenon effect of in high value of advance coefficient and water velocity.

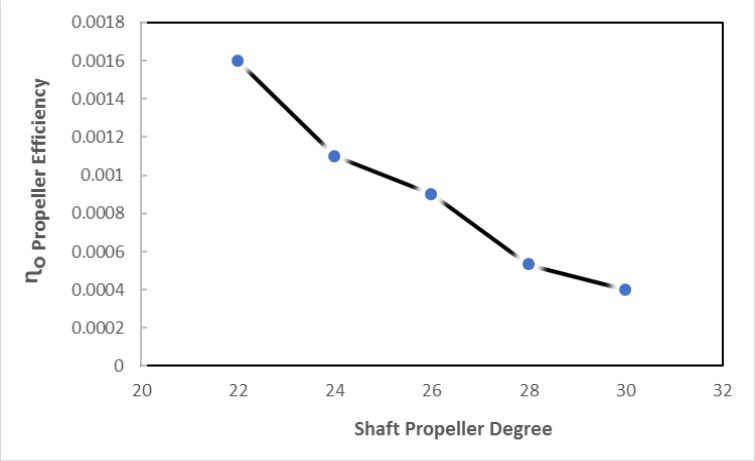

Figure 5 Graph of the propeller efficiency

Figure 5 show the efficiency of the propeller by a variable of shaft angle. The efficiency of the propeller is decreased by reducing the angle of the propeller. The decreased of efficiency is around 5% by two-degree differences. Generally, the results of the experiment were shown the shaft propeller angle affecting the performance of ship propulsion.

5. CONCLUSION

Based on the experimental data results, the characteristics of propulsion can be explained as follow: the best water flow velocity is 0.25 m/s at the angle of 22° of the shaft propeller, the time gained is 1.26 seconds in 0.5 m distance. The optimum power is 1601.01 W, and the efficiency (ηo) is 0.0016. The propeller shaft angle affected the characteristic of propulsion. This shows the fishermen that they must consider the operating procedure and technical aspect of the fishing vessels.

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Design and Build Thermoelectric Cooling Machine on Tawon Car

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Abstract – Cooling machine technology has direct contribution to environmental damage, including ozone depletion and global warming caused by leakage and disposal of synthetic refrigerants (CFC and HFC) to the environment. When CFC has spread to the ozone layer and very easily broken down and then react with the ozone formed by three atoms O (oxygen) which will also be split when there is a stronger appeal than other atoms in the outer. The chemical reaction between atoms is what will produce new molecules, ranging from O_2, O, CO, CO_2, and others. If 0_3 is already divided, its function as a filter solar radiation will be lost. Tawon car is a car assembled in the country, car intrinsically not equipped by the convenience of air conditioning systems, thermoelectric coolers in tawon car is one application of technological innovations thermoelectric, design method used for the process of designing thermoelectric cooler use method VDI 2221. The selected material is aluminum alloy. The result is the use of Peltier elements as cooling engine components by 4 units, pump and fan each of the pieces with the total power output of 200 Watts and a heat exchanger pipe length 2 m, still need more improvement to reach design requirements.

1. METHODS

1.1 Introduction

Cooling machine technology has direct contribution to environmental degradation including ozone depletion and global warming through leaks and discharges of synthetic refrigerants (CFC and HFC) to the environment (McMullan, 2002; Nasruddin, 2003). Release of refrigerant to the environment 60% of service sector (UNEP, 1999).The use of refrigerants usually applied in the vapor compression air conditioning machine on the vehicle or room.

CFC stands for Chloroflourocarbon formed from atoms of chlorine, fluorine, and carbon. The third atom includes atoms that have the number of valence electrons are relatively less stable or is bound by other atoms. When CFC has spread to the ozone layer and very easily broken down and then react with the ozone formed by three atoms O (oxygen) which will also be split when there is a stronger appeal than other atoms in the outer. The chemical reaction between atoms is what will produce new molecules, ranging from O_2, O, CO, CO_2, and others. If 03 is already divided, its function as a filter solar radiation will be lost.

Thermo Electric cooling (TEC), also often called Peltier cooling or solid-state heat pump that utilizes the Peltier effect. When TEC/Peltier current is passed then the device will move heat from one side to the environment and also move the cooler to the system which will be observed. Thermo Electric Cooling (TEC) could become an alternative source of vapor compression refrigeration cycle with the ability more environmentally friendly.

Tawon car is a car assembled in the country by default unit is not equipped by the convenience of air conditioning system (Air Conditioner). Therefore the design of thermoelectric cooler in tawon car is one application of technological innovations thermoelektric based on environmental concerns and as the advanced research of the technology being developed.

1.2 Basic Theory

a. The Basics of Vapor Compression Cooling

The major components of a refrigeration system are the compressor, condenser, expansion valve, and evaporator. The compressor is the heart of a refrigerant system: it uses a small amount of energy to generate the necessary refrigerant flow and subsequent heat transfer as desired. Aspen developed a miniature rotary compressor which efficiently generates a significant cooling capacity within a small volume. The compressor takes in low pressure refrigerant vapor and compresses it to a high pressure and temperature. The refrigerant undergoes an isothermal phase change (gas to liquid) and rejects heat to the ambient environment at a high pressure within the condenser.

The refrigerant throttled to a low pressure and temperature (typically below ambient) through the expansion valve. Refrigerant enters the evaporator primarily as a liquid and again undergoes an isothermal phase change (this time from liquid to gas) as the evaporator absorbs heat from the environment. The refrigerant again enters the compressor as a low pressure vapor restarting the cycle. The refrigeration cycle is demonstrated in the figure below.

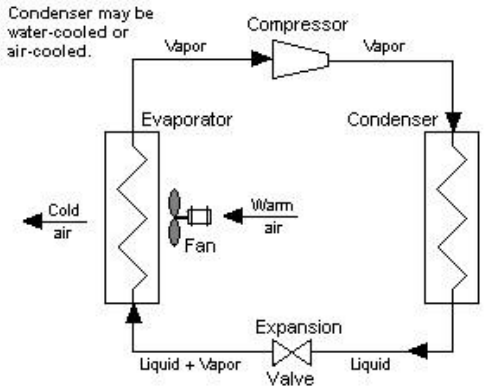

Figure 1 (Refrigeration cycle)

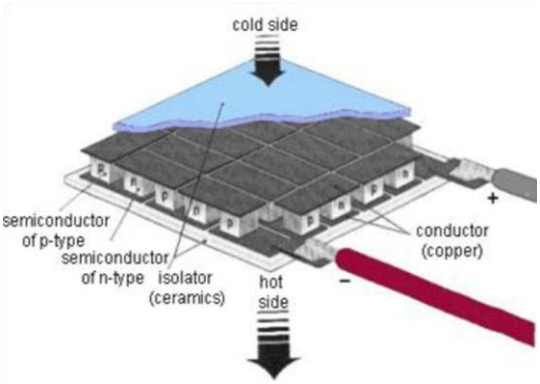

Figure 2 (Flow scheme peltier)

b. Thermoelectric Cooler

Thermoelectric cooling is a cooling device that uses peltier elements in the system as a heat pump. Peltier effect arises when two different metals are connected and both ends of the metal is maintained at different temperatures.

The basic principle of thermoelectric cooling based on the Peltier effect, when a DC current is passed to the element peltier which consists of several pairs of semiconductor cell p-type (semiconductors with high levels of lower energy) and n-type (semiconductor with an energy level higher), will result in one one side of the peltier element to cool (heat is absorbed) and the other side gets hot (heat is released), as shown in Figure 1, the peltier element that becomes the hot or cold depending on the direction of the flow of electric current.

Cold side peltier element to be cold is caused by the flow of electrons from lower energy levels in the p-type semiconductor, to a higher level of energy ie n-type semiconductor. In order for the p-type electron that has lower energy levels can drain the electrons absorb heat resulting in the side to cool. While the heat release to the environment occurs at the connection hot side, where electrons flow from the energy level is higher (n-type semiconductor) to a level lower energy (p-type semiconductor), to be able to flow into the semiconductor p-type, the excess energy at n-type discharge to the environment has become a hot side.

The absorption of heat from the surroundings occurs on the cold side which will then be disposed of on the hot side of the peltier element. So that the calorific value which is released at the hot side is equal to the heat absorbed plus the heat absorbed plus the power supplied to the module.

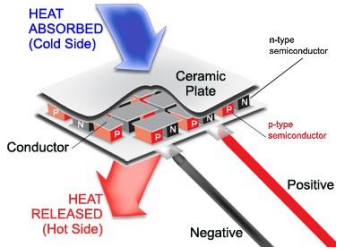

Figure 3 (Electrons flow direction at thermoelectric modules)

2. METHODOLOGY

a. VDI 2221

Once the list of requirements is created, which contains some restrictions that must be met (Demands) and restrictions that are expected to be met (Wishes) limitation demands and wishes, can be internal, namely the limits of the designer's own or external which is the limit of the consumer or users design results.

Variant and the selection of the variants on design ang build thermoelektric cooling machine on the tawon car have been grouped into two variants on the hot side of the peltier element and a variant on the cold side peltier element.

The function of cooling machine in the design can be written as follows:

a. Can operate on the car operating or parked.
b. As temperatures drop in the cabin when the car is parked and the engine driving conditions are not all operating and no passengers.
c. The operator can operate as desired.
d. The energy source is derived from a separate battery from the electrical system of the car.
e. Charging the battery comes from a solar panel that is separate from the car electrical system.

Of the variants that have been made, the selected variant 2 (Variant 2: A1 A2 B3 A4) on the hot side of the peltier element and variant 1 (Variant 1: A1 - A2 - A3) on the cold side peltier element. The variants can then proceed to the next design process, and require further analysis to system design in accordance with the desired specifications. The material chosen by the designer is, with family metals AND class aluminum alloy.

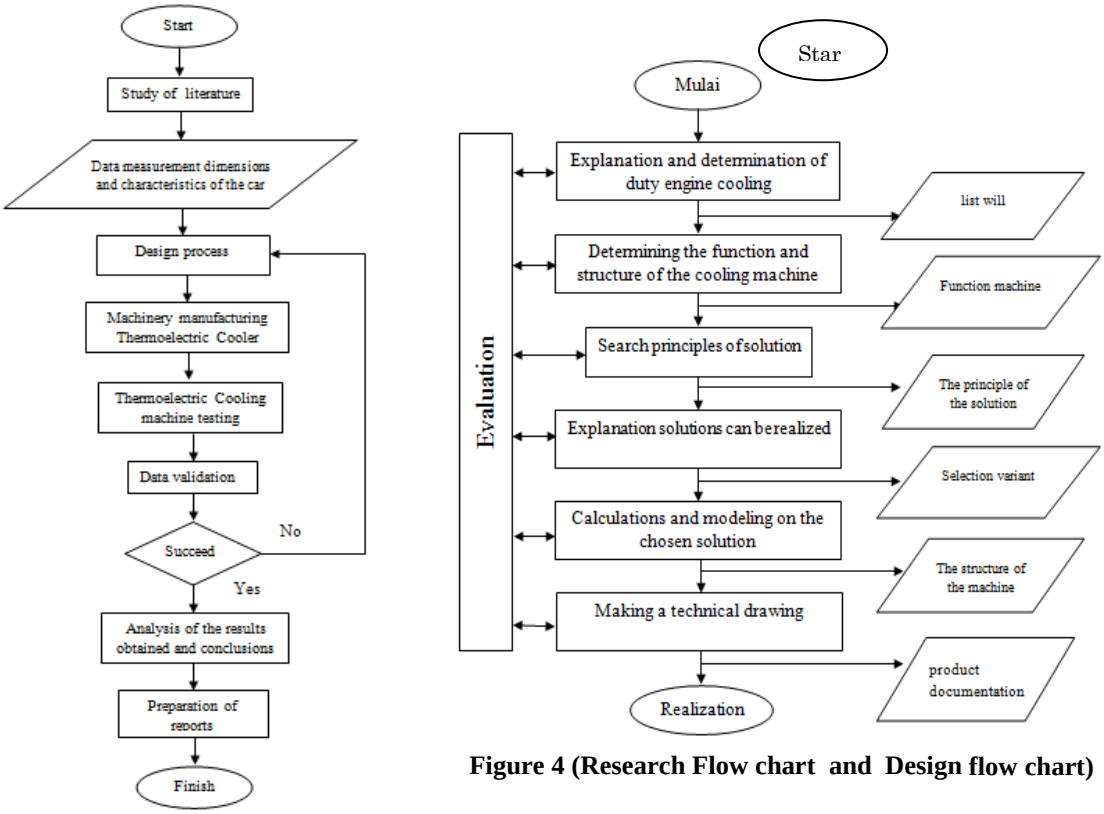

Figure 4 (Research Flow chart and Design flow chart)

3. DESIGN AND ANALYSIS

a. Tempemperature decrease on the car

in the design and analysis results obtained the transmission of heat and radiation, when the condition of the cabin temperature is equal to ambient temperature then the transmission load equal to zero then applies equation

$$Q\,transmision = Q\,radiation$$

The magnitude of the load transmission if $\Delta T = 2$ is:

Table 1 (the thermal load transmission)

MATERIAL	TRANSMISSION LOAD (W)
STEEL-PUTTY	7,795
STEEL-PUTTY- FOAM	19,094
STEEL FOAM(DOOR)	19,549
STEEL FOAM(ROOF)	15,670
STEEL-CARPET	27,964
GLASS	364,59
RADIATION ON THE SURFACE IS NOT THROUGH LIGHT	1540,634
RESULTS	1995,3

b. The number of peltier elements and additional components of cooling machine.

In the selection of the number of peltier cooling element is limited by the amount of power provided by solar panels and batteries, the energy available is limited to 200 watts for cooling components, can be divided into:

Table 2. Components of cooling machine

No	Components	amount	Power (W)	overall power (W)
1	Peltier	4	40	160
2	Water pump coller	1	18	18
3	Fan cool side	2	2,4	4,8
4	Safety Factor load			17,2
	Result			200

c. Cooling box

Based on the data obtained in the market gained sold box dimensions are 70 x 21 x 6000 mm, beam shape chosen because it has a flat surface and in accordance with peltier dimensions. Here is a 3D design of the cooler:

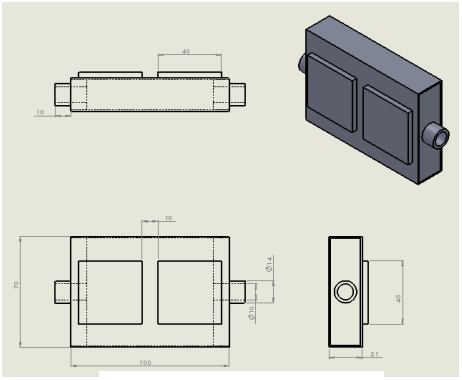

Figure 5 (peltier box)

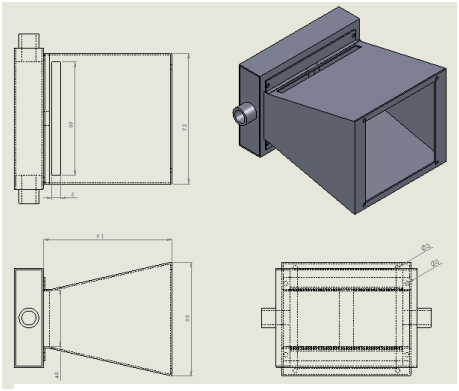

Figure 6 (Construction on cold side)

d. Fan cold side

Fan used to induce airflow to the car cabin and make speed in increased cooling, while the specifications of the fan selected is 22.58 CFM = 0.012 kg / s at air temperatures of 30 ° C, the fan is selected because it has a fairly small, the flow rate is quite large and easily available in the market.

the tool is designed so that air flows on the cold side peltier and cause a decrease in the temperature of the air, here is the design of the fan cold side peltier, from the design can be calculated besaanya drop in temperature experienced by the air:

$$Q = \dot{m} . Cp . \Delta T$$
$$40\ Watt = 0{,}0125\ kg/s . 1005\ J/kg.°C . \Delta T$$
$$\Delta T = 3{,}173\ °C$$

e. Pipe on the hot side and the cooling water and design wiring diagrams

Cooling is performed by a cooling box causes the temperature rise on the hot side and the cooling water, the amount of power transmitted into the hot side of the cooling box by 4 units are: Q_{box} = (peltier units).(calor energy)

$$Qbox = 4 . 20\ Watt$$
$$Qbox = 80\ Watt$$

with a power of 80 watts received on the hot side of the heat exchanger pipe length on cooling machine are:

$$Q = \Delta T/(R_o + R_s + R_u)$$

$$Q = \frac{(30{,}068 - 29{,}531)°C}{(0{,}0079\ \frac{m.°C}{W} .2\ m + 1{,}423 \times 10^{-4}\ \frac{m.°C}{W} .2\ m + 0{,}0041\ m\ °\frac{m.°C}{W} .2\ m)}$$

$$Q = 88\ W$$

On the results of the calculation assumed a heat exchanger pipe length of 2 m, with a record 0.537 ambient temperature must be lower than the temperature of the water and power ditrasmisikan by 80 + 10% safety factor

Here is a 3D design of heat exchanger hot side peltier:

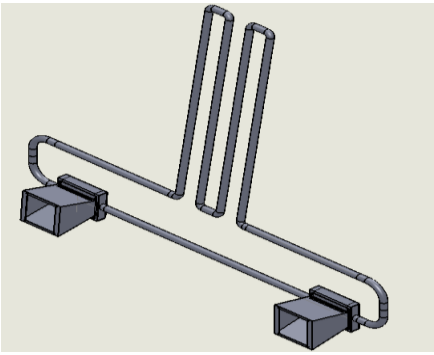

Figure 7 (Construction heat exchanger)

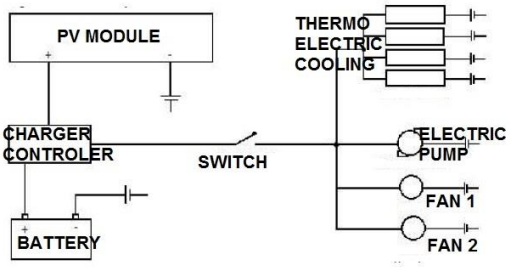

Figure 8 (wiring diagram)

4. CONCLUSION

the result is the use of peltier elements as cooling engine components by 4 units, pump and fan each of the pieces as well as additional safety factors of electrical load with the total power output of 200 watts and a heat exchanger pipe length 2 m.

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Resistance Pattern of *Mycobacterium tuberculosis* to First - Line Anti-Tuberculosis Drugs (ATDs) in Central Java, Indonesia

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Abstract - Multidrug-resistant (MDR) tuberculosis occurred among 2% of all new tuberculosis (TB) cases and 20% of tuberculosis cases with re-treatment. World Health Organization (WHO) reported there were about 6,300 cases of MDR-TB every year. Resistance pattern of tuberculosis to Anti-Tuberculosis Drugs ATDs) vary in one population to another so that MDR data were required in each area. This study was done to evaluate the resistance pattern of Mycobacterium tuberculosis (M. tuberculosis) isolates to the first line ATDs in Health laboratory of Central Java Province, Indonesia. Some isolates (90.5%) showed resistance to isoniazid, (76.2%) were resistant to ethambutol and (61.9%) were resistant to streptomycin. Resistance number to rifampicin occurred among (100%) isolates. In this study, a single resistance of M. tuberculosis isolates was only shown in the resistance to rifampicin, as many as 2 (3:17%). Resistance to two types of drugs was 8 (12.7%) isolates. As much as 4 isolates (6:35%) were resistant to isoniazid and rifampin combination, 3 isolates (4.76%) were resistant to rifampicin and streptomycin, and 1 isolate (1:59%) was resistant to rifampicin and ethambutol. None of the isolates showed resistance to the Isoniazid-Streptomycin combination, Isoniazid-Ethambutol combination, and Streptomycin-Ethambutol combination. There were 8 (12.7%) isolates resistant to the combination of three ATDs, while 17 (27%) of them resistant to Isoniazid, Rifampicin and Ethambutol combination. A total of 31 (49.21%) isolates showed resistance to all first-line ATDs. In conclusion *Mycobacterium tuberculosis* resistance to ATDs in the Central Java Province was dominated by resistance to rifampicin. Resistance to ATDs can be singular or double; with the largest number of cases were resistant to the combination of four types of first line ATDs.

1. INTRODUCTION

Tuberculosis was known as an infectious disease with high prevalence in developing countries. Indonesia is one of the countries with tuberculosis highest burden in Southeast Asia, even in the world (1). Tuberculosis was infected among 660,000 people and estimated 430,000 new cases were found every year. Death caused by tuberculosis was reported in 61,000 cases every year, while Multidrug-resistant (MDR) tuberculosis estimated at 2% of all new cases and 20% of cases with re-treatment (2). Multidrug-resistant tuberculosis is a condition in which the bacteria of tuberculosis were resistant to the drug combination of standard guidelines (2). Multidrug-resistant tuberculosis infection caused types of drugs commonly used appropriately to treatment no longer able to kill bacteria. Patients with MDR tuberculosis showed resistant to first line ATDs at least isoniazid-rifampicin combination, with or without another drug. In cases of MDRTB, then in addition to the possibility of transmission to people around the patient (3) also require much higher costs in the next phase of treatment (4).

Tuberculosis resistance patterns to ATDs vary in one population with other populations (5,6). Various economic, social and demographic factors such as poor education, inaccessible health facilities, smoking; social stigma and social discrimination increase the risk of the MDR tuberculosis spreading (7,8) in a certain area. This is the importance of why each region in the province should have the complete MDR tuberculosis data. Epidemiological data, in which includes ones on resistance of Mycobacterium tuberculosis can help clinicians determine the appropriate treatment for the patient, as well as direct the policy makers to design a treatment program to resolve tuberculosis problems (9).

This study aims to evaluate the resistance pattern of isolates of Mycobacterium tuberculosis to first-line Anti-Tuberculosis drugs in Health laboratory of Central Java Province.

2. METHODS

This was a descriptive retrospective study. All samples provided in the Health laboratory of Central Java Province.

2.1 Procedures

Working procedures of the study started with the planting of the bacteria on the Lowenstein Jensen (LJ) medium for 3-8 weeks. In the event of colony growth of M. tuberculosis, then in said colony, it was continued to test the sensitivity of the bacteria. Sensitivity method used was a modified proportional indirectly by LJ seeding media. Prior to this medium was used for growing bacteria, the fertility test on the media was previously conducted using the bacterium of M. fortuitum within a catalog number of ATCC6841. Each sample was grown on one series of LJ medium containing first line ATDs, such as Streptomycin, Izoniasid, Rifampicin and Ethambutol. Series of tubes inoculated with bacteria were then incubated at a temperature of 37ºC for 3 weeks, and every week, their growth was observed. On control media, bacteria will grow confluently, this was considered to grow 100%. The designation of resistance was done by comparing the percentage growth of bacterias on the drug to control media. Control strains used in this study were BA 66 for a comparison of resistance to streptomycin and rifampicin, and BA 63 for a comparison of resistance to isoniazid and ethambutol. When the number of colonies of bacteria that grow on a medium contained more than 1% ATDs, it was considered resistant isolates.

3. RESULTS AND DISCUSSION

During the period of April 2015 to May 2016 in the Health laboratory of Central Java Province, grown a number of 63 isolates of M. tuberculosis. From all the isolates, bacterial sensitivity test was conducted to first-line antituberculosis drugs, such as Streptomycin, Isoniazid, Rifampicin and Ethambutol. The sensitivity test results are as set forth in Figure 1. Some 57 (90.5%) isolates showed resistance to isoniazid, a number of 48 (76.2%) isolates showed resistance to ethambutol and a total of 39 (61.9%) isolates were resistant to streptomycin. Meanwhile, the highest number of resistance was shown by isolates to rifampicin, occurred at 63 (100%) isolates.

In this study all of the isolates were cultured (100%), and the biggest number of resistance was to rifampicin. The following resistance was to isoniazid, ethambutol and at last to streptomycin.

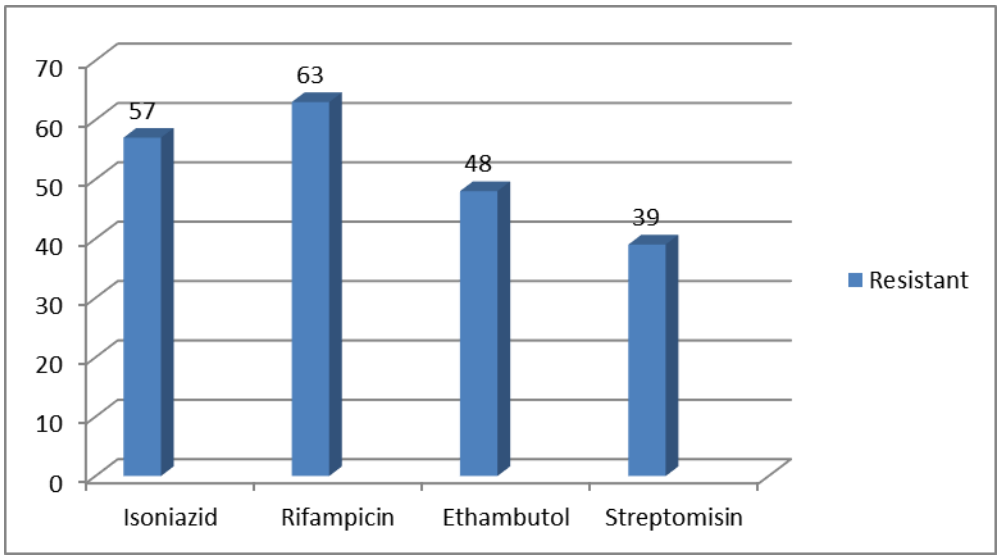

Figure 1. The test results of resistance in Mycobacterium tuberculosis isolates to first-line ATDs.

The exact same results in this study occurred in Mumbai India where isolates of M. tuberculosis resistance to rifampin also reached 100% (10). The previous study conducted by Rintiswati in 1999, showed that resistance to rifampicin occupies the top position of resistance (62.50%) compared to other first-line ATD(s) (11). After six years later, when the same study was conducted, the result was no different, a resistance to rifampicin still ranked first (43.43%) compared to the value of resistance of other drugs (streptomycin: 33.33%., Ethambutol: 26.26%. , isoniazid: 24.24%), although their values are impaired (12).

Resistance patterns of M. tuberculosis isolate to ATD(s) in this study were described in Table 1. In this study, a single resistance of M. tuberculosis isolates was only shown in resistance to rifampicin, as many as 2 (3:17%). The number of resistance to two drugs is not too large, (12.7%) isolates. Some 4 (6:35%) isolates were resistant to isoniazid and rifampin combinations, 3 (4.76%) isolates were resistant to rifampicin and streptomycin, and 1 (1:59%) isolate was resistant to rifampicin and ethambutol. From a number of sensitivity to multiple drug tests, none of the isolates showed resistance to the combination of drugs such as Isoniazid-Streptomycin, Isoniazid-Ethambutol and Streptomycin-Ethambutol.

The resistance to the combination of 3 ATD(s) is as much as eight (12.7%). Most resistance occurred on a combination of drugs such as Isoniazid, Rifampicin, Ethambutol as many as 17 (27%). There was no resistance

to the drug combination of Isoniazid, Streptomycin, Ethambutol and Rifampin, Streptomycin, Ethambutol. A great number, instead, is precisely shown in the resistance number to 4 types of ATDs (Isoniazid, Rifampicin, Streptomycin, Ethambutol). A total of 31 cases (49.21%) showed resistance to all first-line ATD(s). This study showed that resistance could occur on two types of drugs, three types of drugs or against 4 types of first line ATDs combined altogether (13–15). In fact, the results of this research were that the percentage of resistance to 4 types of drugs in the highest rank (49.21%) compared to other dual resistances. This suggests that the risk of resistance to first-line drugs is alarming.

Tabel 1. Resistance patterns of Mycobacterium tuberculosis isolates to first-line ATDs in the period of April 2015 to May 2016 in Central Java (n=63)

Types of ATD	Amount of resistance	% resistance
Single resistance	2	3.17
Isoniazid	0	0
Rifampisin	2	3.17
Ethambutol	0	0
Streptomisin	0	0
2 drugs resistance	8	12.7
Isoniazid, Rifampisin	4	6.35
Isoniazid, Streptomycin	0	0
Isoniazid, Ethambutol	0	0
Rifampicin, Streptomycin	3	4.76
Rifampicin, Ethambutol	1	1.59
Streptomycin, Ethambutol	0	0
3 drugs resistance	22	34.92
Isoniazid, Rifampisin, Streptomisin	5	7.93
Isoniazid, Rifampicin, Ethambutol	17	27
Isoniazid, Streptomycin, Ethambutol	0	0
Rifampicin, Streptomycin, Ethambutol	0	0
4 drugs resistance		
Isoniazid, Rifampicin, Streptomycin, Ethambutol	31	49.21

Resistance is in the category of MDR (resistant to at least isoniazid and rifampicin) occurred in 57 out of 63 isolates. Most TB cases occurred in patients who have previously been treated, uneducated, without health insurance, or from their medical history it was known that they had previously suffered from tuberculosis for at least 3 months. Patients with previous treatment are at risk of getting TB because of inadequate treatment when ATDs are still sensitive or because of relapse after the treatment period were not previously known that they already had suffered from MDR resulting in a wrong treatment at the time. Nevertheless, this re-infection is also a concern to consider (16).

4. CONCLUSIONS

The resistance pattern of M. tuberculosis to anti-tuberculosis drugs in Central Java is dominated by resistance to rifampicin. Resistance to ATDs can be singular or double; with the largest number of cases was resistance to the combination of four types of ATDs.

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Insulin-like Effects of Anti-diabetic Metals (V, Mo, W, and Cr): An Adipogenesis study

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Abstract – The mechanisms of action of V(IV/V), Mo(VI), W(VI), and Cr(III) compounds in this study are thought to be mainly due to their negative regulation of insulin signalling pathway, by inhibition of protein tyrosine phosphates (PTPs), which dephosphorylate phosphotyrosine residues. Therefore, these metal compounds not only affect the insulin signaling pathway but also affect the whole cellular metabolism. In order to investigate insulin-like effects of V(V/IV), Mo(VI), W(VI), and Cr(III) compounds, the current work involves performing adipogenesis assay, or differentiating 3T3-L1 pre-adipocytes into mature adipocytes under stimulation of these compounds. A range of treatment conditions, 10 μM V(V), 10 μM V(IV), 100 μM Mo(VI), and 100 μM W(VI) stimulated adipogenesis levels in the 3T3-L1 adipocytes to similar degrees as those observed with insulin. These results demonstrated the capacity of V(V/IV), Mo(VI), and W(VI) complexes to have insulin-like effects in differentiating 3T3-L1 pre-adipocytes. However, Cr(III) treatment did not stimulate adipogenesis of 3T3-L1 pre-adipocytes to adipocytes.
Keywords: 3T3-L1, adipogenesis, chromium, molybdenum, tungsten, vanadium, anti-diabetes drugs

1. INTRODUCTION

The prevalence of diabetes has reached epidemic proportions and is continuously increasing worldwide. The number of people with diabetes is projected to grow more than double by 2030 from 171 million in 2000 to 366 million people [1]. The diabetes epidemic particularly relates to type 2 diabetes, which accounts for over 90-95% of all cases of diabetes globally [2]. The pathogenesis of type 2 diabetes remains unidentified since there are many malfunctioning mechanisms that simultaneously occur contributing to the progression of the disease. In addition to genetics, which predisposes individuals to develop this disease, there are also many factors which contribute to its development, including physical inactivity, poor nutrition practices and obesity [3]. If left untreated, type 2 diabetes can lead to a multitude of chronic microvascular and macrovascular conditions, such as retinopathy, nephropathy, neuropathy and cardiovascular diseases [2]; which results in increasing disability, reduced life expectancy, and massive health costs. Therefore, efforts to search for safe and effective treatments for type 2 diabetes have become a top priority for both clinicians and researchers.

Complexes of V(V/IV), Cr(III), W(VI), Mo(VI), Zn(II), and Cu(II) have all been proposed as possible drugs in the treatment of diabetes mellitus [4]. It is now accepted that V(V/IV), Cr(III), Mo(VI), and W(VI) interfere with the numerous phosphorylation/dephosphorylation reactions involved in the glucose metabolism. The biological activities of anti-diabetic metal complexes not only affect the insulin signaling pathway but also affect the whole cellular metabolism.

The insulin-like effects of the anti-diabetic metal complexes used in this study were investigated by growing 3T3-L1 mouse preadipocytes to mature adipocytes with regular adipogenesis cocktails, or insulin only, or with the addition of V(V/IV), Mo(VI), W(VI), and Cr(III) complexes. There are studies reporting the effect of anti-diabetic agents on the process of insulin-induced adipogenesis, most of these agents are troglitazone and they can accelerate the adipogenesis process. The ability of $VOSO_4$ to induce differentiation of 3T3-L1 pre-adipocytes to adipocytes in the absence of insulin has been reported [5]. In addition, to provide a complementary overview the effects of anti-diabetic metals on the adipogenesis process of 3T3-L1 pre-adipocytes, the experiments have also been followed using IncuCyte Zoom™ live-cell imaging system (Essen Bioscience) [6].

2. METHODS

2.1 Chemicals

The following reagents: 1–([4–(xylylazo)xylyl]azo)–2–naphthol solvent red 27 (Oil Red O dye), sodium orthovanadate (Na_3VO_4), [$VO(acac)_2$] (acac = acetylacetonate (1–)), sodium molybdate dihydrate ($Na_2MoO_4{\cdot}2H_2O$), were from Sigma-Aldrich; $Na_2[CrO_4]$ was from Ajax; sodium tungsten dihydrate ($Na_2WO_4{\cdot}2H_2O$) was from AnalaR. Na3[Cr(cit)2] (cit = citrato (3–)) was previously synthesized and characterized by Dr T. H. N. Pham. Water was purified using the Milli-Q technique. The following commercial biochemical: foetal calf serum (FCS, heat-inactivated), L–glutamine solution (200 mM), insulin from bovine pancreas, dexamethasone, 3–*iso*–butyl 1–methylxanthine (IBMX), and 2-deoxy-D-glucose (2-DG), were purchased from Sigma-Aldrich; D-glucose (Merck), Dulbecco's modified Eagle's medium – high glucose (DMEM, containing 4.5 g/L glucose, sodium pyruvate, and without L-glutamine), antibiotic-antimycotic mixture (100 U/mL penicillin, 100 U/mL streptomycin, and 0.25 U/mL amphotericin B), phosphate buffer saline (PBS) were from Gibco. For cell experiments, the 3T3-L1 pre-adipocytes (mouse embryonic fibroblast-adipose like cell line, CL-173) were purchased from the American Tissue Culture Collection (ATCC, Manassas, VA).

2.2 Procedures

The 3T3-L1 preadipocytes were seeded into a 24-well plate, and the differentiation was started 2 d after cells reached 100% confluency. The cells were treated with 10 μM $Na_3[VO_4]$, 10 μM $VO(acac)_2$, 100 μM $Na_2[WO_4]$, 100 μM $Na_2[MoO_4]$, 100 μM $Na_3[Cr(cit)_2]$, or 2.0 μM $Na_2[CrO_4]$ in the absence of bovine insulin. The medium was changed every 2 d, and each time the treatments were added. In the positive control, the cells were stimulated by the standard adipogenesis cocktails, while the untreated cells were used as a negative control. After 12 d of differentiation, the cells were stained with oil red O stain to examine the formation of triglycerides and other lipids, which indicated the extent of differentiation [7]. In general, the cells were washed two times with PBS, and fixed with 10% formalin (in PBS, v/v) for 60 min at room temperature. They were then washed with deionized water, and incubated with 60% of 2-propanol (in H_2O, v/v) for 5 min. Next, the cell layers were treated with oil red O solution (1.8 mg/mL) for 5 min, and washed with deionized water. Finally, the stained cells were shaken with DMSO for 1 h to dissolve the absorbed oil red O, and the absorbance of the resulting solution was measured at 490 nm. Results were expressed as mean ± SEM, and the differences between groups were evaluated using Student's *t*-test.

The adipogenesis of 3T3-L1 pre-adipocytes to 3T3-L1 adipocytes has also been followed using IncuCyte ZOOM™ live cell imaging system (Essen Bioscience) [6]. Similar cell preparations as mentioned above were applied. The groups of treatment were: 10 nM insulin, 10 μM $Na_3[VO_4]$, 10 μM [$VO(acac)_2$], 100 μM $Na_2[WO_4]$, 100 μM $Na_2[MoO_4]$, 100 μM $Na_3[Cr(cit)_2]$, or 2.0 μM $Na_2[CrO_4]$. Cells stimulated with standard adipogenesis reagents were used as positive control. The cell culture plates were placed into the IncuCyte incubator at day 0, and kept for 12 d inside the IncuCyte incubator. In this study, the software parameters were set up for proliferation assay for 24-well plate (Corning) with a 10× objective, images were taken every 2 h, with 9 images per well.

3. RESULTS AND DISCUSSION

The absorbance at 490 nm was proportional to the levels of adipogenesis in the 3T3-L1 cells, and stimulated by anti-diabetic metals (no insulin added): V(V), V(IV), Mo(VI), and W(VI) were significantly lower at $p < 0.05$, and at $p < 0.05$ for Cr(III)- and Cr(VI)-stimulated cells, compared to those in control + cell (stimulated by adipogenesis cocktails). The insulin treatment and treatments with V(V), V(IV), Mo(VI), and W(VI), led to similar levels of adipogenesis in 3T3-L1 adipocytes (Figure 1). In contrast, Cr(III) and Cr(VI) complexes did not significantly increase the adipogenesis compared with negative control.

The differentiation of 3T3-L1 pre-adipocytes to adipocytes has also been monitored under a light microscope as cell morphology changed and cells accumulated fat droplets that can be stained by oil red O dye. Figure 2 shows the images of 3T3-L1 adipocytes under the influences of anti-diabetic metals without insulin addition, together with positive and negative controls. As depicted in that figure, the extent of differentiation of 3T3-L1 pre-adipocytes into adipocytes upon conditions of 10 μM $Na_3[VO_4]$, 10 μM [$VO(acac)_2$], 100 μM $Na_2[MoO_4]$, and 100 μM $Na_2[WO_4]$, for 12 d was found to be comparable to the 100 nM insulin treatment. Unstimulated cells (negative control) exhibited no oil droplet formation, while cells treated with standard adipogenesis cocktails appear to be fully differentiated. Cells treated with 100 μM Cr(III) compound only had low oil droplet formations compared to treatments with 10 μM V(V), 10 μM V(IV), 100 μM Mo(VI), and 100 μM W(VI) complexes. Under treatment with 2 μM $Na_2[CrO_4]$, however, 3T3-L1 did not seem to differentiate into adipocytes.

These results demonstrated the capacity of V(V/IV), Mo(VI), and W(VI) complexes to have insulin-like effects in differentiating 3T3-L1 pre-adipocytes. In a recent study, V(V) complexes with Schiff-base organic ligands, in

the range concentrations of 1-20 µM, showed 3T3-L1 adipogenic activity to produce mature adipocytes [8]. Chromium(III) treatment did not stimulate adipogenesis of 3T3-L1 pre-adipocytes to adipocytes to a similar level as was observed those in insulin stimulation. These results indicate that Cr(III) has little or no insulin-enhancing effect, compared with V compounds and other metal complexes in this process.

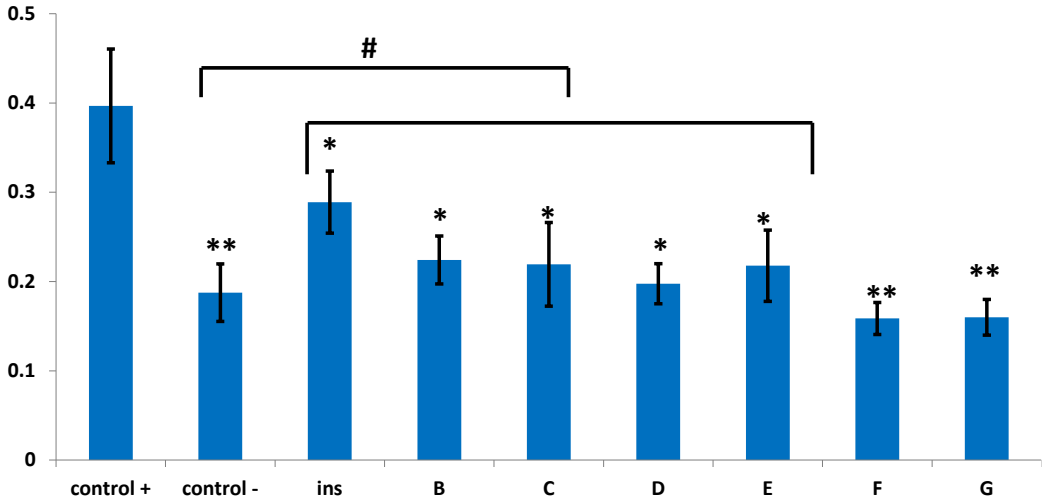

Figure 1 The absorbance at 490 nm of 3T3-L1 adipocytes, stained for lipid with oil red O dye. 3T3-L1 pre-adipocytes were differentiated to 3T3-L1 adipocytes under stimulation of 100 nM insulin or anti-diabetic metals: (B) 10 µM $Na_3[VO_4]$; (C) 10 µM $[VO(acac)_2]$; (D) 100 µM $Na_2[MoO_4]$; (E) 100 µM $Na_2[WO_4]$; (F) 100 µM $Na_3[Cr(cit)_2]$; and (G) 2 µM $Na_2[CrO_4]$, for 12 d, at 310 K. 3T3-L1 pre-adipocytes were also differentiated under stimulation by standard adipogenesis cocktails (control +), and under maintaining medium as control – (DMEM-high glucose, 10% FCS v/v). Significant differences were based on Student's t-test using a two-sample independent test: ** p <0.01, and * p < 0.05, decreased compared to control +, $^{\#}$ p < 0.05, increased compared to control –.

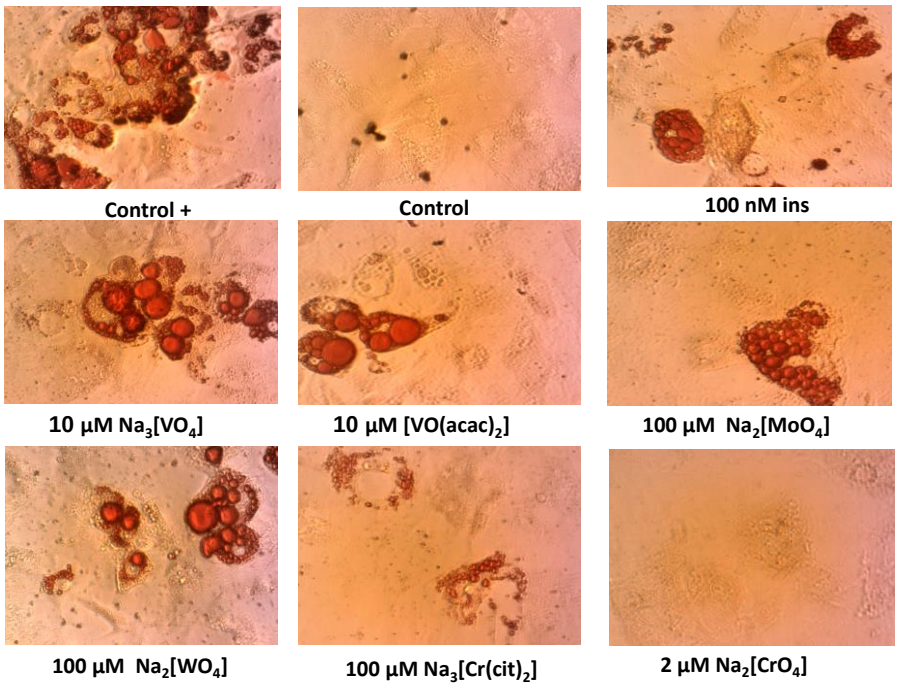

Figure 2 Typical images under light microscope (40x objective) of lipid droplets in 3T3-L1 adipocytes under stimulation of 100 nM insulin or anti-diabetic metals: 10 µM $Na_3[VO_4]$; 10 µM $[VO(acac)_2]$; 100 µM $Na_2[MoO_4]$; 100 µM $Na_2[WO_4]$; 100 µM $Na_3[Cr(cit)_2]$; and 2 µM $Na_2[CrO_4]$, for 12 d, at 310 K. The 3T3-L1 pre-adipocytes were also differentiated under stimulation by the standard adipogenesis cocktails (control +), and under maintaining medium as control – (DMEM-high glucose, 10% FCS v/v).

The IncuCyte ZoomTM live-cell imaging system was also used in adipogenesis assays to follow the differentiation of 3T3-L1 pre-adipocytes to adipocytes and it is also possible to capture cell images in the bright phase contrast mode. Figure 3 displays typical images from 3T3-L1 adipocytes taken from the IncuCyte ZoomTM system, showing the last scan image of the assay, with a wider view than in the oil red O staining figures. These experiments suggested that low insulin concentrations (10 nM) did not differentiate 3T3-L1 pre-adipocytes significantly. Moreover, the 10 µM V(V) and 10 µM V(IV) treatments even resulted in higher lipid droplets formations than insulin treatment at that concentrations, although no treatment reached a similar level as those obtained in treatments with standard adipogenic cocktails.

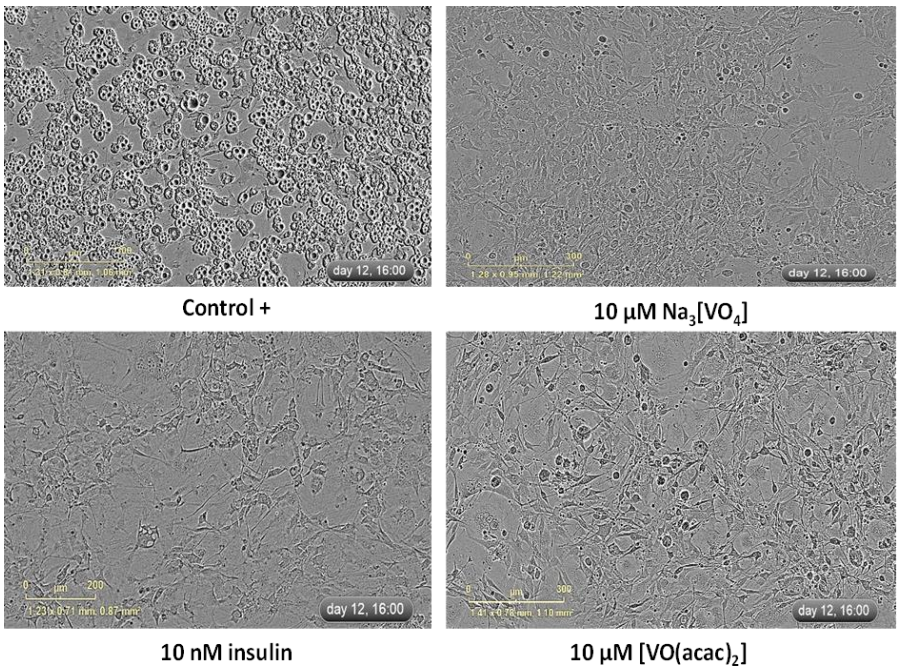

Figure 3 Typical bright phase images taken the IncuCyte ZoomTM live-cell imaging system (10x objective) of 3T3-L1 adipocytes under stimulation of 10 nM insulin, 10 µM Na3[VO4], and 10 µM [VO(acac)2], for 12 d, at 310 K. The pictures were taken at final scan image of the experiment. The cells stimulated with standard adipogenesis cocktails were used as control +.

4. CONCLUSIONS

The current work has demonstrated the capacity of V(IV/V), Mo(VI), and W(VI) compounds to have insulin-like effects in differentiating 3T3-L1 pre-adipocytes. However, Cr(III) compound has little to no insulin-enhancing effect, compared to those metal complexes. Overall, results for this assay suggest that the adipogenic actions demonstrated by V(V/IV), Mo(VI), W(VI) compounds that mimic insulin action can open new understandings of their mechanism of action for type 2 diabetes treatment.

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Sensitivity Enhancement of *Mycobacterium tuberculosis* Detection Based on Nucleotide Amplification of *esxA* Gene in Sputum Specimen of MDR-TB Patients

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Abstract – Multidrug-resistant tuberculosis (MDR-TB) has become a global issue that need to be handled with the right strategy. esxA gene is one of the most important virulence factors of *Mycobacterium tuberculosis* complex (MTBC) which is also known as conserved gene. However, mutation in target gene of MDR-TB patients could impair fitness cost of bacteria, thus sequence variation might possibly rise in esxA gene. The purpose of this study was to evaluate the accuracy of PCR based amplification of esxA gene nucleotide region method in MDR-TB. Total 97 sputum samples from patients with pulmonary tuberculosis in Dr. Soetomo Hospital, Surabaya, Indonesia were collected via consecutive sampling from August 2016 to November 2016. All samples were detected using GeneXpert. Total 20 samples out of 97 samples were detected as MTBC positive, while MDR was then detected using PCR targeting esxA gene. Primers were designed using Clone Manager 6, version 6.00. Amplicons confirmed positive indicated by DNA bands appeared at 580 bp. Total 20 sputum samples identified by PCR showed positive results, in concordance with the results of GeneXpert. Five of positive PCR products were sent for sequencing. Sequence of five samples were analyzed and found to have 100% homolog with *M. tuberculosis* H37Rv (NC_000962.3). GeneXpert method had been declared as the gold standard method of nucleic acid amplification, however it needed high cost. Developing a diagnostic method using PCR based on esxA gene could become an alternative way in determining MTBC diagnosis.

1. INTRODUCTION

Multidrug-resistant tuberculosis (MDR-TB) can occurred when *Mycobacterium tuberculosis* (*M. tuberculosis*) infected host has built a resistance on rifampicin or isoniazid as strongest anti-tuberculosis drug resistance. MDR/RR-TB is one of the global issue that lead to mortality and required right strategy to handle it. WHO reported as much as 10,4 million of tuberculosis (TB) cases occurred globally with mortality rate of 1,4 million in 2015 [1]. In addition, there were 132,120 cases of detected and notified of MDR-TB or rifampicin resistant (RR-TB) globally in 2015 [1].

Indonesia was ranked as second highest TB Burden country in the world back in 2015. Incident rate of all TB cases, including HIV comorbid TB, in Indonesia on 2015 were about 395 cases per 100.000 population. In Indonesia, estimated incidence of MDR/RR-TB cases among notified pulmonary TB on 2015 were about 10.000 cases. New cases of MDR/RR-TB in Indonesia on 2015 was about 2,8% with 32.000 incidence cases of MDR/RR-TB in the same year [1]. Based on Ministry of Health Indonesia, there were 1.860 of confirmed MDR/RR-TB cases in Indonesia on 2015 [2].

East Java province is one of the province in Indonesia with highest TB Burden in Indonesia, sitting at second place after West Java. Case detection rate in East Java was about 52% with number of positive Acid Fast Bacilli (AFB) was 21.306 cases [3] and number of notification case rate was about 110 cases per 100.000 citizen [2]. Based on those epidemiology data, an accurate, rapid, and sensitive diagnosis tool to detect *Mycobacterium tuberculosis* complex (MTBC) was required.

esxA gene is one the most important virulence factors of MTBC, also known as conserved gene. Previous studies stated that esxA gene was a potential target for TB diagnosis [4,5,6]. However, mutation of genes involved in anti-tuberculosis drug resistance, such as rpoB (responsible for rifampicin resistance) or katG (responsible for isoniazid resistance), could impair fitness cost of bacteria, thus sequence variation could possibly rise in esxA gene [7]. If there are possibility of sequence variation in esxA, then variation of gene expression could also rise. Based on background, present work was focused on using esxA gene as a target gene in polymerase chain reaction (PCR) method for TB diagnosis. The purpose of this study was to evaluate the accuracy of PCR-based amplification of esxA gene nucleotide region method in MDR-TB.

2. METHODS

2.1 Sample collection

Sputum samples from patients with pulmonary tuberculosis in Dr. Soetomo Hospital, Surabaya, Indonesia was collected via consecutive sampling from August 2016 to November 2016. As many as 97 samples were detected using GeneXpert. Total 20 samples out of 97 samples were detected as MTBC positive and also confirmed as MDR-TB. This study has received ethical clearance from ethical committee in health research deaprtment of Dr. Soetomo Hospital General Hospital, Surabaya, Indonesia no 541/Panke.KKE/IX/2016.

2.2 Procedures of sample detection

Total 20 sputum samples were first decontaminated using 4% NaOH by Alkali Petrof method recommended by WHO [8,9]. DNA from suspension of decontamination process was extracted using Qiagen kit [10]. Primers used in this study was designed using Clone Manager 6, version 6.00. PCR solution contained 20 µl nuclease-free water, 1 µl primer forward, 1 µl primer reverse, 3 µl DNA template, and 25 µl KAPA2G Fast Ready Mix PCR Kit as PCR Master Mix. Amplification in thermal cycler was initiated from pre-denaturation at 95°C for 3 minutes, then continued to denaturation at 95°C for 10 seconds; annealing at 58.3°C for 10 seconds; extension at 72°C for 15 seconds; reaction was conducted for 35 cycles and ended with final extension for 10 minutes at 72°C. Positive amplicons confirmation was indicated by DNA bands appeared at 580 bp. Five PCR products out of 20 positive samples were sent for sequencing process in 1st BASE. Sequence results were analyzed by Bioedit version 7.2.5.

3. RESULTS AND DISCUSSION

The average age of 20 patients in Dr. Soetomo Hospital which saw their sputum colected on August to November 2016 was 45,75 ± 9,22; patients with range of age between 50-59 had highest percentage among others (45%). In addition, male patients (65%) has higher percentage than female patients (35%) (Table 1).

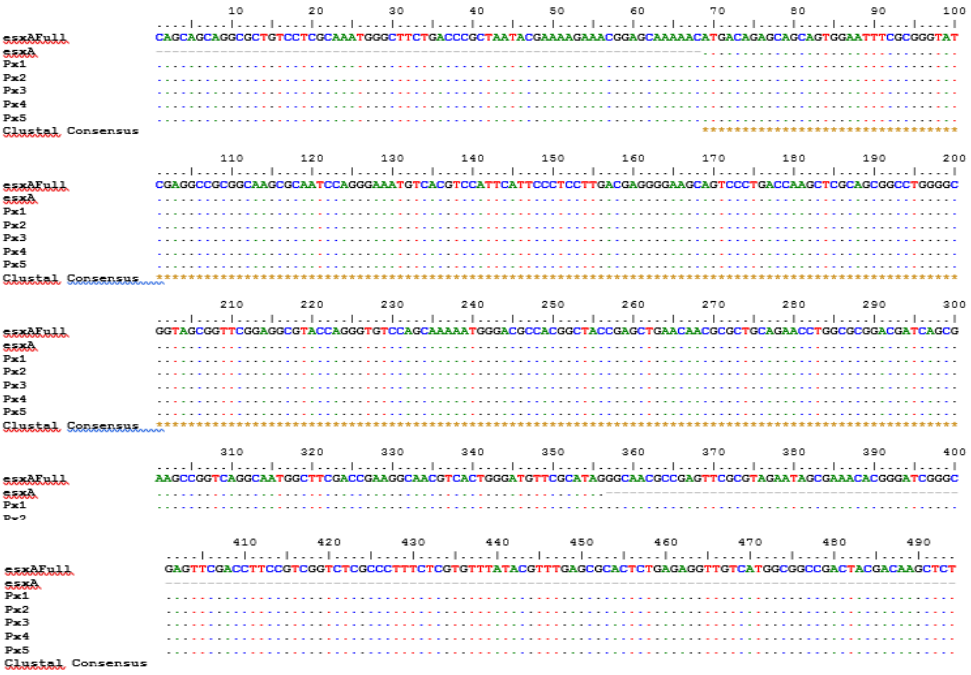

Figure 1 (Multiple alignment result showed 100% homology between samples and *M. tuberculosis* H37Rv (NC_000962.3). Px1, Px2, Px3, Px4, Px5 are samples.)

Some studies revealed that patients with MDR-TB mostly were infected productive age. Research in Tehran showed that MDR-TB patients were distributed most widely in age of 20-40 and >60 years old [11,12]. Many other studies also explained that men had higher risk to be infected with MDR-TB than women. One of the reasons why MDR-TB tend to infect male rather than female patients was because men had more tendency to do outdoor activities compared to woman [13]. Total 20 sputum samples identified by PCR showed positive results, in concordance with the results of GeneXpert. Several researches showed that esxA had 100% sensitivity and 100% specificity as target gen in PCR method [5].

Sequence of five positive samples were analyzed and found to have 100% homology with *M. tuberculosis* H37Rv (NC_000962.3) (Figure 1). Result of multiple alignment by Bioedit version 7.2.5 showed that no mutation and variation found in DNA sequence. Study conducted by Davilla *et al*. also showed that there was no variation on esxA gene examined from 88 clinical isolates [14]. Previous research also proved that esxA gene was conserved, specific, and had potential as the gene target for TB diagnosis [4]. Result showed that esxA gene was unique and a conserved target for detecting MTBC. GeneXpert method had been declared as gold standard method of nucleic acid amplification [1], however it needed high amount cost and PCR method based on esxA gene could be one of the alternative method in detecting MTBC.

Table 1 (Characteristic of MDR-TB patients in Dr. Soetomo Hospital on August to November 2016)

Characteristic	MDR-TB patients n=20
Age (mean ± standard deviation)	45,75 ± 9,22
Range of age: 20-29	1 (5%)
30-39	6 (25%)
40-49	5 (25%)
50-59	9 (45%)
Sex:	
Males	13 (65%)
Females	7 (35%)

4. CONCLUSIONS

All 20 sputum samples identified by PCR showed positive results, in concordance with the results of GeneXpert. Sequence of five samples out of 20 positive samples were analyzed and found to have 100% homolog with *M. tuberculosis* H37Rv (NC_000962.3). Identification of esxA gene on sputum samples using PCR showed high accuracy. In addition, developing a diagnostic method using PCR based on esxA gene could become an alternative way in determining MTBC as TB diagnosis.

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Bacteria Confirmations Using Detection of Specific 16S rRNA gene of *Mycobacterium tuberculosis Complex* in Sputum Specimen for Pulmonary TB

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Abstract – *Mycobacterium tuberculosis Complex* (MTBC) still was found to be primary isolate of pulmonary infection in human. Indonesia was ranked as the second country with highest tuberculosis (TB) disease. New smear-positive cases and all cases of TB suspect in Indonesia increased from year to year. In 2015, all cases of TB amounted to 117 cases per 100,000 people. 16S rRNA gene was chosen for detection because this gene was conserved. 16S rRNA was typically used for phylogenetic analysis of various organisms. The purpose of this research was to study the suitability of PCR method using 16S rRNA gene for identification of MTBC as a confirmatory test after conventional test using standard microscopic detection of acid-resistant bacteria. Sputum sample from pulmonary tuberculosis patients at Dr. Soetomo General Hospital, Surabaya Indonesia was collected randomly from September to November 2016. The sample was examined by PCR with a target of 1537 bp 16S rRNA gene for detection and identification of MTBC, which then compared to conventional method using Ziehl Neelsen (ZN) staining. Total of 30 samples from pulmonary tuberculosis patients were analyzed for MTBC diagnosis purpose. Results of PCR product were 100% positive of 16S rRNA gene, indicated by band at size 1537 bp. While using smear method, only 63.33% of all sample found positive. Detection of MTBC in the sputum of pulmonary TB patients using conventional test still required confirmation with molecular test using nucleic acid amplification method with specific DNA target of 16S rRNA gene from *Mycobacterium tuberculosis Complex*. In other site, this method using 16S rRNA target was able to be tool potentially of species and strains differentiation of isolates and clinical specimens.

Key words: 16S rRNA gene**,** *Mycobacterium tuberculosis Complex*, PCR method, sputum, pulmonary tuberculosis

1. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis Complex* (MTBC). The disease usually not only attack to pulmo (pulmonary tuberculosis), but also attack extra-pulmo, which then called extrapulmonary tuberculosis [1]. According to WHO, there was 9 million new TB cases with 1,5 million mortality caused by tuberculosis in 2013 and 9,6 million new TB cases with 1,5 million mortality in 2014 (WHO, 2015; WHO, 2014). Prediction about new TB cases in 2014 about 2,9 million cases (223 of 100.000 population) and in 2105 was estimated to be 2,8 million cases every year (217 of 100.000 population) [2].

This disease usually appears several years after infection when immune decreased (immunocrompromised) [3]. MTBC was major group of human pulmonary infection which had various characteristics. They were an aerobic group of acid-fast, slow-growing bacteria, and difficult to distinguish among species [4].

Genes of small subunit ribosomal including 5S, 16S, and 23S, and each of them was highly conserved. Out of the three genes, 16S rRNA is the one of mostly used in the molecular epidemiology because its specificity to identify species in phylogenetic analysis [5,6].

Acid Fast Basilli smear (AFB) is commonly used for TB infection screening. This method can be used for finding bacterial form of *Mycobacteria* and counting bacterial number in the sputum specimens. But, this method still have low sensitivity (<50%) for confirming of MTBC infection [7,8,9].

Nucleic acid amplification using PCR method was used for microbial identification because of higher accuracy and efficiency. Therefore, this study aimed to compare suitability of MTBC identification using PCR method using specific DNA target of 16S rRNA and AFB method. This method able to be tool potentially of species and strains differentiation from isolates and clinical specimens [10].

2. METHODS

2.1 Samples Collection

Sputum specimens from pulmonary tuberculosis patients in Dr. Soetomo General Hospital, Surabaya Indonesia were collected randomly from September to November 2016. This research was granted ethical clearance which approved by Ethical Committee in Health Research of Dr. Soetomo General Hospital Surabaya no. 537/ Panke. KKE/ IX/ 2016.

2.2 Acid Fast Bacilli (AFB) Smear

AFB smear was started by decontamination of sputum specimens by processing with NaOH 4%. Then, sputum was smeared in slides with circular area of 2x3 cm^2. First, AFB smear was stained using Carbol Fuhsin, then decolorated with acid alcohol, and the last, slide was stained with Metilen Blue as counterstain. MTBC was characterized by bacili form, red color, with size of 0.4 x 3 μm [11].

2.3 DNA extraction and PCR method

Sputum of pulmonary tuberculosis patients using Qiagen extraction kit (DNeasy® Cat. No. 69504) to get DNA extract. DNA Extract was amplified using PCR (MJ Mini™ Termal cycle BioRed). Primer was designed using target of 1537 bp in specific region of 16S rRNA gene of MTBC H37Rv ATCC 27294 using GENETIX program. Another component was PCR Mix (KapaBiosystem® Ready Mix). Amplification was initiated with denaturation at 94ºC for 20 seconds, followed by annealing at 53,8ºC for 10 seconds, and extension at 72ºC for 30 seconds. Temperature which used for PCR was result of optimization of the same sample.

2.4 Electrophoresis

PCR product was visualized in ultrapure agarose gel 2% (Invitrogen, USA), stained using EtBr and electrophoresed by UV transluminator. The positive control was 16S rRNA gene of *Mycobacterium tuberculosis* H37Rv strains ATCC 27294, while the negative control was PCR mix (KapaBiosystem® Ready Mix) without DNA. Positive amplification result was indicated by specific band in 1537 bp .

3. RESULTS AND DISCUSSION

Result of pulmonary tuberculosis samples smeared using AFB showed 19 samples positively classified as *Mycobacterium* species, while 11 samples were negative. Meanwhile, using PCR with 16S rRNA target gene resulted positive in all samples. Both of MTBC-positive samples using PCR and AFB smear test were presented on Table 1.

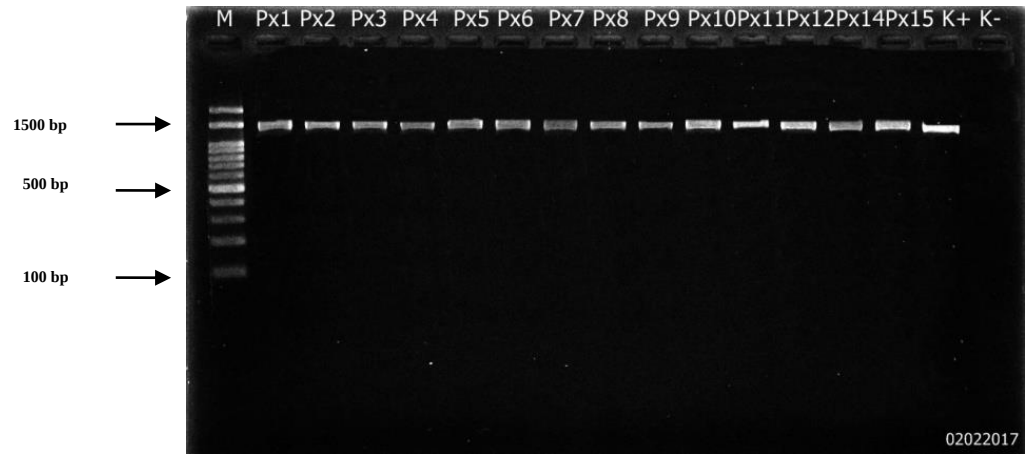

Figure 1. PCR product using target of specific 16S rRNA gene region (Doc16S_Spu_Pub_1, 2016). Result was visualized in agarose gel electrophoresis. Specific target was indicated by 1537 bp band. Px were positive samples; K+ = positive control; K- = negative control; M = marker ladder.

Table 1. Determination of MTBC which identified by PCR using specific target of 16S rRNA genes and AFB smear method among the sputum specimens from the pulmonary TB patients in Dr. Soetomo General Hospital, Surabaya on September-December 2016.

Method	Total samples (N)	MTBC-positive Sample(%)	MTBC-negative Sample (%)
PCR	30	100 (100)	0 (0)
AFB smear	30	19 (63,33)	11 (36,67)

Two method of samples identification showed difference results. Discrepancy of results from both methods showed 36.67% in negative result of AFB smear. Negative result in AFB smear still was detected positive in PCR method. All sputum specimens of pulmonary tuberculosis were detected as positive using PCR method (100%). Table 1 presented comparison of tuberculosis detection between AFB smear and PCR method.

AFB smear was a screening method which used to identify form and number of bacteria in the sputum of pulmonary tuberculosis. Weakness of AFB smear including low sensitivity of only 20-75%, also needed expertise to process sputum of pulmonary TB, staining method had many steps that needed right time calculation, smear was susceptive to lose after washing, and then could not differentiate between *Mycobacterium* genus, so this method was not specific for species identification purpose [8].

Different with AFB smear, PCR method used 16S rRNA gene which was more specific for identifying pulmonary tuberculosis infection in sputum of TB patients. 16S rRNA was universal gene in bacteria (procaryote) which consisted of functional gene followed by conserved and variable region [12]. This method used primer which designed specifically for target gene in 16S rRNA of MTBC. Thus, the positive result would indicate MTBC infection.

PCR method succeedly confirmed samples appeared negative in AFB smear to be positive. This fact was crucial, mainly related to inaccuracy risk for determining management of TB treatment, such as in the case of negative AFB smear from sputum, but manifested clinical symptoms of TB infection. Therefore, PCR method had advantage over AFB smear, which showed more accurate result, so patients infected by MTBC could be continue for treatment.

4. CONCLUSIONS

PCR method can be used TB detection in sputum of pulmonary tuberculosis patients as confirmation for AFB smear test. Analysis using 16S rRNA gene of MTBC could be continued for sequencing purpose to phylogeny analysis of MTBC and also could be used to biomarker of molecular epidemiology.

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Sequences DNA Analysis of the Specific Region eccB5 Gene of Mycobacterium tuberculosis complex Isolates from the Sputum of Pulmonary TB Patients

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Abstract – Tuberculosis is one of an infectious disease caused by the *Mycobacterium tuberculosis complex* (MTBC). The molecular development of detection methods such as nucleic acid amplification is one of the important choices of the examination for detection of MTBC. The *eccB5* gene is one of esx conserved component of ESX-5 system and play role of virulence factor of MTBC. The aim of this study research is analyzing DNA sequencing and homology of MTBC specific region of *eccB5* gene used for specific target detection of nucleic acid amplification to differentiate between MTBC with *non-tuberculosis mycobacteria* (NTM). The samples of clinical isolates of *Mycobacteria* were collected from suspected pulmonary TB patients at Dr. Soetomo Hospital from September 2016 until December 2016. The result showed from 10 isolates of *Mycobacteria* tested, declared that 7 isolated were positive of MTBC with the 1521bp and the 3 were negative bands that were possible of NTM. The percent identity showed 99% until 100% of MTBC compared with *Mycobacterium tuberculosis* H37Rv (NC_000962.3).

Keywords : Pulmonaty TB, MTBC, eccB5 gene, Sequencing, Homology.

1. INTRODUCTION

Mycobacterium tuberculosis complex (MTBC) are group causes of TB. Some species of MTBC are *Mycobacterium tuberculosis, Mycobacterium bovis*, *Mycobacterium africanum* dan *Mycobacterium microtii* [1,2]. Distribution through TB in Africa and Asia. The total incidence of TB cases in 2014 more than in 2013. Estimated incidence of TB cases in 2014 in Africa and Asia are in the range of 300-499 and 2500 cases per 100,000 population [3]. Virulence factor is important to playrole of infection in host. One of the virulence genes is *eccB5* gene. *eccB5* gene is esx conserved component gene of ESX-5 system of MTBC. The molecular nucleic acid amplification is one of the examination for detection of MTBC.

2. METHODS

Sample: Samples were collected from Dr. Soetomo Hospital in September 2016 until December 2016 from suspected pulmonary TB patients. The clinical specimens were cultured in *Lowenstein-Jensen* (LJ).

DNA extraction : The DNA extraction using of TE boiling extraction method.

The nucleic acid amplification : PCR with the primer pairs were designed by software clone manager 9. PCR products were sequenced by 1st Base (Selangor, Malaysia) and sequence analysis using Genetyx-Win program ver.10.0 (Genetyx Corporation, Tokyo, Japan).

3. RESULT AND DISCUSSION

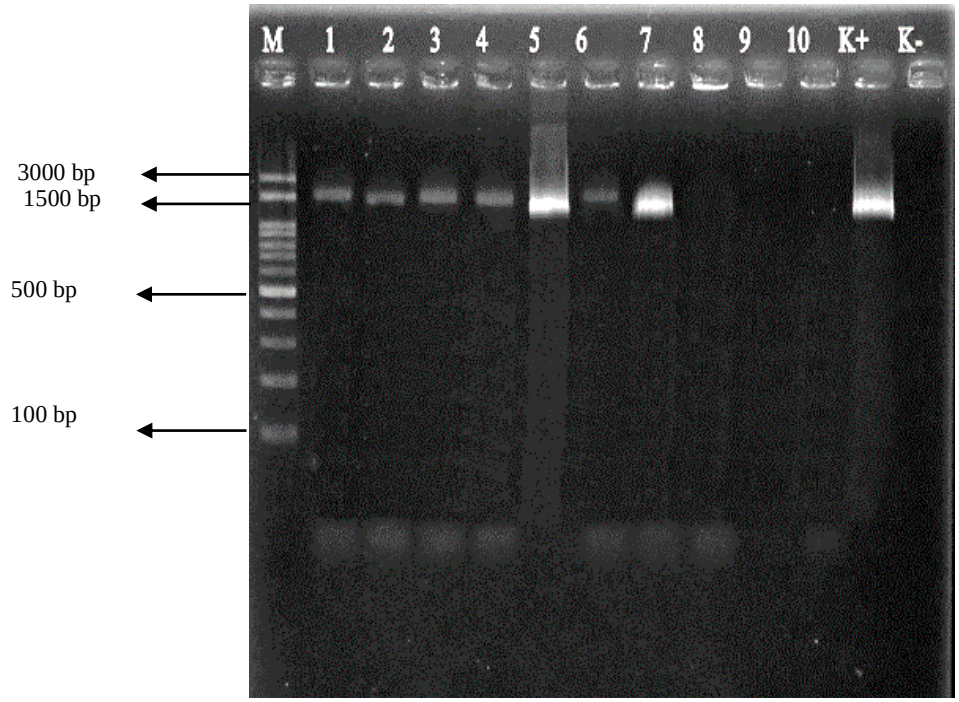

Figure 1. The PCR product of *eccB5* gene of *Mycobacteria* isolates from the sputum of pulmonary TB patients

The result in this study research showed that the conserved of *eccB5* gene and could be differentiated with NTM using this specific DNA target of *eccB5* gene. From the total samples of 10 isolates of *Mycobacteria* tested, declared that 7 isolated were positive of MTBC with the 1521 bp and the 3 were negative bands that were possible of NTM. Bioinformatic analysis showed that the percent identity showed 99% until 100% of MTBC compared with reference strain of *Mycobacterium tuberculosis* H37Rv (NC_000962.3). This study evaluated of validity of *eccB5* gene as the target amplification using PCR technique. Based on this result, *eccB5* gene is spesific and conserved in MTBC. $EccB_5$ is one of conserved membrane protein encoded by *eccB5* gene in gene cluster of ESX-5 system [4,5,6]. Di Luca *et al.,* describe that *eccB5* gene in was one of virulence factor of MTBC, influenced viability; stability of the cell wall of *Mycobacterium tuberculosis* [5]. The *eccB5* gene also could play a role of infection in a host cell; and also induces damage host cell [4,5,6].

4. CONCLUSION

The *eccB5* gene showed the positivity of MTBC with the 1521 bp and could be differentiated between MTBC with NTM. From the bioinformatic analysis of sequences showed that 99% until 100% of MTBC compared with *Mycobacterium tuberculosis* H37Rv (NC_000962.3).

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Ethyl Acetate Fraction of Kalanchoe pinnata (Lmk)Pers Reduces the Production of IFN-α by Dendritic Cells in Pristane-induced Lupus-like Disease Mice

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Abstract – IFN-α has a central role in lupus pathogenesis. On the previous study, the ethyl acetate fraction of *Kalanchoe pinnata* (Lmk) Pers leaves (EF-KP) resulted in the modulation of the T regulatory function and the histology of the kidney. In this study, we observed the effect of the EF-KP on the IFN-α secreted by dendritic cells. The model used was Pristane-induced lupus-like disease mice according to Reeves method (2009) [1]. The experimental groups consisted of 3 groups, a positive control group that received placebo, EF-KP group, and negative control group that received cyclophosphamide. The treatment lasted for 3 weeks. At the end of the experiment, the semiquantitative method was used to observe the proteinuria as a general parameter. Then, the dendritic cells were isolated from the femur. The cells were measured by means of flow cytometry method. The proteinuria level of the positive control group was 79,00±33,81 mg/dL. The level of proteinuria of EF-KP group was 42,22±33,36 mg/dL, meanwhile, the negative control group had the level of 36,50±35,59mg/dL. This decrease indicates the repairing effect on of the kidney function. In the IFN-α measurement, we found the relative percentage of CD123+IFN-α+ reduced significantly in the EF-KP and the negative control groups. The result shows the reduction of the relative percentage of CD123+IFN-α+ in the EF-KP group. The decrease is lower than the positive control. It means that the EF-KP is able to inhibit the overexpression of the IFN-α which is produced by the dendritic cells. This inhibition could inhibit the lupus immune-pathogenesis [2], and also the proliferation of the autoreactive B and T cells.

Keywords: *Kalanchoe pinnata*, CD123+IFN-α+, lupus, proteinuria, treatment

1. INTRODUCTION

IFN-α has a central role in lupus pathogenesis. On the previous study, the ethyl acetate fraction of *Kalanchoe pinnata* (Lmk) Pers leaves (EF-KP) resulted in the modulation of T regulatory function and the histology of the kidney. According to the immunosuppressant activity in delayed-type hypersensitivity model which is reported by Bergmann (1994) [3] and in Pristane-induced lupus mice which are reported by Indriyanti (2011) [4], the EF-KP is suggested to influence on the IFN-α secretion. It also inhibits the inflammatory pathway indicated by using some biomarkers, such as TNF-α and TGF-β [5,6]. The early mechanism of Pristane is the effect on the plasmacytoid dendritic cells, which is dominantly present in the marrow-bone of the femur of the mice [2]. The dendritic cell markers are CD11c [1] and CD123. Therefore, this research purpose was to determine the effect of the EF-KP on the IFN-α which was secreted by dendritic cells in lupus mice.

2. METHODS

2.1 Materials

The *Kalanchoe pinnata* (Lmk) Pers fresh leaves were obtained from cultivation farm in Trenggalek, East Java. The botany identification of this plant was performed by Conservation Unit of Indonesian Institute of Science, Purwodadi with the identification number of 0284/IPH.06/HM/II/2015. The chemicals used in the fractionation were obtained from Merck through PT. Dianum as Indonesian supplier.

The female Balb/c mice aged 4 weeks were received from LPPT Gadjah Mada University, Indonesia. These mice were pathogen free species with the certificate number of 352/LP3HP/29/VII/2015. They were housed, randomized, and handled by using standard maintenance on the Guide of the Care and Use of Laboratory Animal 8th edition, published by National Research Council [7].

TMPD (Pristane) with the code number of Sigma-P2870 was obtained from the Sigma-Aldrich supplier at Singapore. Anti-CD123 and anti-IFN-α (Biogenesis), and also materials used in the flow cytometry assay were obtained from Biology Molecular Laboratory, Brawijaya University.

2.2 Procedures

The identity of the ethyl acetate fraction of *Kalanchoe pinnata* (Lmk) Pers (EF-KP) used in this research was provided as a chromatogram profile which was obtained by means of UPLC-QTOF-MS/MS tandem system. The UPLC instrument used was a UPLC Acquity SDS (Waters). The column used was Acquioty UPLC BEH C18 1.7um; 2.1x50 mm; flow rate 0.3 mL/minute. The injected volume was 5 uL (5000 ppm), and the temperature of 40ºC. Eluents A: H2O+formic acid 0.1%; B: acetonitrile +formic acid 0.1%. The MS instrument used was XEVO-G2QTOF (Waters) with a positive ESI (resolution mode). The capillary voltage of 3 kV; sample cone voltage 38 V; desolvation T 300ºC; source T 110ºC; desolvation gas 500L/h; cone gas 16L/h. Then, the single compound profile was determined by means of densitometry method (Camag 3).

In this study, we observed the effect of the EF-KP on the IFN-α secreted by dendritic cells. The model used was Pristane-induced lupus-like disease mice according to Reeves (2009)[1]. The experimental groups consisted of 3 groups; a positive control group that received placebo, an EF-KP group, and a positive control group that received cyclophosphamide [8]. The treatment lasted for 3 weeks. At the end of the experiment, the semiquantitative method was used to observe the proteinuria as a general parameter. Then, the dendritic cells were isolated from the femur of the mice. The cells were measured by means of flow cytometry method. The data was analyzed by facilitating of BD CellQuest program. Finally, the data was calculated statistically by means of SPSS 22 version. The ethical clearance of this research was approved by local ICUC of Veterinary Medicine Faculty of Universitas Airlangga on January 12, 2016, with the certificate number of 526-KE.

3. RESULTS AND DISCUSSION

a. The identity of the EF-KP

Kalanchoe pinnata (Lmk) Pers has a lot of active compounds [9] which are predicted to result in many beneficially effects for lupus. The activities are immunosuppressant and anti-inflammatory [3,10], anti-allergy [11], immunomodulatory [12], and antioxidant [13]. No toxic effects occur during acute and subchronic toxicity test [14] and it is safe for the maternity since it could inhibit the uterus contraction [15-17].

The profile of the EF-KP which was tested in this research is shown in **Fig 1**.

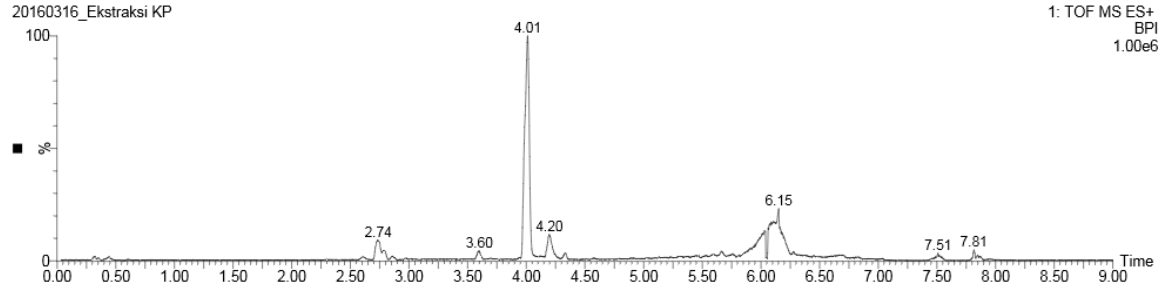

Fig 1. The chromatogram of the EF-KP

The liquid-liquid extraction (LLE) fractionation reduced a lot of compounds of the crude extract. According to the literature, the crude extract contains quercetin in the form of its glycosides. In the instrument, the glycosides are fragmented become its glycone and aglycone. The peaks at the retention time of 2.79 and 2.74 are predicted as the quercetin (aglycone). However, not all of the glycosides would be hydrolyzed well so that the active marker of rutin is better quantitated than the quercetin. The multiple peaks in the chromatogram made the quantitation of the marker invalid. In this research, we used the densitometry method to find the pure and single peak of rutin marker.

The rutin separation was performed based on the Indonesian Herbal Pharmacopeia procedure. The rutin spot was obtained on the HP-TLC plate (Silica gel 60 F_{254}) with the eluent of ethyl acetate P: n-butanol P: formic acid P (5:3:1). The EF-KP solution tested was 1.25% of the EF-KP in the ethanol P. The standard used was rutin 0.1% in the ethanol P, meanwhile, the detection reagent was sitroborat solution LP. The results of the densitometry assay are shown in **Fig 2.**

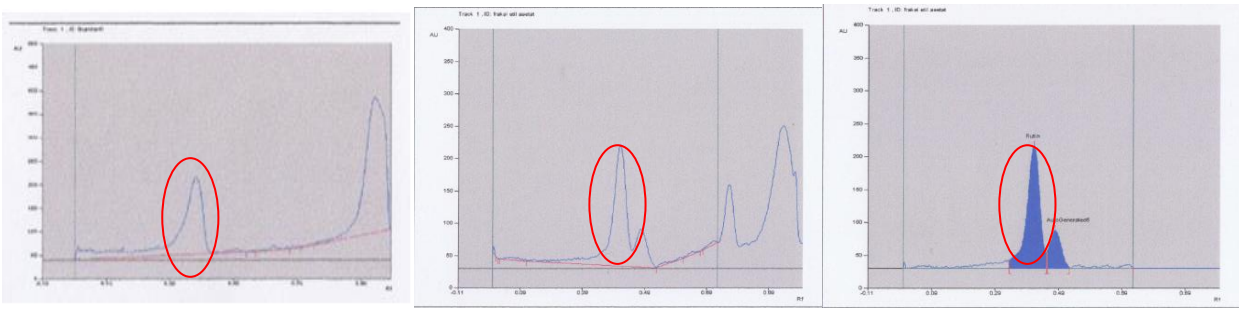

Fig 2. The rutin chromatogram of the rutin standard (a), EF-KP (b), and EF-KP (highlighted) (c)

The chromatograms were obtained by means of a densitometer (Camag 3). The results show the rutin peak in the EF-KP is not well separated to another peak. Thus, the optimization was continued so the quantitation of the rutin cannot be performed at present.

b. The *in vivo* experiment in lupus mice

This experiment was time-consuming because of the Pristane induction time that was lasted for 6 months to collect the mice which had the same baseline of proteinuria at the value of ++ (100 mg/dL) based on previous research [1,18,19]. The proteinuria level was the easiest parameter that could be observed without harming or killing the mice. Therefore, we use it as a general parameter which shows the kidney function repairing process in each experimental group. Then, the specific parameter observed was the CD123+IFN-α relative percentage. The data is described below.

The proteinuria level of the positive control group was 79.00±33.81 mg/dL. The level of proteinuria of EF-KP group was 42.22±33.36 mg/dL, meanwhile, the negative control group had the level of 36.50±35.59mg/dL. This decrease indicates the repairment of the kidney function.

Then, in the IFN-α measurement, we found the relative percentage of CD123+IFN-α+ reduces significantly (**Fig 3**) in the EF-KP and the negative control groups.

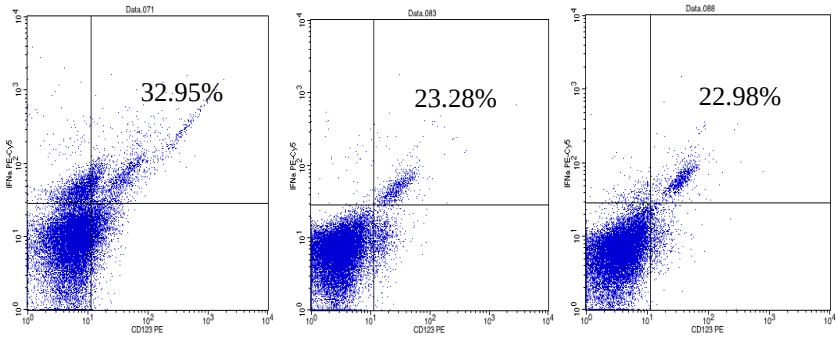

Fig 3. The profile of relative percentage of CD123+IFN-α+ analyzed by means of flow cytometry method in the femur of the positive control group (a), EF-KP group (b), and negative control group (c).

The result shows the reduction of the relative percentage of CD123+IFN-α+ in the EF-KP group. The decrease is lower than the negative control. It means that the EF-KP is able to inhibit the overexpression of the IFN-α produced by dendritic cells. This inhibition could inhibit the lupus immunopathogenesis [2], and also the proliferation of the autoreactive B and T cells.

Over-expression of the type I interferon, a kind of cytokine, usually correlates with a viral infection or autoimmune disease. There is a missing link between the type I interferon with a nucleic acid or antibody. However, the nucleic acid and the immune complex bind the *toll-like receptor* (TLR) lead to an innate immune activation. The effect is followed by the overexpression of IFN-I and IFN-II [20]. The IFN-α over-expression is mostly responsible for autoimmune pathogenesis [21,22].

Generally, the interferon which is correlated to the immune-modulation is IFN-gamma, meanwhile, the IFN-α is expressed dominantly in the viral infection. However, the Pristane effect demonstrates a different result. The IFN-α is the dominant interferon which impacts on many lupus biomarkers which result in some types of lupus-specific antibodies [1,2]. The IFN-α is secreted by some types of cells. According to the Pristane mechanism, the specific type of cell which products IFN-α is a dendritic cell. The dendritic cell surface markers are CD11c [1,22] and CD123. The cell is placed inside the femur bone.

This research is focused on the IFN-α inhibition by using the EF-KP as a drug candidate. The EF-KP activities such as anti-inflammation and antioxidant are the main consideration of the decision. The result shows the

reduction of the relative percentage of CD123+IFN-α+ with the value of 32.95±8.25% (placebo group), 23.28±9.31% (EF-KP group), and 22.98±10.39% (cyclophosphamide group). There is a significant ($P<0.05$) reduction of the relative percentage of the CD123+IFN-α+ in the group which received the EF-KP and another one which received cyclophosphamide. The result shows the EF-KP abilities to inhibit both the IFN-α and other biomarkers near the IFN-α pathway. Additionally, the production of antibodies would be decreased [23].

4. CONCLUSIONS

The EF-KP ability to inhibit the IFN-α pathway is predicted to be potential to inhibit the autoantibody production, causing the reduction of immune complexes. The further research is needed to observe the reverse effect caused by the immune-homeostasis mechanism.

5. ACKNOWLEDGEMENTS

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Total Antioxidant Activity and Anthocyanin Content Of Purple Uwi Banggai (*Dioscorea alata*)

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Abstract – On this research, qualitative and quantitative analysis of anthocyanin - functioning as antioxidant-, as well as total antioxidant activity from tuber and peel of purple Uwi Banggai with variety of purple intensity were conducted. The method used is Harborne method for qualitative analysis of anthocyanin, *pH differential* method for quantitative analysis of anthocyanin, and DPPH for total activity of antioxidant. Before all analysis were performed, extraction with maseration method were done on samples in order to gain extract. The result of this research showed that all samples contained anthocyanin. The anthocyanin content of tuber varied from 7.31 mg / 100 mg (mauve) to 168.01 mg / 100 mg (dark purple) while for peel were 39.82 mg / 100 mg (mauve) to 183.30 mg / 100 mg (dark purple). Total antioxidant activity on tuber varied from 1044.36 mg/L (mauve) to 127.29 mg/L (dark purple) while for peel were 263.12 mg/L (mauve) to 113.76 mg/L (dark purple). Peel of purple uwi Banggai had the highest antioxidant activity. Overall, purple uwi Banggai had quite high anthocyanin content and medium antioxidant activity and also had the potency to become a good source of carbohydrate for food, with the advantage of having antioxidant compounds, especially to prevent degenerative illness. The waste peel of purple uwi Banggai also could be utilized to obtain its anthocyanin content.

1. INTRODUCTION

From many kinds of tubers growth in Indonesia, yam or uwi (*Dioscorea alata L.*) has a good potency to be developed as a source of carbohydrate and bioactive compound. Nevertheless, the cultivation of Uwi in Indonesia have not yet maximized due to the lack of information of Uwi's nutritional value and also its bioactive compound content. One type of Uwi existing in Indonesia is Uwi Banggai (*Dioscorea alata*), which have many variety and color like white, yellow, and purple **[1]**. Uwi Banggai contains anthocyanin when its color is purple. Anthocyanin is a bioactive- compound belong to flavonoid group. In addition to its function as natural dye that yields purplish red color, anthocyanin also has a role as antioxidant **[2]**. The aim of this research was to gain information about the content of anthocyanin in several varietis of purple uwi Banggai, and also its total antioxidant activity. That information would be a benefit for adding knowledges among people consuming uwi Banggai, and also for the government to become more better in knowing the advantages of uwi Banggai especially the purple variety.

2. METHODS

2.1 Chemicals

The reagent used in this research were methanol, sodium hidroxide, chloride acid, DPPH (2-diphenyl-1-picrylhydrazil), buffer solution of potassium chloride pH 1, buffer solution of sodium acetate pH 5, and vitamin C.

2.2 Procedures

The population of objects for this research was uwi Banggai cultivated in Kepulauan Banggai Sulawesi Tengah. Uwi Banggai used as the objects were three types purple uwi Banggai generally consumed by people of Banggai Kepulauan. The selection of objects were selective random on purple uwi Banggai with variety stated before, having purple tuber and peel with different purple color intensity.

First, tuber were peeled. Tuber and peel then were washed to eliminate any dirt. Extraction with maseration method were performed on clean tuber and peel of purple uwi Banggai. Afterwards, tuber and peel were weighed approximately 30 – 50 gram, mashed and soaked for 48 hours with 75 – 100 mL methanol. Filtering were conducted so that filtrate and residue be separated. Filtrate was collected and residue were soaked again in methanol for 24 hours. The solution then were filtered and the filtrate obtained was mixed with the first filtrate.

The Total filtrate was vaporized using vacuum rotary evaporator until methanol dissapeared. On the extract, qualitative and quantitative analysis of anthocyanin were performed, as well as total antioxidant activity. Haborne method was used for qualitative analysis of anthocyanin, pH differential method was used on quantitative analysis of anthocyanin **[3]** while DPPH method was used on total antioxidant activity analysis **[4]**

3. RESULTS AND DISCUSSION

The physical appereance of purple uwi Banggai is shown in picture 1. Qualitative analysis of anthocyanin was performed to identify the existence of anthocyanin on tuber and peel of purple uwi Banggai. From the experiment, it was obtained that all of objects research contained anthocyanin. The color appearance of each sample depended on its purple color intensity. The more intense the color of purple uwi Banggai, the darker the result of analysis. The final color of sample solution was red with the additon of HCl and became green with the further additon of NaOH. This test was based on physical property of anthocyanin which was red-pink colored in pH 1-6 (acid) and green colored in pH 8-14 (base) [5].

The summary of anthocyanin content and total antioxidant activity of purple uwi Banggai are presented in table 1. A1 and A2 code represented the tuber and the peel of one variety of sample that had colored dark purple tuber and colored mauve peel. B1 and B2 code represented the tuber and the peel of one variety of sample that had colored mauve tuber and dark purple colored peel. C1 and C2 code represented the tuber and the peel of one variety of sample that had colored dark purple tuber and peel.

Figure 1. Uwi Banggai (*Dioscorea alata*)

Table 1. The Average of Anthocyanin Content and Total Antioxidant Activity of Purple Uwi Banggai

Sample	Anthocyanin Content (mg/ 100 gram)	Total Antioxidant Activity (mg/L)
A1 (colored dark purple Tuber)	168,01	133,81
B1 (colored mauve Tuber)	7,31	1044,36
C1 (colored dark purple Tuber)	145,80	127,29
A2 (colored mauve peel)	39,82	263,12
B2 (colored dark purple peel)	73,60	141,32
C2 (colored dark purple peel)	183,30	113,76

Natural color purple – red on part of plant is commonly derived from anthocyanin and betacyanin content [6]. Sample A and C had a quite high anthocyanin content that was 168,01 dan 145,80 mg/100 g. While the sample of peel having the high anthocyanin content wa sample C as much as 183,30 mg /100 g. It was shown that the peel could have anthocyanin higher than the tuber. This results matched with some research that showed the peel of purple sweet potato had higher anthocyanin content compared to its tuber [7]. It could be concluded that there was a potential usage of uwi Banggai peel to be extracted for its anthocyanin content. Nevertheless, the tuber and the peel of uwi Banggai colored mauve had low anthocyanin content. This result matched with its low purple color intensity on tuber, peel, filtrate from maseration process, and extract. Anthocyanin color was influenced by the concentration and types of anthocyanin [8].

The highest antioxidant activity was shown by sample C. The purple color intensity and anthocyanin content of tuber from sample C was also the highest. Generally, it was stated that the tuber and peel of purple uwi Banggai had medium antioxidant activity. Antioxidant on plant comes from several active compounds, and divided into two types, which classified as nutrient (vitamin A, vitamin E, Vitamin C, Selenium, Zinc, etc), and as non nutrient (phenolic compound and flavonoid compound) **[9]**. Red or purple colored fruit like dragon fruit and bit, have high anthocyanin and antioxidant activity because it hasd both types of antioxidant, while carbohydrat source of food commonly have low anthocyanin content and antioxidant activity. This research had showed that purple uwi Banggai had the advantages over others types of carbohyrates source of food due to its higher anthocyanin content.

4. CONCLUSIONS

It was concluded that purple uwi Banggai (*Dioscorea alata*) as tuber and peel, had a good potency to become a carbohydrate source of food that also good for health especially to prevent degenerative illness, unlike other carbohydrate source of food, due to its quite high anthocyanin content -functioning as natural dye and antioxidant-, and also medium activity antioxidant. Waste product of uwi Banggai in the form of peel also can be processed to obtain its anthocyanin extract.

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Black Soybean (*Glycine soja* L.) Natto decreased the Lipid Metabolism and repair Endothelial Injury of Atherosclerosis Mice-model

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Abstract – High cholesterol levels in the blood cause hypercholesterolemia. Hypercholesterolemia is a risk factor for atherosclerosis causes. Atherosclerosis is densing and the narrowing of artery's wall by high concentrate of LDL in the blood. The macrophages will phagocyte oxidized LDL to form a foam cells. Stacking foam cell along with the proliferation of smooth muscle cells can trigger the formation of fibrous plaque. Fibrous plaque cause narrowing of the blood vessels. The purpose of the research was to know the influence of black soybean *natto* on the lipid profile, hepatic *Malondialdehide* (MDA) levels, the amount of foam cell and the density of aorta's wall in Atherosclerosis subject mice. The object of the research used 25 male *Swiss* mice's. Mice's were divided into five groups with five replications. Treatment group includes normal diet (K-), atherogenic diet (K+), atherogenic diet + *natto* 200 mg/ml (P_1), atherogenic diet + *natto* 400mg/ml (P_2), and atherogenic diet + *natto* 800 mg/ml (P_3). Black soybean *natto*extract administered orally every day until 4 weeks. At the end of the treatment mice were dissected for blood serum, hepar, and aorta taken. Lipid profile is measured by enzymatic test using spectrophotometer. MDA levels were measured using *the Thiobarbituric Acid Reactive Substances* (TBARS) technique with spectrophotometer. The aorta was measured by treating it on cross-section with Hemotoxilyn Eosin staining to count the number of the foam cell and the wall density. The result showed that the black soy natto effected on the lipid profile, MDA levels,amount of foam cell and the aorta's wall density in Atherosclerosis mice. Isoflavones are included in the flavonoid group which is a polyphenolic compound. Primary antioxidants such as soybeen acts as a free radical acceptor mechanism which can inhibit free radical oxidation process. The mecanism of isoflavones in soybeen is reduce free radicals by donating H ion and as a scavenger directly. The presence of free radicals reduction by isoflavones may prevent LDL oxidation process so that the number of oxidized LDL in the intima will decrease. A decrease in the number of oxidized LDL will reduce the number of foam cell.

1. INTRODUCTION

High cholesterol levels in the blood cause hypercholesterolemia. Hypercholesterolemia is a risk factor for atherosclerosis causes]. Atherosclerosis is densing and the narrowing of artery'swall by high concentrate of LDL in the blood. The macrophages will phagocyte oxidized LDL to form a foam cells. Stacking foam cell along with the proliferation of smooth muscle cells can trigger the formation of fibrous plaque. Fibrous plaque cause narrowing of the blood vessels [1]] Natto is a Japanese traditional food such as soy fermentation of bacteria Bacillus subtilis [2]. Natto extract has antioxidant activity and can reduce the monocyte adhesion to the endothelium wall, so it can inhibit the formation of plaque on the wall of the aorta [3]. Another alternative that has been used as a substrate natto is fermented black soybean varieties detam 2. Black soy contains glycosides and aglycone isoflavones. The content of aglycone isoflavones daidzein and genistein in black soy natto higher than unfermented black soybean [2]. Soy isoflavones can reduce lipoproteins oxidation [6], decrease LDL in dyslipidemia rats [7] and losing weight, decrease total cholesterol, triglycerides, and LDL [5].

2. METHODS

The research conducted in Biology Laboratory, Animal Physiology Sub Laboratory Universitas Negeri Malang, from November 2014 until January 2015. The object for the test is 25 male Swiss mice aged 7 week and weight ±30 gram. Mice were divided into five groups with five replications. Treatment group includes normal diet (K-), high-fat diet (K+), high-fat diet + natto 200 mg/ml (P1), high-fat diet + natto 400 mg/ml (P2), and a high-fat diet + natto 800 mg/ml (P3). Black soybean natto extract administered orally every day until 4 weeks. Atherogenic diet and administration of natto extract orally performed simultaneously every day for 4 weeks. Atherogenic diet treatment is done by injection of adrenaline on the first day followed by providing quail egg yolk and palm oil are blended at Hi-Gro Medicated feed 551. At the end of the treatment mice were dissected for blood, liver, and

aorta taken. Then, blood centrifuged to obtain serum for measuring lipid profile. Lipid profile is measured by enzymatic test using Diasys FS reagent and spectrophotometer. The liver will be used to measure the MDA levels with Thiobarbituric Acid Reactive Substances (TBARS) technique. The aorta was measured by treating it on cross-section with HE (Hemotoxilyn Eosin) staining to count the amount of the foam cell and the wall density. The results will be analyzed using statistic test one way Anova and followed by Duncan test.

3. RESULTS AND DISCUSSION

Lipid Profile

Black soybean Natto contains isoflavones and anthocyanins which include on flavonoid group that can decrease cholesterol levels [9]. Flavonoids able to inhibit the enzyme activity acylCoA: Cholesteryl aciltransferase that play a role in the decreasing process of cholesterol esterification in the intestine and liver, and also the enzyme 3-hydroxy 3-methylglutaryl CoA (HMG CoA) that play a role in the inhibition of cholesterol synthesis. Inhibition of HMG-CoA reductase enzyme causes the inhibition of cholesterol synthesis, it will cause a reduction of blood cholesterol levels [10]. Beside that, in the other research show that black soybean anthocyanin extract can reduce weight, total cholesterol, triglycerides, and increasing HDL cholesterol [8].

Table 1 Mean and Error Standard Of Lipid Profile

Treatment*)	Result			
	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
K-	113,25 ± 7,17 b	83,5 ± 2,04 a	60,52 ± 2,49 a	37,05 ± 5,2 b
K+	167,5 ± 13,01 a	89,25 ± 4,81 a	59,50 ± 2,08 a	92,08 ± 14,85 a
P_1	156,6 ± 12,93 a	88 ± 4,28 a	60,82 ± 6,44 a	68,98 ± 4,39 a
P_2	169 ± 7,12 a	62,33 ± 3,69 b	61,74 ± 6,15 a	75,55 ± 7,89 a
P_3	176,25 ± 2,78 a	92,8 ± 2,85 a	68,02 ± 1,44 a	89,18 ± 3,18 a

*) K-= Normal diet; K+= Atherogenic diet; P_1= Atherogenic diet + *natto extract* 200 mg/ml; P_2= Atherogenic diet + *natto extract* 400 mg/ml; P_3 = Atherogenic diet + *natto extract* 800 mg/ml

The content of anthocyanins and isoflavones such as genistein and daidzein in the black soybean natto is also capable of lowering triglyceride levels. Black soybean Natto with a concentration of 400 mg/ml could reduce levels of triglycerides in mice with high-fat diet. The content of isoflavones able to inhibit enzymes involved in lipogenesis [4]. Anthocyanins can also inhibit Fatty Acid Synthase (FAS) that plays a role in the metabolism of fatty acids [6]. Both the content of flavonoids in black soybean natto also can lower triglyceride levels through the mechanism of the increased activity of the enzyme lipoprotein lipase (LPL). Increased activity of the enzyme LPL, will hydrolyze carrying VLDL triglycerides into fatty acids and glycerol. Fatty acids then absorbed by the muscles and other tissues are oxidized to produce energy and by adipose tissue is stored as energy reserves. A decrease in fatty acid will cause a decrease in triglycerides.

Giving black soybean natto also has a tendency to increase HDL levels when compared to the treatment without giving black soybean natto. Increased HDL levels are influenced by the content of isoflavones contained in black soybean Natto. Soy Isoflavones are phytoestrogens which also has a structure similar to estrogen, so it works similar with estrogen. Estrogen can increase the levels and the production of apolipoprotein A-1, which is the main constituent of HDL[3]. An increase of apolipoprotein A-1 will trigger an increase in HDL. Isoflavones are found in black soybeans also plays a role in inhibiting the absorption of cholesterol that comes from food intake and cholesterol synthesized by the liver. Giving black soybean natto with a concentration of 200 mg/ml had a tendency to lower LDL levels in the mice blood serum. Phytosterols will compete and replace cholesterol in micelles [7]. Phytosterols of black soybean natto intake will reduce the amount of cholesterol absorption by the intestine [8] Isoflavones also have an effect on LDL receptors. Isoflavones can increase the regulation of LDL receptor activity, so that the levels of LDL in the blood will be reduced as will be bind with LDL receptor [7].

MDA Level

Results of the study of black soybean natto able to reduce levels of MDA.

Table 2 Mean ± Error Standard Mice MDA Level

Treatment	Mean MDA Level (ng/mL)
K+	923 ± 46,56 a
P_3	752,5 ± 16,38 b
K-	734,5 ± 58,97 b
P_2	710 ± 27,14 b
P_1	666 ± 16,17 b

Keterangan:
K- = Normal diet
K+ = Atherogenic diet
P_1 = Atherogenic diet + *natto extract* 200 mg/ml
P_2 = Atherogenic diet + *natto extract* 400 mg/ml
P_3 = Atherogenic diet + *natto extract* 800 mg/ml

MDA decrease seen after administration of natto extract of black soya in the treatment group natto extract, MDA levels in the treatment group is lower when compared to the positive control group with giving a high-fat diet. Giving black soybean natto with a concentration of 200 mg / mL is able to reduce levels of MDA is better that is equal to 666 ng/mL.

Decreased levels of MDA caused by the activity of antioxidants in black soybean natto. Black soy contains isoflavones in natto and nattokinase containing enzymes produced in fermentation by the bacteria Bacillus subtilis that have antioxidant properties. Natto is a food rich in antioxidants [6]. Foods rich in antioxidants can reduce formation of free radicals due to the presence of toxic compounds such as ROS and peroxidation so it decrease the levels of MDA. The antioxidants in black soybean natto able to reduce formation of ROS. Mechanism of isoflavones compounds in black soy natto reducing formation of radicals and ROS in two ways to donate hydrogen ions from the bond and as a scavenger (peradam) free radicals directly. At the 5.7-dihidroksil meta structure in ring A on isoflavones are able to donate hydrogen ions to form compounds that are more stable and are formed fenoksil less reactive radicals [16], while the 4'-hydroxyl group in ring B compound isoflavones act as a scavenger of ROS. Hydroxyl in ring B can donate hydrogen ions to hydroxyl radicals and peroxyl radicals that will both stabilize and form a more stable radical flavonoids. Chemical bonds in soy isoflavones daidzein or genistein kind to be donated ion is shown in Figure 1.

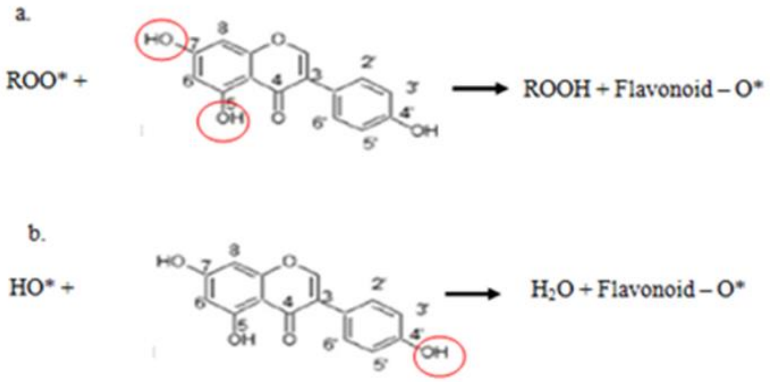

Figure 1 a. Isoflavones in Donating Hydrogen Ion b. Isoflavones as Scavenger (Source: Tamam, 2013)

The bond between the radical compound with isoflavones resulting radical compounds are not reactive anymore and ROS will not oxidize lipoprotein in LDL and reduce the formation of lipid peroxides. Reduction oxidation process has resulted in reduced peroxidation products such as decreasing MDA levels.

MDA treatment group extract of natto decreased at all concentrations of 200 mg/mL, 400 mg/mL, and 800 mg/mL, only the treatment extract of natto with a concentration of 200 mg / mL show results MDA levels lower than the other treatment groups, while at higher concentrations natto less effective in lowering levels of MDA because of the ability of antioxidants to donate hydrogen affects its activity [6]. The a molecule will be able to react as if the primary antioxidants can donate a hydrogen atom quickly on lipid radical, and would result in a radical derived from the antioxidant is more stable than the initial lipid radicals. Decreased levels of MDA are better at giving natto concentration of 200 mg/mL, at these concentrations is the most optimal concentration in lower levels of MDA. Conclusions from the study that the giving natto has no effect in lowering levels of MDA.

Amount of foam cell and the aorta's wall density

The lifestyle consumed high fat and high carbohydrates foods can increased levels of cholesterol, triglycerides and LDL in blood [6].

Table 3 Mean ± Standard Error (SE) Amount of foam cell and the aorta's wall density

Parameter	Group				
	K-	K+	P_1	P_2	P_3
Amount *Foam cell*	6,1 ± 1,12 d	38,45 ± 3,31 a	17,17 ± 2,56 bc	12,95 ± 1,29 c	19,85 ± 2,32 b
Aorta's wall density (µm)	41,36 ± 5,81 c	58,86 ± 2,19 a	52,61 ± 2,75 ab	43,49 ± 2,14 bc	52,71 ± 2,08 ab

Note:
K- = Normal diet
K+ = Atherogenic diet
P_1 = Atherogenic diet + *natto extract* 200 mg/ml
P_2 = Atherogenic diet + *natto extract* 400 mg/ml
P_3 = Atherogenic diet + *natto extract* 800 mg/ml

Excessive number of LDL in the blood caused atherosclerosis [9]. Atherosclerosis is the densing and the narrowing of artery's wall caused by high concentrate of LDL in the blood. High concentrate of LDL in the blood will trigger oxidative stress and caused endothelial dysfunction [10]. Endothelial dysfunction will uptake of monocytes and LDL into the intima. Monocytes will differentiate into macrophages and secrete chemical

compounds cytokines. Buildup of LDL in the intima be oxidized by reactive oxygen species (ROS) to oxidized LDL. The macrophages will phagocyte oxidized LDL to form foam cells [10]. It will be covered by the proliferating smooth muscle cells of the tunica media to the intima and formed fibrous plaque. Finally the atherosclerosis vessel walls become thicker than normal vessel walls [9].

Natural component antioxidant of soybeen is isoflavones. Isoflavones are included in the flavonoid group which is a polyphenolic compound [20]. Primary antioxidants such as soybeen acts as a free radical acceptor mechanism which can inhibit free radical oxidation process. The mechanism of isoflavones in soybeen is reduced free radicals by donating H ion and as a scavenger directly [7]. The presence of free radicals reduction by isoflavones may prevent LDL oxidation process so that the number of oxidized LDL in the intima will decrease. A decrease in the number of oxidized LDL will reduce the number of foam cell too. This is supported by the statement of Grassi et al., [11] that Natto is also the potential to reduce the adhesion of monocytes to endothelial work by inhibiting chemical compounds MCP-1 (monocyte chemoattractant protein-1), which is secreted by the endothelium. MCP-1 is used to trigger the monocyte adhesion to the endothelium, so that when working MCP-1 inhibited the adhesion of monocytes is also reduced. Natto can reduce the formation of monocytes and macrophages by reducing inflammation smooth muscle cells by cytokines. Cytokines are chemical compounds secreted by monocytes such as IL-1 and TNF-α. Cytokines can trigger proliferation of smooth muscle cells of the tunica media to the intima of blood vessels. The presence of smooth muscle cell proliferation which covers foam cell is called by fibrous plaque [12]. At the higher concentration is 800 mg/ml tend to be ineffective compared to the two other concentrations. This is consistent with the statement Grassi et al (2010) that the consequences of donor H ions of isoflavones will generate new free radicals of isoflavones, resulting in large number may increase the number of new free radicals.

4. CONCLUSIONS

The result showed that the black soy natto effected on the lipid profile, MDA levels, amount of foam cell and the aorta's wall density in Atherosclerosis mice.

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Preparation and Characterization of Reserpine-High-Risk Drug into Sub-Micron Particles for a Safe Drug with Minor Side Effects

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Abstract – The major effort to decrease the side effects of drugs is the development of delivery processes the active-pharmaceutical-ingredients (API). Delivery processes of API are growing in line with advances in nanotechnology. Reserpine which is one of the antihypertensive drugs is rarely manufactured by pharmaceutical companies because of its side effects that were difficult to control. On this occasion, the researcher was motivated to lay the early foundation in the delivery of reserpine by presenting it as sub-micron particles. The method for preparation of particles was an emulsion solvent evaporation. The amount of reserpine were weighed as much 4 mg and poly(lactide-co-glycolide) (PLGA) were 50 mg with three variations of the stabilizer weight of poly(vinyl-alcohol) (PVA) of 40, 80, and 120 mg as formula F1, F2, and F3. Selection of the best formula was based on the percentage of encapsulation efficiency (% EE) indicated that F2 and F3 were 71% and 80%. Further F2 and F3 were characterized by using their physical properties. Three kinds measurements for characterization of physical properties were applied i.e transmission electron microscopy (TEM), particles size analyzer (PSA), and atomic forced microscopy (AFM). These three sophisticated instruments provided an overview of the particles which were spheric shape, smooth surface, with the size range 302 ± 23 nm, polydispersity index (PDI) 0.31 and zeta potential -8 ± 0.4 mV. Based on morphology, size, PDI, surface appearance, zeta potential, and the % EE, indicated at the initial steps that the particles were potential to be developed further for security purposes for human health.

1. INTRODUCTION

The development dosage form containing reserpine into suspension is an alternative option for the comparison to a tablet, especially for patients with difficulty in swallowing. The creation became suspension is not an easy task to prepare because reserpine is unstable in water. Submicron-sized particles can be applied in the creation of suspension by using the principles of technology particle [1-3].

The development of technology particle has succeeded in manufacturing the coated drug ingredient in biopolymer to produce stable particles in water. This aspect is required by reserpine. Besides, the problem of stability in water, reserpine molecule (Fig.1) has chemical interactions in a free state with compounds of the human body. It is hard to avoid, because of the interaction, reserpine is known as a drug has many side effects. Side effects of reserpine include disorders of the central nervous system (CNS), sedation and unable to concentrate or perform complex activities [4].

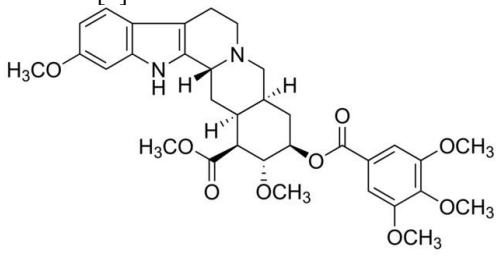

Figure 1. Chemical structure of reserpine

With the coating technique, it is expected that reserpine side effects can be reduced as the hallmarks of targeted drug delivery in the prevention of disease [5,6].

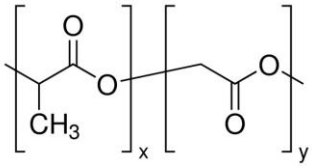

Figure 2. Chemical structure of PLGA

For coating procedure, the pharmaceutical biodegradable polymers have been used such as poly (lactide co-glycolic acid) (PLGA) Fig 2. The drug coating will be optimized when the active substance is coated by the polymer even equipped with a stabilizer such as polyvinyl alcohol (PVA) Fig.3. To improve the stability of the particles, the use of more than one layer has been reported [7] by Nafee 2008.

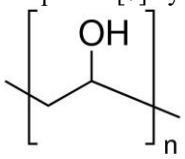

Figure 3. Chemical structure of PVA

2. METHODS

2.1 Chemicals

The chemicals used in this study were namely reserpine (Sigma-Aldrich), ethyl acetate (Merc), ethanol 96% (Merc), aqua bidest, PLGA (Sigma-Aldrich), and PVA (Kuraray).

2.2 Procedures

Dissolving of Materials

Reserpine powder was weighed 2.7 g put in a glass beaker and dissolved in 8 mL ethyl acetate. Furthermore, reserpine was stirred for 4 hours at a speed of 75 rpm. The solution was allowed to stand up to the rest of reserpine soluble homogeneous powder. The phase of reserpine in solution was pipetted as much as 500 mL for each containing 4 mg of reserpine. PLGA powder weighed as much as 500 mg inserted into a glass beaker and dissolved in 15 mL ethyl acetate. Furthermore, PLGA was stirred for 4 hours at a speed of 75 rpm. The solution was allowed to stand up to the rest of the PLGA powder settles. PLGA solution formulation stage pipette 2 mL each containing 50 mg of PLGA for each formula.

Preparation of PVA 2.5% using PVA powder weighed 2.5 g put in a glass beaker and then dissolved in 100 mL of distilled water at a temperature of 60ºC. Then, the mixture was stirred for 24 hours at a speed of 75 rpm to produce a white cloudy PVA solution and was homogenized by vortex. PVA solution formulation stage was pipetted of 1.5 mL each carries 40 mg PVA (F1), 2 mL bring 50 mg PVA (F2), and 2.5 mL of carrying 60 mg PVA (F3).

Formulation

In this study there are three formulas and differentiated on the use of PVA as a stabilizer. F1 using PVA 1.5 mL, 2 mL F2 using PVA, and F3 using PVA 2.5 mL.

Particles PLGA-Reserpine Formation.

Preparation and characterization of particles by Mardiyanto, 2015. Starting with the preparation of particles: reserpine solution of 60 mL contains 4 mg of reserpine was mixed with a solution of PLGA as much as 2 ml contains 50 mg of PLGA using a magnetic stirrer (mass 1). PVA solution 2.5%, as much as 1.5 mL for formula 1, 2 mL of 2.5% PVA for formula 2, and 2.5 mL of 2.5% PVA for formula 3 were prepared on a magnetic stirrer (mass 2). Mass 1 was dropped using a micropipette 10 mL into the mass 2 by using a magnetic stirrer. Forwarded homogenization process was set for 1 hour at a speed of 750 rpm. The process of particle homogeneity was also done using ultra Turrax with a speed of 12,000 rpm for 30 seconds. The emulsion was diluted by adding 30 mL distilled water and evaporated for 24 hours over a magnetic stirrer to remove organic solvents.

Determination of Percentage Encapsulation efficiency (% EE)

The suspension of particles was inserted into the centrifuge tube and was centrifuged for 15 minutes, at a speed of 13,000 rpm. Determination of % EE was done by measuring the absorbance of each formula with UV-Vis spectrophotometer at a wavelength of 358 nm. A calibration curve was made on a series of reserpine concentrations of 10, 20, 30, 40, 50 μg / mL of the stock solution.

Determination of Diameter, PDI, and Zeta Potential

The equipment was used in this test was PSA. Measurement of the average diameter, particle size distribution, and zeta potential using DLS method. The suspension of particles that have done through the stage of purification wass taken about 2 mL, and then inserted into the cuvette Zetasizer. Measurement of particle diameter, PDI, and zeta potential do with the scattering angle 90º.

Determination of Particle Shape and Morphology

Determination of particle shape and morphology using a TEM. Particles suspension were diluted with aquabidest. After that the sample was placed on a TEM grid which made of copper, then the electron beam passes through the sample and was captured by the detector with the acceleration voltage of 100 kV or more.

3. RESULTS AND DISCUSSION

Preparation of PLGA resulted in the conduction of solvation by adding ethyl acetate then was stirred about 5

minutes in which the PLGA was dissolved in ethyl acetate in the form of a clear viscous solution. Results were obtained in the form of a clear solution, it happened because of PLGA dissolves in one semi-polar solvent ethyl acetate (Mardiyanto, 2015). Reserpine is also soluble in ethyl acetate. PVA powder that had been prepared, further was mixed with distilled water. Dissolving PVA in hot distilled water was taken about 24 hours to produce a homogeneous solution became a bit white turbid and it happens because of decreasing temperature.

During the drop by drop process (Fig.4), the initial emulsion was formed then became more turbid according to the amount of PLGA-reserpine which was dropped. Stirring for 1 hour was a precondition before come into homogenizing step. Removing organic solvent after dilution of homogenizing emulsion (Fig.5) was recognized by the tracing odor of ethyl acetate.

Figure 4. Process of drop by drop to perform emulsion

Data of % EE of formula 1, 2 and 3 was processed based on the amount of reserpine that were not fit into particles to obtain an efficient yield. This yield was visibly different from one formula to others: formula 1 of 60,75 % ± 1,5, formula 2 of 73 % ± 2,6, and formula 3 of 65 % ± 3,8 but statistical tests were still needed. So that the obtained data were performed first by Shapiro-Wilk normality test, and resulted that the data are normally distributed. Then the normally distributed data were analyzed using ANOVA (one-way) to see the difference between formula 2 and 3 were chosen by further follow-up testing done with Tukey Post Hoc test and LSD (Least Significant Difference) in SPSS 21®.

(a) (b) (c)

Figure 5. Resulting particles in aquadest

Not only data of % EE, to see the difference between a formula, but also the particle of around 300 nm in diameter by PSA data (Fig.6) were processed first by the statistical test, and the data were known to be uneven distributed. Further data were transformed into a logarithm so that data could normally be distributed.

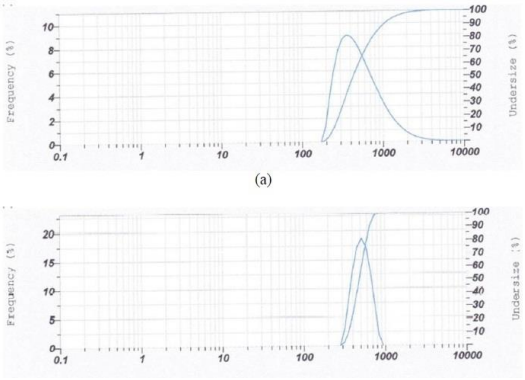

Figure 6. Avarage diameter by PSA measurement

Normally distributed data were statistically analyzed using independent t-test in SPSS 21® indicated there was no significant difference between formula 2 and 3. Obtained image of TEM (Fig.7) and AFM (Fig.8) were processed by using ImageJ® program with same contrast and visualization of pict area.

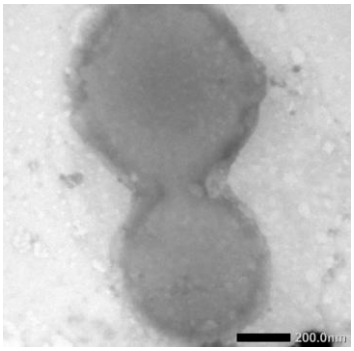

Figure 7. TEM image of particles

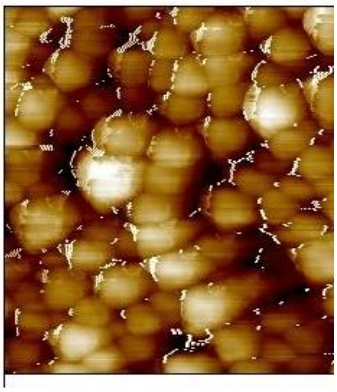

Figure 8. AFM image of particles

4. CONCLUSIONS

The stable colloid system of three formula was resulted using reserpine into emulsion solvent evaporation method. % EE of formula 1, 2 and 3 were 60,75 % ± 1,5, 73 % ± 2,6, and 65 % ± 3,8. The particle of around 300 nm in diameter and negatively charge were recognized by PSA. Obtained image of TEM and AFM were represented the homogenous and spherical particles.

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Subcloning of Haloacid Dehalogenase Gene from *Klebsiella pneumoniae* Strain ITB1 into pET-30a Expression Vector

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Abstract – Organohalogen, including monocholoroacetic acid (MCA), comprise a major compound used in manufacture of dyes, drugs, pesticides, and other industrial products. The waste of these compounds is stable, therefore become environmental pollutants. Such degradation may occur via enzymatic dehalogenation that is catalyzed by haloacid dehalogenase. Previous studies had successfully isolated and characterized the haloacid dehalogenase gene from *Klebsiella pneumoniae* strain ITB1, named *hakp1*. This gene will be subcloned into pET-30a expression vector to further improve and analyze its expression. In order to get a correct orientation of subcloning, a specific primer with addition of restriction sites on both ends need to be designed and used on PCR gene amplification prior to subcloning into the pET-30a. Specific primer for this purpose has been designed, which are 5′-GAATTCATGATCCGCGCCATCGTG-3′ as forward primer and 5′-AAGCTTTCATGCTGGGATCTGCTCC-3′ as reverse primer with addition of *Eco*RI and *Hind*III recognition site respectively. These restriction sites were added based on the nucleotide sequence and restriction site information of *hakp1* gene and pET-30a vector. Amplification of *hakp1* gene was performed by PCR using the chromosome of *Klebsiella pneumoniae* ITB1 as template. The 0,7 kb obtained amplificon was ligated into pGEM-T vector, transformed into competent *E. coli* TOP10, and plated out on blue-white screening medium to produce pGEM-hakp1 recombinant clone. The pGEM-hakp1 plasmid were isolated, confirmed by size screening, restriction analysis, re-PCR, and sequencing. The fragment was then subcloned into pET-30a expression vector employing the two created restriction sites, and transformed to *E. coli* BL21 (DE) for further studies.

1. INTRODUCTION

Organohalogen compounds, including monochloroacetic acid (MCA), comprise a major group of compounds used in the manufacture of dyes, drugs, pesticides, and other industrial products [1]. These compounds are persistence to the environment, thus can accumulate in the ground water, spread over the biosphere, seep into the ground, and caused long-term pollution due to its stability [2]. Organohalogen compounds were harmful to human health and other organisms due to its toxicity. These compounds are also possible to be transformed into other toxic metabolites that may cause damage to the liver, nervous system, kidney dysfunction, decreased immunity, and may develop cancer in long-term exposure [3].

An increasingly popular approach to bioremediation of organohalogenated compounds is biological dehalogenation[4]. The carbon-halogen bond is enzymatically cleaved for dehalogenation by specific dehalogenases. Haloacid dehalogenases, or halidohydrolases, are hydrolytic enzymes that cleave the halogen-carbon bonds in halogenated aliphatic acids, whether mono- or disubstitute, yielding a hydroxy- or an oxoalkanoic acid[5]. Some bacterial strains are able to produce haloacid dehalogenase that that catalyze the dehalogenation and transform organohalogen compounds into harmless and non-toxic metabolites. These bacteria are potentially used as bioremediation agents to overcome organohalogen pollutants [6].

Haloacid dehalogenase genes have been isolated and characterized from several microorganisms such as *Bacillus cereus* (*bcfd1*)[7], *Rhizobium* sp. RC1 (*DehL)*[8], *Pseudomonas putida* (*dehH109*)[9], and *Xanthobacter autotrophicus* GJ10 (*dhlB*)[10]. A problem in using a wild-type bacterial dehalogenase for industrial biotransformation is often because of their basal level availability and its low efficiency in reducing organohalogen pollutants. The development of molecular biotechnology provides a solution to increase dehalogenases production by cloning the gene into an expression system for high enzyme productivity. Dehalogenase genes from these bacteria have been successfully cloned into expression vector which creates high producing recombinant clones with high dehalogenase activity.

Previous studies had successfully isolated and characterized the haloacid dehalogenase gene from *Klebsiella pneumoniae* strain ITB1 that cloned into pGEM-T vector, named as pGEM-*hakp1*[11]. This vector will actually

not make a good expression because does not contain a ribosomal binding site essential for translation. In order to get good expression, the *hakp1* gene should be subcloned into an expression system that has a strong and regulatable promoter, pET-30a vector, which is performed in this research.

2. METHODS

2.1 Bacterial Strains and Chemicals

All bacterial strains were obtained from Biochemistry Laboratory ITB. *Klebsiella pneumoniae* strain ITB1, was used as source of chromosomal DNA where the haloacid dehalogenase *hakp1* gene was isolated through PCR. *E. coli* TOP10 and *E. coli* BL21 (DE) were used as bacterial hosts.

The PCR GoTaq® Green Master Mix, restriction endonucleases *Eco*RI and *Hind*III, *T4 DNA Ligase*, and 1 kb DNA Ladder were purchased from Promega. Oligonucleotide primers were ordered from Macrogen, South Korea. Isopropyl-B-D-Thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-B-D-galactoside (X-gal) from Promega were used as supplement on screening positive recombinant clones. Ampicillin, tetracycline, and kanamycin from Bioline were used as antibiotic to screen positive recombinant clones. Bacto Agar, natrium chloride, yeast extract, and tryptone from Amresco were used as ingredient of Luria-Berthani (LB) medium.

2.2 Procedures

2.2.1. Primer Design and Amplification

A pair of primer was designed based on haloacid dehalogenase gene sequences from *Klebsiella pneumoniae* ITB1[11] with the addition of *Eco*RI site on forward primer and *Hind*III site on reverse primer. The *hakp1* nucleotide sequence was obtained from GenBank with accession number KX898150 which has been analyzed using SnapGene program to obtained suitable restriction sites. These designed primers would amplify *hakp1* gene suitable to be subcloned into pET-30a expression vector.

The amplification was performed by PCR using 25 µL solution of PCR GoTaq® Green Master Mix Kit (Promega) which template and primer composition according to company protocol. PCR reaction was initiated with predenaturation at 94^0C for 4 min, followed by 34-cycles of denaturation at 94^0C for 30 s, annealing at 60^0C for 30 s, extension at 72^0C for 1 min., and ended by final extension at 72^0C for 5 min. The PCR product was confirmed by electrophoresis on 1% agarose gel.

2.2.2. Subcloning and Selection Process

The hakp1 PCR product was ligated into pGEM-T linear vector using *T4 DNA ligase*. The ligation product was transformed into competent *E. coli* TOP10 cells using heat shock method [12]. The obtained transformants were plated out on LB medium supplemented with 100 µg Ampicillin, 0.1 mM IPTG, and 20 µg/mL X-Gal for blue-white screening. The recombinant pGEM-*hakp1* plasmid was then isolated using lysis alkaline method [12] and confirmed by size screening, re-PCR, and sequencing analysis. The presence of *Eco*RI and *Hind*III sites on recombinant pGEM-*hakp1* was also confirm by restriction analysis.

The *hakp1* gene from pGEM-*hakp1,* obtained by double digest with *Eco*RI and *Hind*III, was ligated to linear pET-30a obtained from previous digestion with the same restriction enzymes. The ligation product was transformed into competent *E. coli* BL21 (DE) by heat shock method and plated out on LB medium supplemented with 50 µg Kanamycin to screen positive recombinant clone. The recombinant pET-*hakp1* plasmid was then isolated and confirmed by size screening, re-PCR, restriction analysis, and sequencing.

3. RESULTS AND DISCUSSION

3.1 Primer Design

Nucleotide sequence of haloacid dehalogenase gene from *Klebsiella pneumoniae* ITB1 has been submitted to the GenBank and could be access with accession number KX898150. This *hakp1* fragment is a 690 bp, from start codon ATG to stop codon TGA. This fragment contains 31 restriction sites as identified by mapping with *SnapGene* Program (**Figure 1**), but none of these were suitable for subcloning into pET-30a. Therefore, additional restriction site need to be added for correct expression orientation in pET-30a. The *Eco*RI site was added on the forward primer, which was right after the promoter, and *Hind*III site was added on the reverse primer. This designed would made the start codon located exactly after the promoter, and would produce right expression system of *hakp1*.

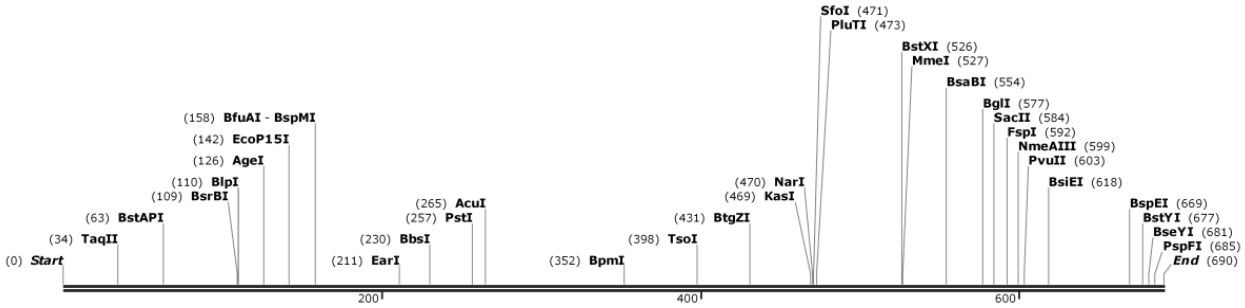


Figure 1. Restriction map of *hakp1* gene

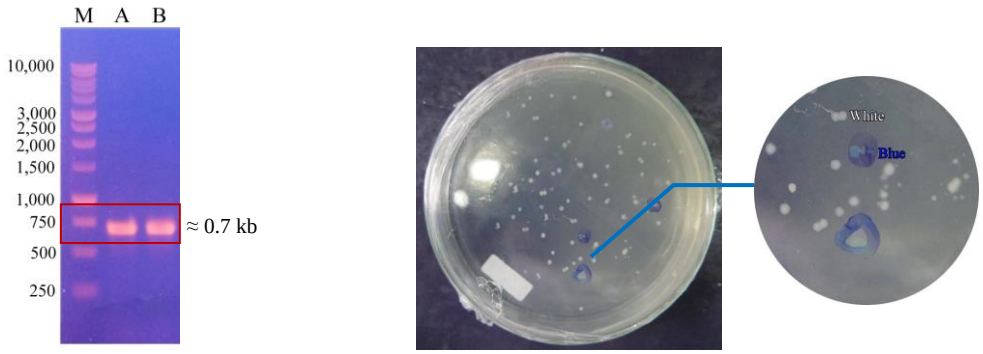
The designed primers are depicted in **Table 1**. The *Eco*RI and *Hind*III sites are underline. The melting point (Tm) and GC percentage of each primer are shown**.**

Table 1. Primers for amplification of *hakp1* gene with addition of *Eco*RI and *Hind*III restriction sites.

Primer	Nucleotide Sequences (5'-3')	Length	Tm(^{0}C)	%GC
Forward	GAATTCATGATCCGCGCCATCGTG	24	66.9	54.2
Reverse	AAGCTTTCATGCTGGGATCTGCTCC	25	67.4	52

3.2 Amplification and Cloning of *hakp1* Gene

Amplification of *hakp1* gene was done by PCR using designed primers (**Table 1**) and chromosomal DNA from *Klebsiella pneumoniae* strain ITB1 as template. This process yielded a 0.7 kb band fragment on 1% agarose gel electrophoresis (**Figure 2.a.**), which is in line with *in silico* analysis using *SnapGene* and *BlastPrimer* program. Ligation of *hakp1* amplicon into pGEM-T could be directly performed without prior treatment due to its complementary overhang ends. The pGEM-*hakp1* recombinants were transformed into *E. coli* TOP10 for blue-white screening (**Figure 2.b.**) in which the recombinant clones revealed as white colonies.



(a) **(b)**

Figure 2. Amplification of *hakp1* gene and transformation of pGEM-*hakp1* plasmid into *E. coli* TOP10. (a) Electropherogram of *hakp1* amplicon; M: Benchtop 1 kb DNA ladder, A-B: 60^{0}C and 61^{0}C annealing. (b) Blue-white screening of *E. coli* TOP10 transformants.

3.3 Confirmation of pGEM-*hakp1* Recombinant

The presence of pGEM-*hakp1* recombinant in white transformants was confirmed by size screening, re-PCR, and restriction analysis. Plasmids from several white transformants were isolated and electrophorized in the agarose gel, in which its size could be compared to plasmid isolated from blue transformant (**Figure 3.a.**). It can be seen that the recombinant pGEM-*hakp1* (line W1-W5) showed larger size compare to pGEM (line B1-B2). These plasmids were re-PCR using the same primers (**Table 1**), and the produced amplicons were electrophorized (**Figure 3.b.**). As expected, the pGEM-*hakp1* recombinant yielded a 0.7 kb amplicon (line W1-W5), whereas the pGEM did not produce any (line B1-B2).

Restriction analysis were performed by digesting pGEM-hakp1 recombinant plasmids using *Eco*RI and *Hind*III, to ensure that these enzymes cut pGEM-*hakp1* recombinant plasmids and producing the 0.7 kb fragment. The electropherogram of these analysis is shown in **Figure 3.c.** It could be seen that digested pGEM-*hakp1* produced two bands, 3 kb of pGEM and 0.7 kb of *hakp1* gene fragment (DG1-DG5), whereas the undigested plasmid showed only one band (UD). The sequences of pGEM-*hakp1* (data not shown) confirmed the presence of *Eco*RI

and *Hind*III sites on both ends of *hakp1* gene. The plasmid map of pGEM-*hakp1* recombinant is depicted in **Figure 3.d**.

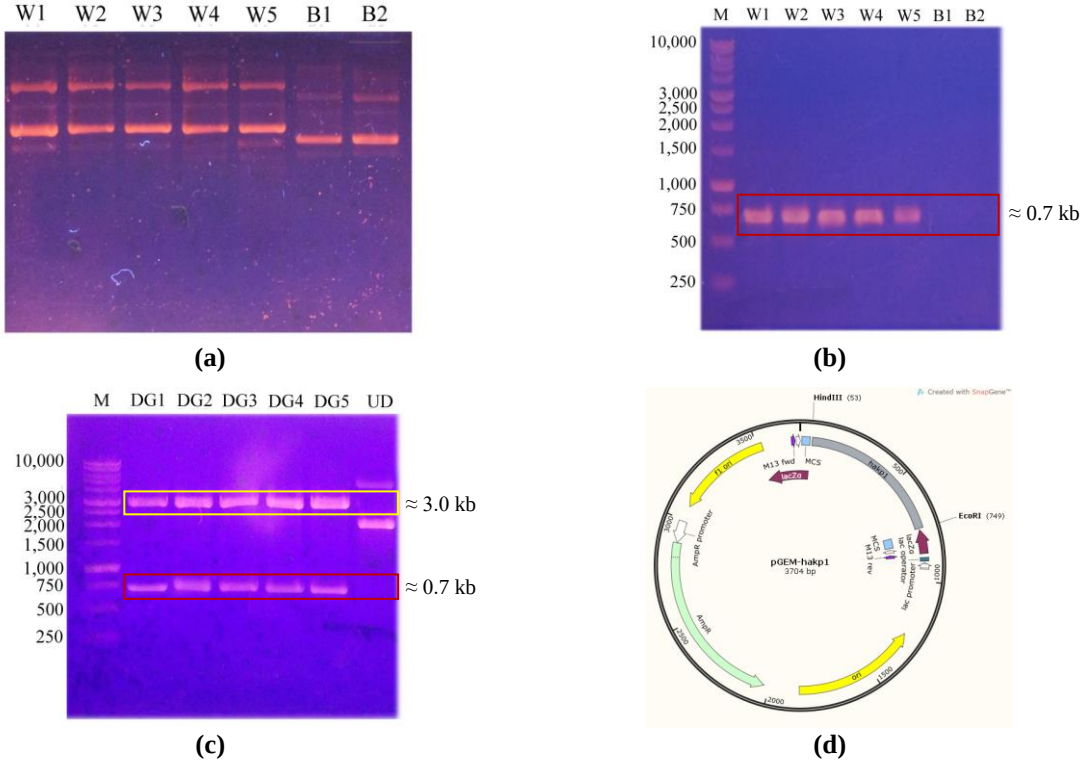


Figure 3. Analysis of pGEM-*hakp1* recombinants. (a) Size screening of pGEM-*hakp1*(W1-W5) and pGEM (B1-B2). (b) Re-PCR of pGEM-*hakp1*; M: Benchtop 1 kb DNA ladder, W1-W5: pGEM-*hakp1*, B1-B2: pGEM. (c) Digested pGEM-*hakp1* with *Eco*RI and *Hind*III; M: Benchtop 1 kb DNA ladder, DG1-DG5: Digested pGEM-*hakp1*, UD: Undigested pGEM-*hakp1*. (d) Map of pGEM-*hakp1*.

3.4 Subcloning *hakp1* Fragment into pET-30a Expression Vector

Digestion of pGEM-*hakp1* recombinant and pET-30a vector by *Eco*RI and *Hind*III were ligated using *T4 DNA ligase* and transformed into competent *E. coli* BL21 (DE). The obtained transformants on kanamycin plate is shown in **Figure 4.a**. The *E. coli* BL21 (DE) colonies harboring either pET or pET-*hakp1* recombinant clone would survived on this medium due to the presence of kanamycin resistance gene, therefore, size screening should be performed. Further restriction analysis and re-PCR on pET-*hakp1* were also performed for confirmation.

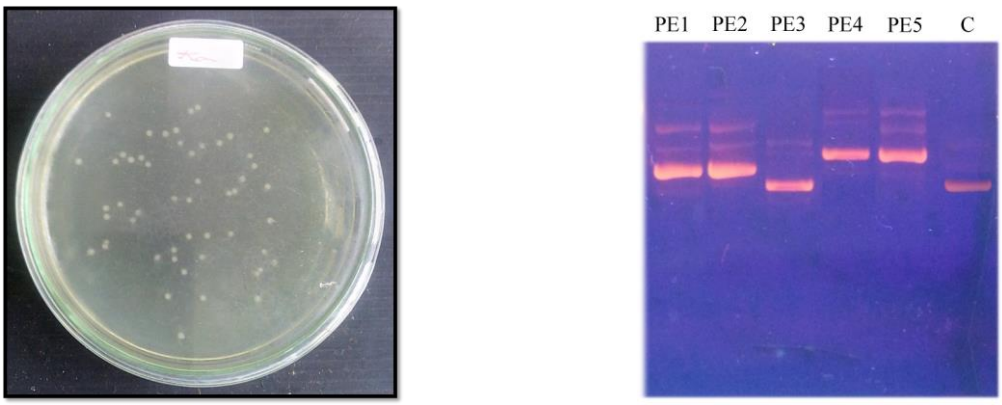
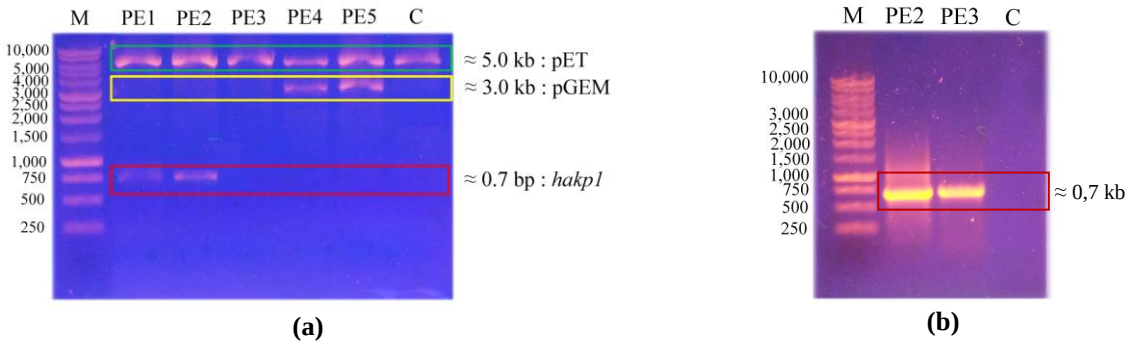


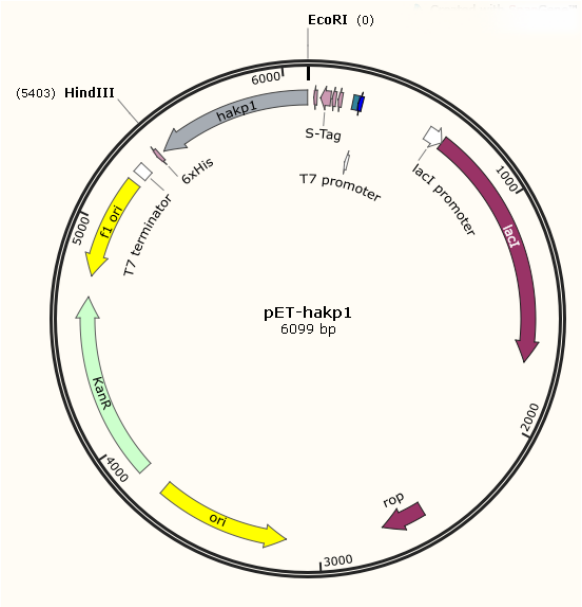
Figure 4. Subcloning of hakp1 fragments into pET-30a. (a) Transformants of *E. coli* BL21 (DE) on kanamycin plate. (b) Size screening of isolated pET plasmid; PE1-PE6: plasmid isolated from transformants, C: pET-30a.

Electropherogram of isolated plasmids from *E. coli* BL21 transformants showed three different size of plasmid (**Figure 4.b.**). The largest bands (line PE4-PE5) might appeal to pET-pGEM, the medium bands (line PE1-PE2) appeal to pET-*hakp1*, and the small one (PE3) appeals to pET-30a (**Figure 3.b.**). Restriction analysis confirmed that two isolated plasmids showed two bands with size 5 kb of pET and 0.7 of *hakp1* (line PE1-PE2), one

plasmid showed two bands with size 5 kb of pET and 3 kb of pGEM (line PE4-PE5), and the other only showed single band with size 3 kb of pET (line PE3) (**Figure 5.a.**). Re-PCR of pET-*hakp1* recombinant plasmid yielded a 0.7 kb band on 1% agarose gel electrophoresis (**Figure 5.b.**) Mapping of pET-*hakp1* plasmid sequence showed *hakp1* gene was correctly inserted into pET expression system with the initial codon on downstream of T7 promoter (**Figure 5.c.**).



(a) **(b)**



(c)

Figure 5. Confirmation of pET-*hakp1* recombinant plasmid. (a) Digested isolated pET plasmid (PE1-PE5) and pET-30a (C) by *Eco*RI and *Hind*III. (b) Re-PCR of pET-*hakp1*; M: Benchtop 1 kb DNA Ladder, PE2-PE3: pET-*hakp1*, C: pET-30a. (c) Map of pET-*hakp1*.

4. CONCLUSIONS

The designed primers have been successfully adding suitable restriction sites to *hakp1* gene that encode haloacid dehalogenase from *Klebsiella pneumoniae* strain ITB1. This gene has been subcloned into pET expression system to produce pET-*hakp1* recombinant plasmid. Size screening, restriction analysis, and re-PCR confirmed the results. The formed pET-*hakp1* recombinant cloned is now ready for further expression studies.

5. ACKNOWLEGMENT

We would like to acknowledge Lembaga Pengelola Dana Pendidikan (LPDP) Indonesia for full financial support on this research.

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Study on The Effect of Anti-oxidant Properties of Various Vegetables on The number of Free Radicals in The Instant Noodles

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Abstract – The substance contained in instant noodles containing and lead to the emergence of free radicals. The study was conducted to determine the role of antioxidant contained in vegetables which are usually mixed vegetables in instant noodles. The results showed that organic tomatoes have higher capacity than carrots, cucumber, and broccoli in reducing the number of free radicals in instant noodles.

1. INTRODUCTION

The development of food consumption, especially instant noodle, showed a significantly increasing rate. However, many problems arise due to consumption of instant noodles. In addition to the processing of instant noodles that is easy and practical, the price of instant noodles is relatively economical and readily accessible by all people. In the instant noodles are some of the materials include tartrazine (yellow dye), preservatives, MSG (Monosodium Glutamate), sodium alginate and CMC (Carboxymethylcellulose). The materials contain or generate the emergence of free radicals. Some studies have shown, however, that consuming less common fruits and vegetables contribute much more to the reduction of free-radical processes, most likely because they contain a lot of non-vitamin antioxidants, such as polyphenols and anthocyanins [1].

2. METHODS

The sample of Instant noodles cooked then add spices and antioxidants found in various vegetables. Natural antioxidants that are used include broccoli (*Brassica oleracea var. Italica*), cucumber (*Cucumis sativus*), tomato (*Solanum Lycopersicum*), and carrots (*Daucus carota*). Vegetables inserted into the juicer, then processed to separate the pulp and juice. The total volume of each antioxidant extracts obtained from the process of 150 ml. Then extract natural antioxidants are grouped into five variations of natural antioxidant solution that is 20%, 40%, 60%, 80% and 100% by volume solution of 50 ml each. ESR (Electron Spin Resonance) Leybold-Heracus used to detect the presence of free radicals in the sample and calibrated using DPPH.

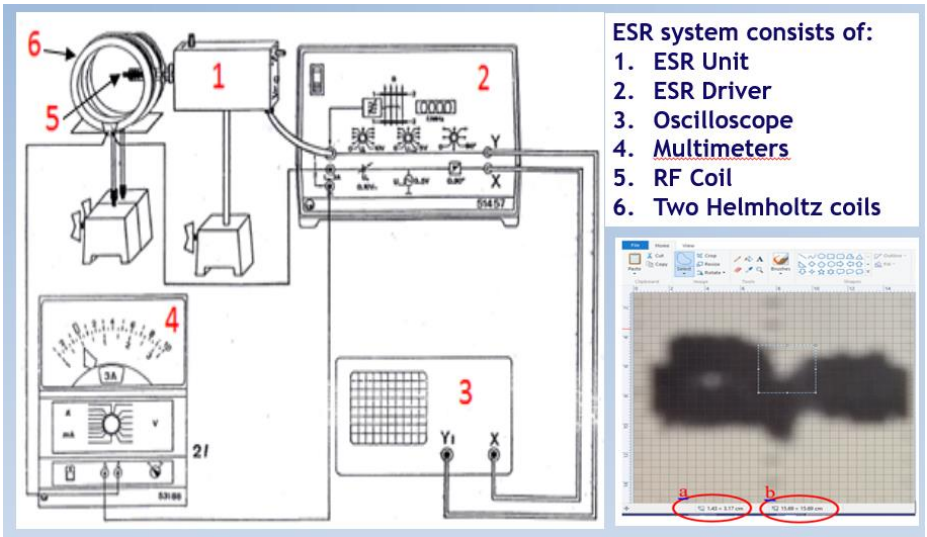

Figure 1 System Acquisition Data of ESR Equipment

3. RESULTS AND DISCUSSION

The results show that increasing number of antioxidants can decrease the concentration of free radicals. Tomatoes are the type most effective natural antioxidants to reduce free radicals type O_2^- and Fe_3^+ when compared with cucumber, carrots, and broccoli. Tomatoes contain bioflavonoids (including lycopene, α-carotene, and ß-carotene), vitamin C and vitamin E, which can reduce the amount of free radicals [2][3].

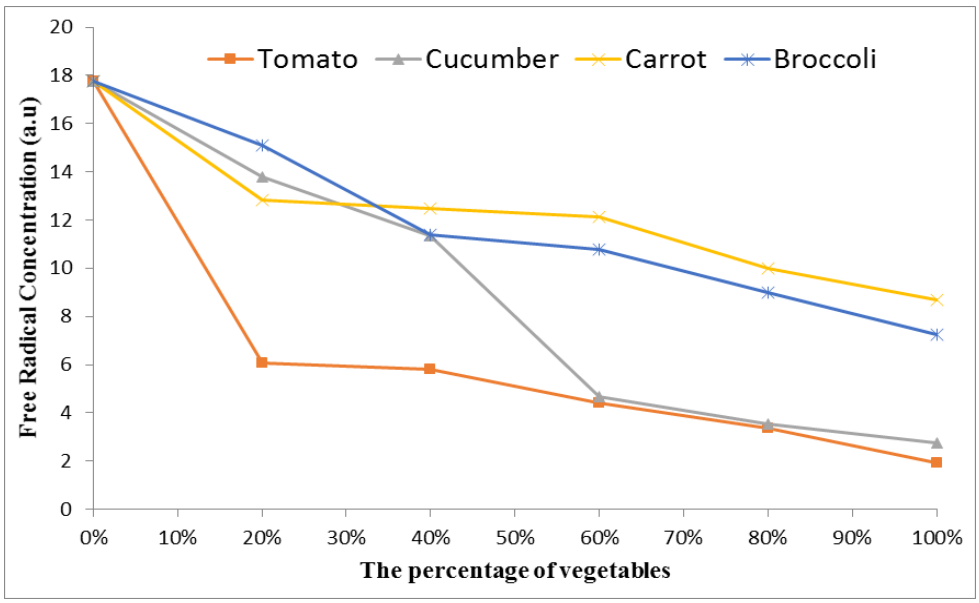

Figure 2. Anti-oxidant effect of various vegetables on the number of free radicals

The active compounds found in tomatoes can release electrons to donate to the free radical type of O_2^- and Fe_3^+. The compound will react in a chain so that the free radical will stable and form H_2O. Meanwhile, Vitamin E and vitamin C is a substance that has several OH groups that can release hydrogen atoms and electrons to reduce the number of free radicals.

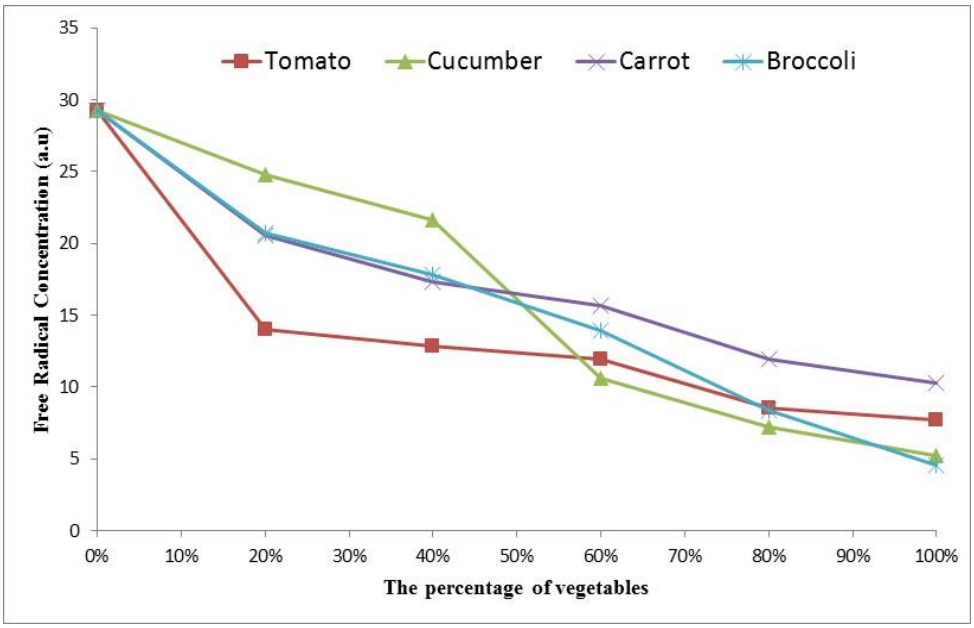

Figure 3. Anti-oxidant effect of various vegetables on the number of Fe3+ free radicals

Additionally, vitamin E and vitamin C can also reduce free radicals caused by reactive oxygen species. The alleged effect of reducing the risk for many diseases is not only due to the effect of individual antioxidants, such

as α-tocopherol, ascorbic acid, or β-carotene but also may be the result of antioxidant compounds not yet known or synergy of several different antioxidants present in fruits and vegetables.

4. CONCLUSIONS

The substance contained in instant noodles containing and lead to the emergence of free radicals. Instant noodle seasoning will trigger the appearance of free radicals O_2^- and Fe_3^+. The results showed that organic tomatoes have higher capacity than carrots, cucumber, and broccoli in reducing the number of free radicals in instant noodles.

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Pairwise Sequence Alignment between Hepatitis B Virus and Hepatocellular Carcinoma Using Dynamic Programming Approach

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Abstract – This paper aims to analyze the Hepatitis B Virus (HBV) and Hepatocellular Carcinoma (HCC) through pairwise DNA sequence aligmnment. Hepatitis B virus (HBV) is a DNA virus which can cause acute and chronic hepatitis in humans. Mutation of the virus DNA in X region has a potential role in HCC. Bioinformatics has role including analyzing the development of a virus through DNA sequence alignment. The similar sequences indicate that they have similarity of physical-chemical properties. It is purposed to align global sequence using Needleman Wunsch algorithm as dynamic programming approach. Global sequence alignment creates end-to-end alignment even though there is a difference in some region. The method is applied to DNA sequence of 858 HBV and 12 HCC patient in region-X of genotype A. As a result, the similarity of pairwise sequence alignment is achieved of 96.55% with default parameter value (match=2, mismatcch=-3, gap opening=-5 and gap extension=-2). Furthermore, the number of sequences does not effect to the similarity.

Key words: *HBV, sequence alignment, dynamic programming, Needleman Wunsch*

1. INTRODUCTION

Hepatitis B virus (HBV) is a DNA virus which can cause acute and chronic hepatitis in humans. The chronic hepatitis can further progress to liver cirrhosis and carcinoma of the liver (hepatocellular carcinoma, HCC)[1]. Bioinformatics have roles include mapping and DNA analysis, protein sequencing, aligning different DNA, constructing and performing models of protein structures in three dimensions [2]. However, the main goal of bioinformatics is to know the biological process including analysis the development of the virus through the similarity rate of DNA sequence alignment as the purposed of this research.

There are two methods of sequence alignment including optimal global sequence alignment and optimal local sequence

alignment. There are several algorithms of sequence alignment using dynamic programming approach and heuristic approach. The Needleman-Wunsch algorithm for optimal global sequence alignment [3] and Smith-Waterman [4] for optimal local sequence alignment are based on dinamic programming approach algorithm. While the algorithm that uses heuristic approach is FASTA [5]and Basic Local Alignment Search Tool (BLAST) [6]. BLAST algorithm does not produce optimal alignment but computational speed is faster than an algorithm that uses dynamic programming approach (Kasap, et al., 2008) [7]. Several algorithms have been developed to perform sequence alignment but getting the results are not always optimal, such as Simple Graphical Alignment Tree algorithm (GSA), and Super Pairwise Alignment (SPA).

2. METHODS

2.1 Genome Sequencing

DNA sequencing is the process of ordering nucleotide bases which consist of adenine, guanine, cytosine and thymine (A, T, G, C) in a DNA molecule. However, genome sequencing is determining the nucleotide sequence of bases in DNA or genome in the body of an organism. Sequencing result has performed a sequence of letters which states nucleotide bases in particular DNA, such as AGTCCGCAGGCTCGGT.

Genome sequencing is always compared to the encoding process, and it is not immediately able to provide genetic information. It needs to translate the genome sequencing result to understand how to work, what the genes make up the genome, how different genes can relate and coordinate. Genome sequencing is now a process that is critical in scientific research and technology, especially in the field of health and biotechnology. The

slight mutation of DNA sequences can cause abnormal genes and then, they make a disease. Thus, in the field of health, one of which genome sequencing is applied to diagnosis cancer. Cancer is caused by abnormal DNA base composition contained in the body cells

2.2. Sequence Alignment

Sequence Alignment is the process whereby a sequence compared by looking for patterns of the most common characters and inter-related sequences. Pairwise sequence alignment is a alignment process between two sequences based on similarity in order to search on the database and multiple sequence alignment. This similarity can be expressed in percentages. It means that the sequences have similarities to phisical-chemical properties. Another term, the degree of similarity in amino acid sequence is similar between the two protein sequences [8]. The similarity may indicate a functional relationship, or the structural and evolutionary relationships [9]

It is necessary to shift sequence at certain positions in order to get the maximum similarity such that sequence alignment can be performed. There are two method of sequence alignment, ie global and local alignment. The global alignment method is to compare the whole sequences. This method is suitable to perform sequence alignment that has a high degree of similarity of the whole part of the sequence. Another hand, the local alignment method is to compare a local area or part of the sequence.

2.2.1. Global Alignment

Global alignment creates end-to-end alignment even though there is a difference in some region. This approach suitable for aligning similar sequence [9]. As illustration, it can be showed in Fig.1.

```
EARDFNQYYSSIKRSGSI
 :...:..:::::::...
EPKLFIQYYSSIKRTMGH
```

Fig. 1. Global alignment

2.2.2. Local Alignment

Local alignment only aligns the most similar region within a sequence. There is no need to align all part of sequence, only the region with big similarity according to some criteria [9]. By using the same sequence is as Fig. 1, local alignment becomes as follow:

```
EARDFNQYYSSIKRSGSI
       :::::::
EPKLFIQYYSSIKRTMGH
```

Fig. 2. Local alignment

2.3. Dynamic Programming

The Dynamic Programming solves the original problem by dividing the problem into smaller independent subproblems then combines all sub solution become the final solution [2]. These techniques are used in many different aspects of computer science. Needleman-Wunsch is one of the algorithms for sequence alignment which is defined by dynamic programming approach. This method is purposed in this research to apply the pairwise sequence alignment between HBV and HCC.

Needleman-Wunsch Algorithm

Needleman-Wunsch algorithm is a dynamic programming method to find global optimal sequence alignment and it allows the gap. This is an algorithm that computes the similarity between two sequences by making MxN matrix (M is the length of the first sequence, N is the length of the second sequence) and then fills the entire matrix to perform alignment. This algorithm always produces optimal solution for the alignment. After filling the entire matrix, this algorithm will do a backtracking form end to end point to build the aligned sequence [3].

As illustration, there are two protein sequences of length m and n where a = a_1a_2 ... a_m and b = b_1b_2 ... b_n. To find a parallel sequence with the highest score, it must be constructed a matrix F where F (i, j) is the value of the best sequence alignment between $a_{1:i}$ and $b_{1:j}$. First, it is constructed matrix F and is filled in F (0,0) = 0, F (i, 0) = -i and F (0, j) = j. Furthermore, it is applied as Eq.1

$$F(i,j) = max\begin{cases} F(i-1,j-1) + s(a_i, b_j, p) \\ F(i-1,j) \\ F(i,j-1) \end{cases} \quad \text{Eq.1}$$

Given $s(a_i, b_j, p)$ as the scheme function if $a_i = b_j$, then it is returned to score a match. However, if $a_i \neq b_j$, , then it is returned to score a mismatch. The final score can be obtained by looking the value of F(M,N). The alignment is built by doing backtrack from F(M,N) to F(0,0). The procedure is compared to the value on F(i,j) to its top left and diagonal using equation (1). For example, if F(i,j) = F(i,j-1), then backtrack is recorded an insertion in b_j [7] To get the better performance, the data of an insertion, deletion or gap can be recorded during filling the matrix. Sometimes, several paths can be generated from this process. This indicates that there is more than one alignment that can be generated to the same score.

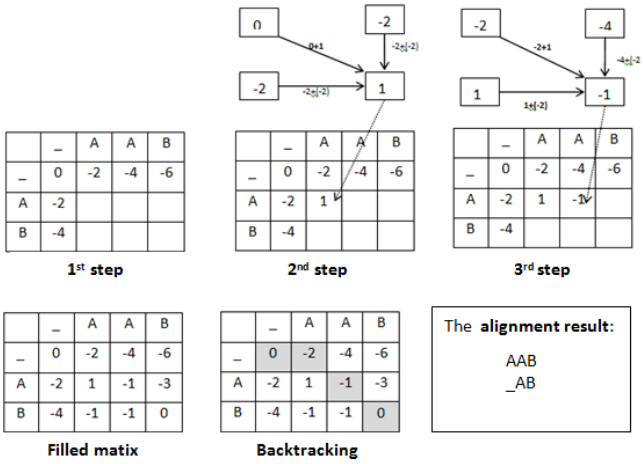

Fig.3. The steps of Needleman Wunch Algorithm (match=1, mismatch=-2, gap=-2)

3. RESULT AND DISCUSSION

This research tested HBV DNA sequences, with specific genotype-A in X region. The data is downloaded from HBV database at url: https://hbvdb.ibcp.fr/HBVdb/HBVdbDataset?view=/data/nucleic/fasta/A_X.fas&seqtype=0gnl|hbvcds|AB014370_X_P-A. There are 858 HBV DNA sequences and 12 HCC DNA sequences. Then, they are totally 10296 in pairs. As illustration, the data ser of two sequences alignment is as follows.

```
ATGGCTGCTAGGCTGTGCTGCCAACTGGATCCTTCGCGGGACGTCCTTTGTTTACGTCCCGTCGGCGCTGAATCCCGCGG
+++++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
ATGGCTGCTAGGCTGTACTGCCAACTGGATCCTTCGCGGGACGTCCTTTGTTTACGTCCCGTCGGCGCTGAATCCCGCGG
12345678901234567890123456789012345678901234567890123456789012345678901234567890
```

In order to know the characteristics for HBV and HCC, it is applied to pairwise sequence alignment using Needleman-Wunsch algorithm based on percentage of similarity. It is used the default parameter (match=2; mismatch=-3; gap opening=-5; gap extension=--2). The experimental result showed that the similarity rate of 96.548% and score rate=849.8. As an illustration, the performance result for several sequences algnment in pairs is shown in Table 1.

Table 1. Performance Result of Sequence Alignment

Sequence #	Score	Similarity (%)
1	900	98,71
2	860	96,99
3	875	97,63
4	900	98,71
5	900	98,71
6	905	98,92
7	895	98,49
8	895	98,49
9	895	98,49
10	840	96,13

Furthermore, this research is applied to various number of data sequences in order to know the effect to performance, i.e. score and similarity. The result shown that the number of data is not effect to the performance measure either scoring or simiarity as in Fig.1 and Fig. 2.

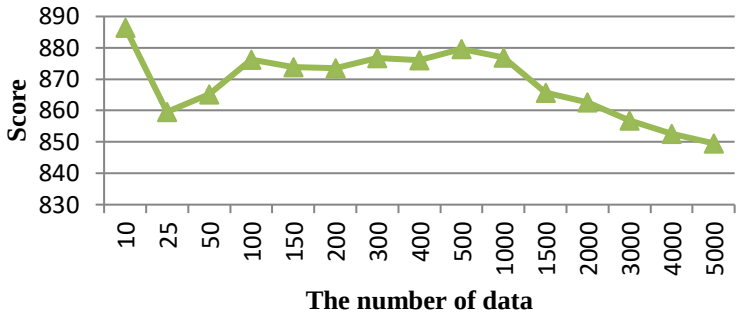

Figure 1. The performance result of score against the number of data

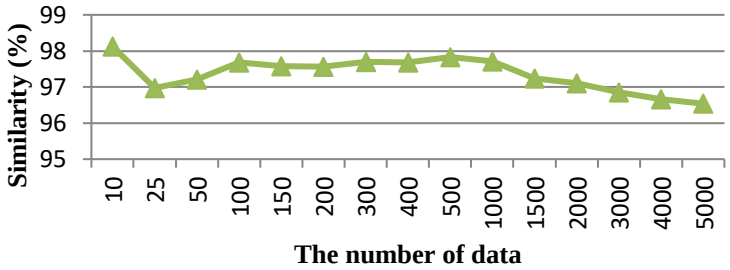

Figure 2. The performance result of similarity rate against the number of data

4. CONCLUSSION

Needleman-Wunch, is a diynamic programming method, has been successfully implemented to perform the pairwise DNA sequence global alignment between HBV and HCC:

1. The principle of Needleman-Wunsch algorithm is using matrix to align the sequences in pairs, including initialization of matrix, filling matrix using maximum scoring and trce back to perform the sequemce result. The matrix region is filled DNA scoring for marking and backtracking process.
2. The test result of pairwise DNA sequence alignment between hepatitis B virus and hepatocellular carcinoma showed that the both sequences are obtained similarity rate of 96 547% in average. It means that the both sequences have similarity in phisical-chemical properties.
3. The number of data is not effect to either score value or similarity rate of sequences.

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Preparation of Radiolabeled (Gd-DOTA)n-PAMAM trastuzumab: an Analogue Candidate Targeted Contras Agent

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Abstract – Magnetic resonance imaging (MRI) is a modality commonly used for diagnosis for many diseases including cancer. In order to get a good image of anatomy and the physiological processes of the body of patients, a certain amount of contrast agent has to be administered to the patient prior imaging. Recently, Centre for Radioisotope and Radiopharmaceutical Technology has been able preparing a candidate of a new targeted contrast agent (Gd-DOTA)n-PAMAM G3-trastuzumab. As a candidate of new contrast agent it has to undergo several physico-chemical and biological tests. These tests can be performed relatively easy if (Gd-DOTA)n-PAMAM G3-trastuzumab labeled with a radioactive element such as ^{153}Gd, the most suitable element as it is chemically similar to Gd. However, due to the difficulty in a procurement of ^{153}Gd, an alternative for radiolabeling of (Gd-DOTA)n-PAMAM G3-trastuzumab has to be established. This report, therefore, will introduce the use of ^{125}I for radiolabeling of Gd-DOTA-PAMAM G3-trastuzumab. The use of ^{125}I, a low energy gamma emitter, for radiobeling of (Gd-DOTA)n-PAMAM G3-trastuzumab is possible since a part of its building block is trastuzumab, a monoclonal antibody. This antibody provides tyrosyl functional group that one of its protons could be substituted by ^{125}I. The iodination reaction was performed by addition of Iodogen as oxidator. The (Gd-DOTA)n-PAMAM G3-trastuzumab labeled with ^{125}I has been successfully prepared with radiochemical purity of 97.7 ± 0.9% after purification using a Sephadex G25M column. Its preliminary bio-distribution test showed that there was no significant uptake in thyroid, iodine targeted organ and its MRI seems function well. Therefore Gd-DOTA-PAMAM G3-trastuzumab-^{125}I might be used as an analogue of (Gd-DOTA)n-PAMAM G3-trastuzumab in its physicochemical and biological tests.

1. INTRODUCTION

Magnetic resonance imaging (MRI) is a modality which commonly used for diagnosis many diseases including cancer. In order to get a good image of anatomy and the physiological processes of the body patients, a certain amount of contrast agent has to be administered to the patient prior imaging [1][2]. Contrast agent consists of paramagnetic metallic ions such as gadolinium (Gd), and manganese (Mn) which are complexed with certain ligands to form paramagnetic metallic complexes. There is also iron (Fe) based contras agent in the form of iron oxide super paramagnetic. However, the most common agents used in MRI procedure are Gd-based contrast agents. A few of them are Magnevist® (Gadopentetate dimeglumine, Gd-DTPA), Dotarem® (Gadoterate, Gd-DOTA) dan Omniscan® (Gododiamide, Gd-DTPA-BMA) and Gadovist® (Gadobutrol, Gd-DO3A-butriol). There has been some limitation on MRI contrast agents which are currently available in market. Most of them are non-specific and have low molecular weight. Therefore there are quickly excreted from the body through kidney, as a consequence for a relatively longer MRI procedure, some dose of contrast agents has to be re-administered.

Currently, there has been a lot of interest in developing a new type of contrast agent which is specific, higher relaxation time and can detect diseases on a cellular level. This can be done by increasing the number of Gd in a contrast agent and conjugating it to a molecule or bio-molecules which as has target molecule/ receptor on certain diseases. Recently, Centre for Radioisotope and Radiopharmaceutical Technology, National Nuclear Energy Agency (PTRR – BATAN) has been able preparing a candidate of a new targeted contrast agent [3]. It is a gadolinium-1,4,7,10-tetraazacyclododecane - 1,4,7,10-tetraacetic acid (DOTA) – polyamide amine (PAMAM) dendrimers Generation 3 – trastuzumab [(Gd-DOTA)n-PAMAM G3-trastuzumab] which has been reported to be quite stable. PAMAM G3 has 32 amines functional group that can be used to attach < 32 DOTA which later can complex Gd. Trastuzumab is a monoclonal antibody which has been used for immunotherapy of a poor prognosis of breast cancer which has over-expressed human epidermal growth factor receptor type 2 (HER-2/ neu) [4]. Trastuzumab is DNA recombinant humanized monoclonal antibody (mAb) which binds to extracellular of HER-2/neu. By combining these three building blocks, it is expected that the new contrast agent

would have high relaxing time and highly specific for MRI procedure for breast and other cancers which have over-expressed HER-2 [5] .

As a candidate for a new targeted contrast agent, it has to be undergone several tests in order to assess their efficacy and safety for use in human subjects. Early testing as such physicochemical and biological tests would be performed with laboratory investigation. These testing would use extensive chemical and animal studies in order to acquire a better understanding of how the drug works. These testing would be much easier if the tested drug is labelled with radioactive, so by virtue of its radioactive decay it can be used to explore the stability, bio-distribution, binding to its receptor and other tests by tracing the path of the radioisotope.

Radiolabeling $(Gd\text{-}DOTA)_n$-PAMAM G 3.0-Trastuzumab with radioactive atom which is similar to the one in a contrast agent would be an ideal as it does not change its chemical structure. In this project, due to the difficulty in procurement of ^{153}Gd, an alternative for radiolabeling of (Gd-DOTA)n-PAMAM G3-trastuzumab has to be established. As alternative radiolabeling with other radionuclide which is not expected to change its physico-chemical and biological behaviour need to be establised. This report therefore will introduce the use of ^{125}I for radiolabeling of (Gd-DOTA)n-PAMAM G3-trastuzumab. The use of ^{125}I, a low energy gamma emitter, for radiobeling of (Gd-DOTA)n-PAMAM G3-trastuzumab is possible since a part of its building block is trastuzumab, a monoclonal antibody.

2. METHODS

2.1 Chemicals

Reagent and material used in this study included (Gd-DOTA)n-PAMAM-trastuzumab and $Na^{125}I$ (prepared by PTRR), Iodogen (Peirce Lab.), $CHCl_3$ (Sigma-Aldrich), Sephadex G25M column (GE Health Care) blocked with *bovine serum albumin* (BSA, Sigma-Aldrich) and then primed with phosphate buffer saline (PBS) 0.01 M pH 7,2 and Whatman 3 paper (Merck-Millipore).

2.2 Procedures

(Gd-DOTA)n-PAMAM-trastuzumab was prepared according to producer reported by Rahmania *et al* [3]. In brief, NHS-DOTA was conjugated to PAMAM to result (DOTA)n-PAMAM. This conjugate was then activated with Trout's reagent to give DOTA-PAMAM-SH. This intermediate product was then conjugated with trastuzumab which has been activated with SMCC (trastuzumab-SMCC) resulted in (DOTA)n-PAMAM-trastuzumab which was then complexed with Gd^{3+} to give (Gd-DOTA)n-PAMAM-trastuzumab.

Preparation of Iodogen tube
Aliquot of Iodogen (1 mg/ mL in $CHCl_3$) was pipetted into a glass tube (1 ∅ x 8 L cm). Iodogen was then dried out from its solvent ($CHCl_3$) by purging with N_2 gas to result in a tiny white sheet on the base of the tube. This tube was ready to be used for a iodination process.

Iodination of (Gd-DOTA)n-PAMAM-trastuzumab with ^{125}I.
(Gd-DOTA)n-PAMAM-trastuzumab was added into the Iodogen tube which was followed by addition aliquot of $Na^{125}I$ solution. The reaction mixture was vortex slightly and then let to react for five minutes. Aliquot of KI (1 mg/mL) was added and the reaction was continued for another five minutes. Iodination of (Gd-DOTA)n-PAMAM-trastuzumab with non radiactive was performed in similar manner with iodination with ^{125}I.

Purification of ^{125}I-labelled (Gd-DOTA)n-PAMAM-trastuzumab .
Purification of ^{125}I-labelled (Gd-DOTA)n-PAMAM-trastuzumab was performed by loading the reaction mixture on top of a Sephadex G25M column. The column was then eluted with PBS 0.01 M pH 7.2 and the fractions were retrieved (250 µL/ fraction). Each fraction was then measured for its activity using dose calibrator (Capintec USA) and for its protein content. The protein content was measured by taking 10 µL of the sample from each fraction which was then mixed with protein dye (diluted 1/4 with H_2O). Fractions that gave a blue colour indicated that they contained protein (mAb), and these fractions were then measured for their radiochemical purities. Radiochemical purity of (Gd-DOTA)n-PAMAM-trastuzumab-^{125}I was measured using chromatography system where Whatman No. 3 was used a static phase while the mixture of methanol : water (3:1) was used as mobile phase.

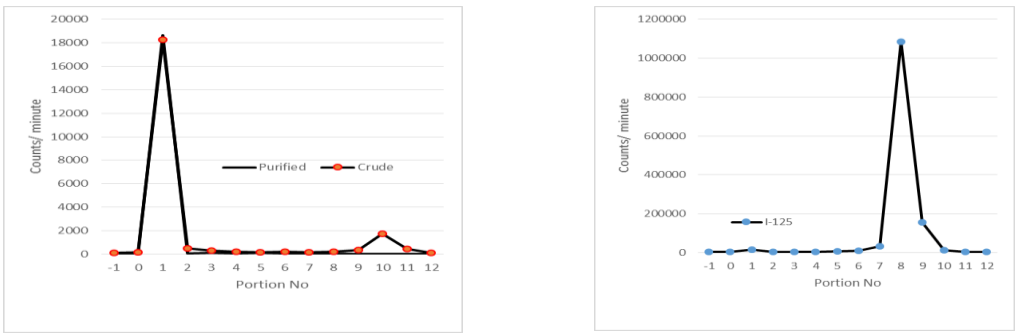
Biodistribution Study
Preliminary biodistribution was performed after gaining an Ethical Approval from Health Research Ethic Committee, Faculty of Medicine, Padjadjaran University, Bandung (No. 080/UN6.c.32/KEPK/PN/2015). Aliquot of (Gd-DOTA)n-PAMAM-trastuzumab-^{125}I (100 µCi) was injected intravenously to two groups of mice (3 mice/ group). After 6 and 24 hours the mice were sacrificed and their organs of interest were dissected and then counted using a gamma counter (ÇAPRAC-t).

MRI Procedure
MRI procedure was performed by injecting aliquot of (Gd-DOTA)n-PAMAM-trastuzumab-I in a rat intravenously. At a designed time images were then taken **(**MSCT 128 Slices Dual Sources**)**.

3. RESULTS AND DISCUSSION

(Gd-DOTA)n-PAMAM-trastuzumab is a candidate of contrast agent [3]. Unlike many contrast agents which are currently available in the market, this contrast agent is a targeted one due to the trastuzumab which is one part of a whole building block. Trastuzumab, a monoclonal antibody, is an anti for breast, ovarian and other cancer which over expressed human epidermal receptor type 2 (HER-2).
Trastuzumab with molecular weight of ~ 14,5 kDalton consists of amino acid chains to form four polypeptide chains, two identical heavy chains (H) and identical light chains (L), bound by sulphide bridges to form a symmetrical structure (Fig. 1.A). It is reported that there are 62 tyrosil groups (Fig. 1.B) in trastuzumab, 15 in a complementarily determining regions (CDR) 6 in each V_{Kappa} and 9 on VH [6]. Some of these tyrosil groups might be available for iodination.

The use of (Gd-DOTA)n-PAMAM-trastuzumab-^{125}I where one or two of protons on tyrosil group on meta position on tratuzumab substituted with ^{125}I is expected to be able in providing an analogue of (Gd-DOTA)n-PAMAM-trastuzumab. Radiolabeling of (Gd-DOTA)n-PAMAM-trastuzumab with ^{125}I was performed under a controled environment to result in (Gd-DOTA)n-PAMAM-trastuzumab-^{125}I with its integrity still intact. The obstacle of that ^{125}I might be released from trastuzumab during *in vitro* and *in vivo* testing could be assessed through biodistribution test. In addition, the *in vivo* behaviour of ^{125}I which was well documented could also be used as a reference [4].
Radiolabeling of $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab with ^{125}I was carried out using Iodogen as oxidator [7], with mol ratio of $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab to ^{125}I/ I of 1 : 1, giving an efficiency of radiolabeling of 88,3 ± 3,9%. These results showed that there was 12.7% of unbound ^{125}I/I in the reaction mixture. This results also indicated that there was in average less than one molecule of ^{125}I/ I attached to one molecule of $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab. There have been many researchers reported that radiolabelled or radioiodinated monoclonal antibody which has a limited number of number radioactive/ iodinated molecule attached to its maintained its immunoreactivity [8] [9]. Therefore the ability of $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab-^{125}I, which is one of amine groups used to attach $(Gd\text{-}DOTA)_n$-PAMAM and one of proton of its tyrosil group substituted with ^{125}I, to bind to its target receptor (HER-2), is expected to be maintained.
Radiochemical purity of a radiotracer or radiopharmaceutical has to meet with a standard requirement in order to avoid the unbound radioactive interfering with the *in vitro* and *in vivo* testing results. The radiochemical purity for a good tracer or radiopharmaceutical could be varied between 85 - 100% [10]. However, higher radiochemical is much preferred as it will give more accurate image. Therefore the $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab-^{125}I with radiochemical purity of 88% was purified through a Sephadex G25M column and its fraction retrieved. Radiochromatogram of $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab-^{125}I prior to and after purification are shown in Figure 2A, whereas Figure 2B shows the radiochromatogram of Na^{125}I for comparison.



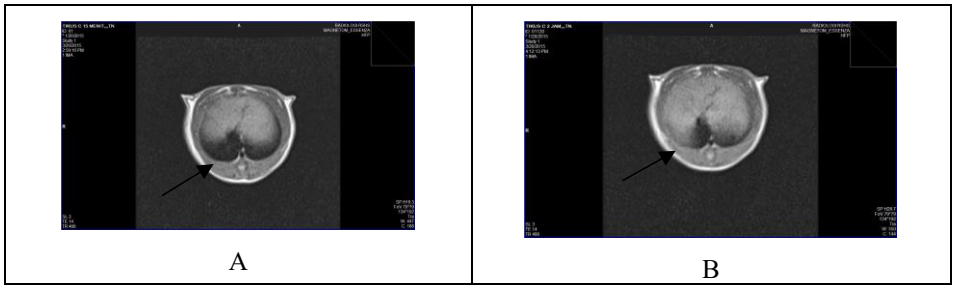
(A) (B)

Figure 2. Typical Radiochromatogram of Crude and Purified $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab-^{125}I (A) and Na^{125}I (B)

It can be seen that $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab-^{125}I has a Rf of ~ than 0.3 (Fig 2A. and 2B) and whereas free ^{125}I has a Rf of 0.9 (Fig 2A). Radiochemical purity of $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab-^{125}I after purification with Sephadex G25M column was found to be 97.7 ± 0.9%. This radiochemical purity meets with the radiochemical purity requirement for a good tracer or radiopharmaceutical.

In order to access the stability $(Gd\text{-}DOTA)_n$-PAMAM-trastuzumab-^{125}I in vivo preliminary biodistribution study and MRI procedure were performed in normal mice and rat respectively. Preliminary biodistribution study

showed there was no significant uptake in the thyroid (< 1%), target organ of ^{125}I, up to 72 hours post injection. Spetz *et all*., reported that nearly 99% of ^{125}I or ^{131}I was uptaken by tyroid when Na^{125}I or Na ^{131}I was injected to the rats [11]. Therefore, this result showed that (Gd-DOTA)$_n$-PAMAM-trastuzumab-^{125}I relatively stabile *in vivo*. Whereas heart MRI images of (Gd-DOTA)$_n$-PAMAM-trastuzumab-I showed that a clearer image after 2 hours post injection (Figure 3B) compared to the image 15 minutes post injection (Figure 3A). This can be presumed that (Gd-DOTA)$_n$-PAMAM-trastuzumab-I still functioned as contrast agent despite the introduction of new atom into its molecule.



A B

Figure 3. MRI images of Heart 15 minutes (A) and 2 hours (B) Post Injection

4. CONCLUSIONS

The (Gd-DOTA)n-PAMAM G3-trastuzumab labelled with ^{125}I has been successfully prepared with a radiochemical purity of 97.7 ± 0.9% after purification using a Sephadex G25M column. Preliminary biodistribution study indicated there was no significant ^{125}I released from (Gd-DOTA)n-PAMAM G3-trastuzumab-^{125}I and its MRI seems functioning well. Therefore (Gd-DOTA)n-PAMAM G3-trastuzumab-^{125}I may be used as an analog of (Gd-DOTA)n-PAMAM G3-trastuzumab in its physico-chemical and biological tests.

5. ACKNOWLEDGMENT

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Preparation of Albumin Microspheres Kits Labeled with Technetium-99m as Radiopharmaceuticals for Lungs Imaging

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Abstract – Technetium-99m (^{99m}Tc) labelled albumin radiopharmaceuticals with particle size of 10-90 µm has been widely used for lung perfusion imaging using SPECT, such as ^{99m}Tc- macroaggregate albumin (MAA). Preparation of ^{99m}Tc-albumin particulate in the form of microspheres (MA) has been developed but its performance need to be studied to be used as an alternative to ^{99m}Tc-MAA. The study has been carried out comprising the preparation of albumin microspheres, formulation of the kits to be labeled with ^{99m}Tc, radiolabeling performance with ^{99m}Tc and stability on storage. Albumin microspheres was prepared by stirring the emulsion of human serum albumin in vegetable oil at certain rpm and time followed by heat denaturation of albumin. Radiochemical purity of ^{99m}Tc labeled MA was analysed using paper chromatography with 85% methanol and saline as eluents, the size and shape of the particles were observed through microscope equipped with haemocytometer. Besides, the study of some techniques used was also carried out including the effect of filtering and the effect of using dilute HCl instead of concentrate one to dissolve tin chloride on the radiolabeling performance. The results showed that the albumin microspheres kit can be highly labeled with ^{99m}Tc with stability of only 2 hours after labeling but the shelf-life can withstand more than 4 months in deep freeze storage. It was concluded that the protocol of preparing MA kits has been successfully obtained.

Keywords: ^{99m}Tc, microspheres, albumin, radiochemical purity.

1. INTRODUCTION

Technetium ^{99m}Tc labeled radiopharmaceutical has been widely used in nuclear medicine for diagnosis of various diseases, such as albumin particulates in the form of macroaggregates, microspheres or nanospheres.

Albumin is a globular protein abundantly found in plasma, which its tertiary structure can be denatured and aggregated by disrupting its weak interactions such as ionic and hydrophobic interactions and hydrogen bonds. Denaturation of albumin can occur by means of heat and chemicals mainly strong acid or bases which alters its pH. Heat gel formation of albumin involves 3 steps, i.e protein unfolding, formation of fibrils aggregates through disulphide and hydrophobic bonding and crosslinking process of the fibrils through non-covalent interactions [1-3].

Albumin microspheres have an ability to incorporate various radionuclides, and radiopharmaceuticals based on human serum albumin have good physiological behaviour and easy to produce. Microspheres of human serum albumin can also be used as carriers for delivery of radionuclides to malignant tumors [4].

Technetium ^{99m}Tc-macroaggregate albumin (MAA) and ^{99m}Tc-microsphere albumin (MA) are commonly used for lungs perfusion imaging, whereas ^{99m}Tc-nanoalbumin is used to detect sentinel lymph nodes in regard with breast cancer diagnosis. ^{99m}Tc-MAA was more popular recently to be used as lungs perfusion imaging using SPECT (Single Photon Emission Tomography) [5-7]. The use of some ^{99m}Tc radiopharmaceuticals have been expanded along with the development of nuclear medicine in the world, such as ^{99m}Tc-MAA and ^{99m}Tc-TSC which were previously used for lungs and liver imaging respectively now is also used to study the speed of gastric emptying [4-11].

Both MAA and MA kits have the same advantage in regard with its biocompatibility and biodegradability compared with non-albumin particulate kits, and MA kits is supposed to give better performance than MAA kits because of its uniformity in particle shape and size. As reported in some journals that the shape of particles in MA kit is microspheres with particle size of 25 µm, and the accepted particle size is within 10-50 µm, whereas MAA particles are irregular with size of 10-90 µm [12, 13]. The difference between particle shape of MAA and MA can be seen in Figure 1.

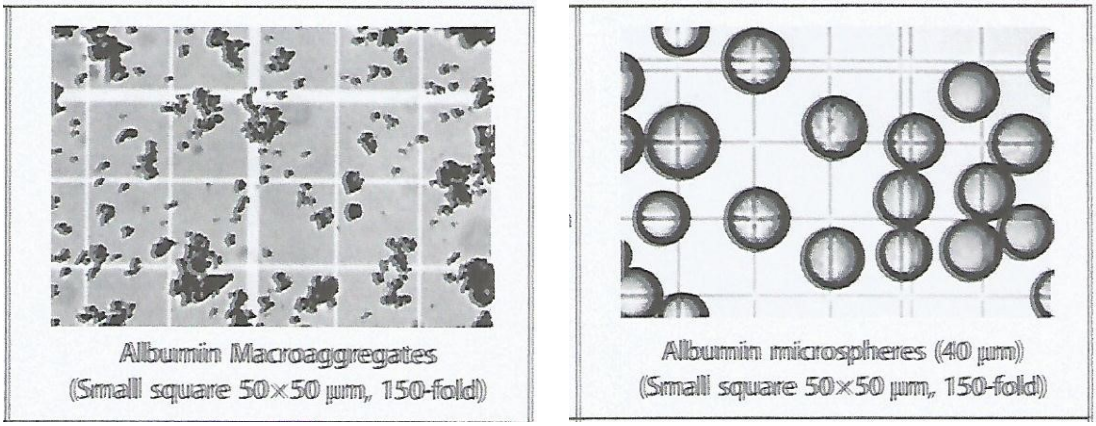


Figure 1. Particle shape of MAA (left) and MA (right) in hemocytometer, examined under microscope with the same multiplication [13]

Study of radiopharmaceuticals formulation and process validation is important since many factors are involved that can alter pharmacokinetic behavior such as variability of particle size within batches in particulate radiopharmaceuticals. Mostly when particle size is less than 5 µm the uptake in the lungs will be low and tends to accumulate in the liver, oppositely, when the size is more than 100µm it can possibly plug the artery. Therefore every possibility that can influence radiopharmaceutical biodistribution shoud be studied [14-16].

Research in albumin microspheres had been initiated in BATAN over ten years ago, but the purpose was to develop radiosynovectomy agent using beta emitting radionuclides, such as samarium-153 (153Sm) and rhenium-186/188 (186Re or 188Re). The results showed that albumin microspheres of expected size can be produced by heating and stirring albumin solution at certain temperature and speed [17], and in the last 2 years the study of MAA kit preparation has been carried out.

The aim of this research is to study the critical steps in the kit manufacturing with the goal of obtaining validated protocol of albumin microspheres kits preparation as a part of development project of albumin based radiopharmaceuticals for lungs imaging as an alternative of MAA kits which is previously available in the market.

2. METHODS

2.1 Chemicals

Human serum albumin (HSA, Sigma-Aldrich), vegetable oil in this case olive oil was chosen, n-hexane (Merck), stannous chloride dihydrate (Sigma-Aldrich), HCl 32N (Merck), sodium diphosphate decahydrate (Merck), polysorbate-80 (Merck), methanol (Merck), water for injection (IPHA), saline (sodium chloride solution for injection, IPHA), nitrogen gas (medical grade), and pertechnetate 99mTc solution.

2.2 Procedures

Steps of work comprise preparation of albumin microspheres, preparation of albumin microsphere kits, labeling kits with ^{99m}Tc, analysis of radiochemical purity and measuring particle size [18].

Albumin powder was disolved in 1 ml of saline to get 20% b/v albumin solution and then added with 100 ml of olive oil. The mixture was stirred for 30 min at 750 rpm, then heated gradually to 140°-160°C to disperse. Suspension was filtered through Whatman-1 paper, the precipitates was washed 3 times with n-hexane. Albumin microspheres obtained was dried and weighed and the particle size was measured under microscope provided with haemocytometer. The particles size should meet the requirement of 5-90 µm.

Preparation of albumin microspheres kits was done by adding a fresh solution of 5 mg/ml stannous chloride in HCl to an aliquot of sodium diphosphate (60 mg/ml) and pH was adjusted to 2. Solution of Sn-pyrophosphate (stannous diphosphate) was added into a vial containing 50 mg of albumin microspheres (MA), stirred in 10 min and then heated in waterbath for 15 min. The mixture was filtered with 5 µm filter (Millipore) and the microspheres was rinsed with Tween-80 (polysorbate-80) and then added with 5 ml of water. The mixture was purged with nitrogen gas during process. The mixture was dispensed 0.5 ml each into vials with rubber stopper and sealed with Al-seal and stored in deep freezer (New Brunswick Scientific).

Three experiments were designed, i.e P1, P2 and P3. Variation was carried out by disolving stannous chloride in concentrated HCl and then diluted with water (P1) compared with ones which was disolved directly in 1N HCl (P2 and P3). Another variation was undergoing filtration (P1 and P2) compared with ones without filtration step (P3) as can be seen in Table 1.

Table 1. Design of experiments

Treatment	P1	P2	P3
$SnCl_2$ was dissolved in conc. HCl, followed by dilution with water	Yes	NA	NA
$SnCl_2$ was dissolved directly in diluted HCl	NA	Yes	Yes
Filtration through 5 µm pore size filter	Yes	Yes	NA

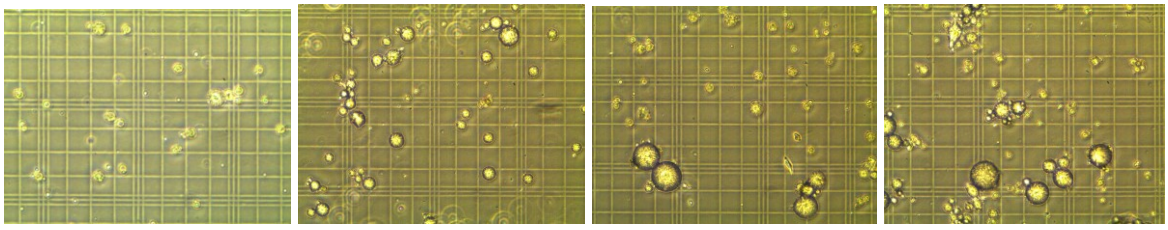
Labeling the kits with ^{99m}Tc was done by adding 3-5 mCi of ^{99m}Tc into a vial containing MA kit, vortexed and allowed for 10 min. Analysis of radiochemical purity of ^{99m}Tc-albumin microspheres was done by spotting small volume of sample onto a strip of Whatman-1 paper sized 1 x 15 cm and eluted in 2 chromatography chambers containing methanol 85% and saline respectively until each eluents reached 1-2 cm from strip edge. Strips were allowed to dry and measured the radioactivity using Single Channel Analyzer (SCA, Veenstra Instruments) [18, 19].

Prior to reconstitution with pertechnetate ^{99m}Tc solution, observation of particle size and shape was carried out using microscope provided with haemocytometer.

Stability test was carried out by labeling the kits with ^{99m}Tc and measuring its radiochemical purity. The sample was kits stored in deep freezer for several months, and kits which have been labeled with ^{99m}Tc and stored in room temperature within several hours.

3. RESULTS AND DISCUSSION

Preparation of albumin microspheres from 3 batches resulted microsphere particles size of 10-25µm in average (Figure 2a), it conforms the requirement that more than 90% of the particles should be spheres and have diameter in range of 5-90 µm and none is bigger than 100 µm [18]. The preparation of albumin microspheres kits, either through filtering (P1 and P2) or without filtering (P3), and whether using concentrated HCl (P1) or 1N HCl (P2) to dissolve the tin chloride did not affect the integrity of the particles (Figure 2b-2d).

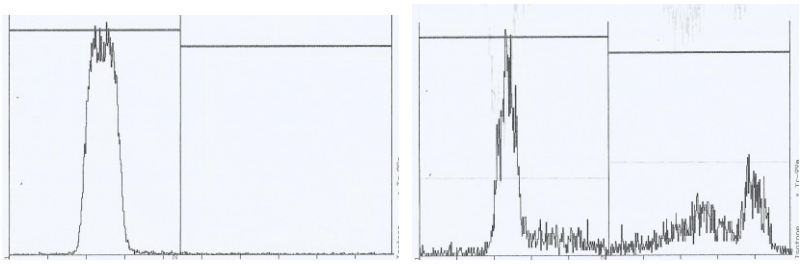


(A) (B) (C) (D)

Figure 2. Albumin microspheres before processed (a) and albumin microspheres after processed as kits (b-d). Albumin microspheres particles of experiment using concentrated HCl and filtering process (b), using 1N HCl with filtering process (c) and using 1N HCl without filtering process (d).

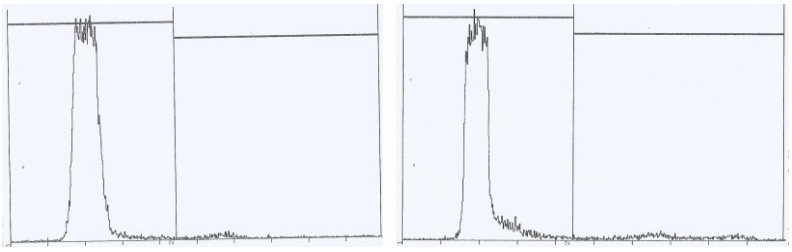
The main parameter of quality is radiochemical purity of ^{99m}Tc-MA as the main product, in which the radiochemical purity must exceed 90% as a requirement for ^{99m}Tc radiopharmaceutical product in general [13, 18]. Analysis of radiochemical purity using paper chromatography systems with methanol 85% as eluent showed single peak for P3 with radiochemical purity of 98%, and 2 peaks for P1 and P2 with radiochemical purity of 96% and 96.2% respectively [20]. When the eluent was replaced with saline the results differed significantly, which showed 2 peaks for most of the samples indicating that another impurity was detected. System using methanol 85% can not separate ^{99m}Tc-pyrophosphate as impurity from ^{99m}Tc-MA as main product (Figure 3a), whereas saline can separate ^{99m}Tc-pyrophosphate from ^{99m}Tc-MA although ^{99m}Tc-pyrophosphate and ^{99m}Tc-pertechnetate as main impurities were close one to each other (Figure 3 b). Overall results of radiochemical purity were higher when analysed using methanol 85% as eluent rather than using saline as eluent (Figure 5 a-b). It indicated that saline is better than methanol 85% to be used in this experiment, because it can separate impurities i.e ^{99m}Tc pertechnetate and ^{99m}Tc-pyrophosphate from ^{99m}Tc-microsphere albumin as the main product.

The experiment using 1N HCl and with filtering process (P2) showed single peak for both eluent systems, indicated that the impurities was relatively low (Figure 4 a-b). Radiochemical purity of P1 and P2 were less than 90% as required, it was likely caused by filtering step which time consuming that led to possibility of contact with air as a source of oxidants.



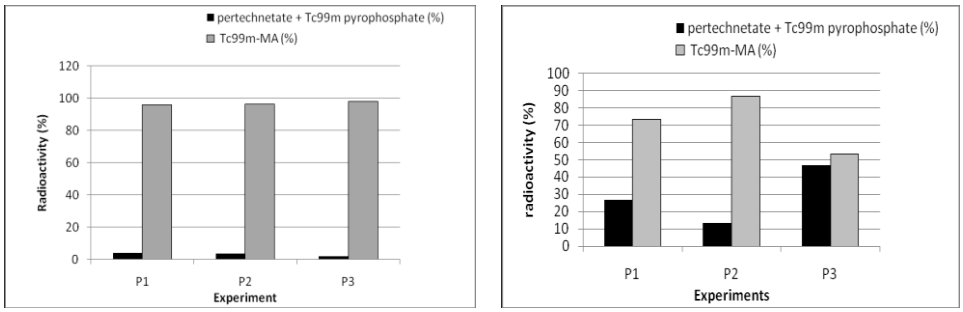
(a) (b)

Figure 3. Radiochromatogram of the same sample using methanol 85% (Figure a) and saline (Figure b)



(a) (b)

Figure 4. Radiochromatogram of the experiment using 1N HCl and with filtering process from the system with methanol 85% (Figure a) and saline (Figure b)



(a) **(b)**

Figure 5. Radiochemical purity of 99mTc-MA using methanol 85% as eluent (Figure a) and saline as eluent (Figure b)

The experiments using concentrated HCl and diluted HCl in the dissolution process of SnCl2 produce a radiochemical purity of 73.5% and 86.8% respectively (Figure 5b, P1 and P2), indicated that the use of HCl 1 N was better than that of concentrated HCl to dissolve $SnCl_2$ which resulted in higher radiochemical purity [11]. The experiment without filtering process showed poor labeling yield of only ~ 50%, it indicated that ^{99m}Tc-pyrophosphate as impurity was still existed in the mixture, and it can be observed when using saline as eluent of radiochromatography system. Methanol 85% which is used as eluent for analysing radiochemical purity of various albumin-based particulate radiopharmaceuticals [18] can only be applied if the filtering process through 5 μm filter is undertaken so there has been no more solute impurity inside the kits.

Stability test conducted on the experiment of selected protocol included stability during storage at -80°C within several months and stability over time after being labeled with ^{99m}Tc, which showed that the kits stored for 4 months still demonstrated high labeling yield whereas after being labeled can stand only less than 2 hours. Kit that has been labeled with ^{99m}Tc showed a significant decrease in radiochemical purity, i.e from 81.5% initially, declined to 65.1% and 57.6% respectively after 2 hours and 5 hours.

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4. CONCLUSIONS

Protocol of albumin microspheres preparation has been obtained both for preparing the microspheres and the kits. Preparation of the kits is better using diluted hydrochloric acid as a solvent for stannous chloride, and filtering process is important step to separate impurity specifically ^{99m}Tc-pyrophosphate from the main product. The kits after stored in -80°C can withstand its stability within several months, but its stability after being labeled is low.

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Synthesis and Characterisation of AuNPs-PAMAM G4- (^{131}I) Nimotuzumab

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Abstract - In our preliminary study, preclinical trials of the targeted CT Scan contrast agent AuNPs-PAMAM G4-Nimotuzumab which consist of biodistribution, clearance and autoradiography has been done by simulation using radioactive compounds ^{198}AuNP-PAMAM G4-nimotuzumab. Biodistribution and clearance of ^{198}AuNPs-PAMAM G4-Nimotuzumab showed high accumulation of radioactivity in the liver, spleen and kidney. To explore the possibility of the release of gold nanoparticles (AuNPs) from the cavity of PAMAM G4, in this study we have been labeling AuNPs-PAMAM G4-Nimotuzumab by using ^{131}I radionuclide. The biological tests (biodistribution) of the compound AuNP-PAMAM G4-(^{131}I) Nimotuzumab were performed in normal mice at the certain interval time. Au-NPs-PAMAM G4-(^{131}I) nimotuzumab was synthesized by reacted AuNPs-PAMAM G4-Nimotuzumab with ^{131}I radionuclide using iodogen method, followed by purification of the complex of Au-NPs-PAMAM G4-(^{131}I) nimotuzumab using Sephadex G25 (PD 10) column. The radiochemical purity of Au-NPs-PAMAM G4-(^{131}I) nimotuzumab was analised by paper chromatography using metanol 75 % as mobile phase and whatman I as stationary phase. The biodistribution studies of the AuNPs-PAMAM G4-(^{131}I)nimotuzumab was carried out using normal mice. Blood, kidney, spleen and liver were collected, weighed, and the radioactivity was measured in a Gammatec II gamma counter. The radiochemical purity of Au-NPs-PAMAM G4-(^{131}I)nimotuzumab before and after purification were 88.22±0.55 % and 96.06±0.75% respectively. Biodistribution of AuNP-PAMAM G4-(^{131}I) Nimotuzumab (% ID/gram organ) in blood, kidney, liver and spleen at 2 hours,24 hours, 48 hours and 96 hours after injection have a similar pattern compared with the ^{198}AuNPs-PAMAM G4 –nimotuzumab compound but its radioactivity was lower. Significant radioactivity in liver and spleen was due to the native of antibodies and or PAMAM dendrimer which is excreted through the reticuloendothelial organs.

Keywords: *gold nanoparticles, CT Contrast Agent, radiochemical purity, Au-NPs-PAMAM G4-(^{131}I)nimotuzumab, Sephadex G25 (PD 10) column.*

1. INTRODUCTION

CT-Scan is an imaging modality that is widely used in many hospitals in Indonesia. This instrument is used to perform imaging on a physiological level of organs or tissues. The use of contrast agents is one option to improve the imaging quality by means of CT Scan. To increase the quality of this modality from physiological level to molecular imaging level on detecting cancer more accurately, the synthesis of AuNPs-PAMAM G4-nimotuzumab compounds as a targeted CT Scan contrast agent has been done which will be used for early detection of lung cancer [1-3].

In our preliminary study, preclinical trials of the targeted CT Scan contrast agent AuNPs-PAMAM G4-nimotuzumab which consist of biodistribution, clearance and autoradiography has been done by simulation using radioactive compounds ^{198}AuNP-PAMAM G4-nimotuzumab [4-7]. The biological tests (biodistribution) of the compound ^{198}AuNP-PAMAM G4-nimotuzumab resulted the accumulation of ^{198}Au radioactivity in the liver, spleen and kidneys. Accumulation in the kidneys is due to the compounds excreted by the kidney while the accumulation in the liver and spleen can be caused by several factors such as lipophilicity, particulate form of the compounds or the aggregation of ^{198}Au particles released from the cavity of PAMAM [8-11].

To investigate the largest possible causes of accumulated ^{198}AuNP-PAMAM G4-nimotuzumab compounds in the liver and spleen, in this study we have been labeling AuNPs-PAMAM G4-Nimotuzumab by using ^{131}I radionuclide [12-14]. Purification of Au-NPs-PAMAM G4-(^{131}I)nimotuzumab compound was done using Sephadex G25 (PD 10) column. Radiochemical purity of this compound was analysed by paper chromatography using methanol 75% as mobile phase and whatman I paper as stationary phase. On this compound, biodistribution test will be performed compared with ^{198}AuNP-PAMAM G4-nimotuzumab compounds and ^{131}I-

nimotuzumab. Point the biological observations in this study mainly focused on some organs such as blood, kidneys, liver and spleen.

2. METHODS

2.1. Chemicals

Materials used in this study included AuNPs-PAMAM G4-nimotuzumab compound and ^{131}I radionuclide which was produced by PTRR BATAN. Saline, distilled water were obtained from IPHA. PD-10 column (Sephadex G25 (PD 10)) were obtained from Pharmacia. A mice of 25 – 35 g weight was used for biodistribution study. Ethylene glycol, $NaIO_4$, ammonium acetate, glacial acetic acid, acetic acid and NaH_2PO_4 (E.Merck) were obtained from Merck. Aquabidest sterile was obtained from PT. IKA, Indonesia. Equipment used in this study were centrifuge, magnetic stirrer, pH meter, analytical balance, micropipette, protein filter 30 kilo dalton molecular weight cut off (30 KD MWCO) and syringes.

2.2. Procedures

The compound of AuNPs-PAMAM G4 –(^{131}I)nimotuzumab solution was made by reacting AuNPs-PAMAM G4-nimotuzumab with ^{131}I radionuclide using iodogen method. Purification of the complex Au-NPs-PAMAM G4-(^{131}I)nimotuzumab was done by using Sephadex G25 (PD 10) column. Radiochemical purity of Au-NPs-PAMAM G4-(131I)nimotuzumab before and after purification using Sephadex G25 (PD 10) column were analysed by paper chromatography using methanol 75% as mobile phase and whatman I as stationary phase. Biodistribution patterns of Au-NPs-PAMAM G4-(^{131}I)nimotuzumab were studied in normal mice at 2 h, 24 h, 48 h and 96 h after injection. Normal mice weighing ~ 30 g were *intra vena* administered with 0.1 ml (~ 100 µCi) of Au-NPs-PAMAM G4-(^{131}I)nimotuzumab via tail vein of the mice. The mice were sacrificed and blood, kidney, lung, spleen, liver were collected and weighed. Radioactivity of organs was measured by gamma counter and percentage of radioactivity per gram (% ID/g) was determined.

3. RESULTS AND DISCUSSION

Labeling of Au-NPs-PAMAM G4-nimotuzumab with ^{131}I radionuclide has been done using iodogen method. Purification by sephadex G25 (PD 10) column was performed to separate the conjugate of AuNPs-PAMAM G4 –(^{131}I) nimotuzumab from free ^{131}I as impurity.

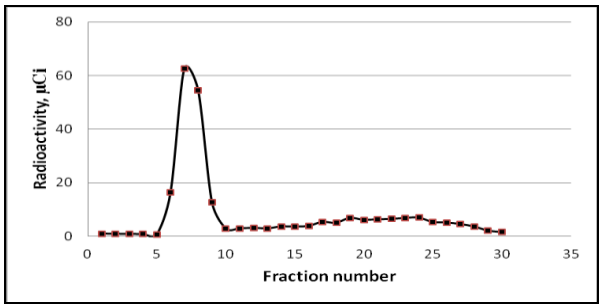


Figure 1. Elution pattern of AuNPs-PAMAM G4 –(^{131}I) nimotuzumab on sephadex G25 (PD 10) column .

As shown in Figure 1, elution pattern of AuNPs-PAMAM G4 –(^{131}I) nimotuzumab on sephadex G25 (PD 10) column indicated that more than 65% of radioactivity came from AuNPs-PAMAM G4 –(^{131}I) nimotuzumab. Fraction number 6, 7, 8 and 9 were combined and radiochemical purity was determined as % radiochemical purity of the compound after purification (Figure 2C).

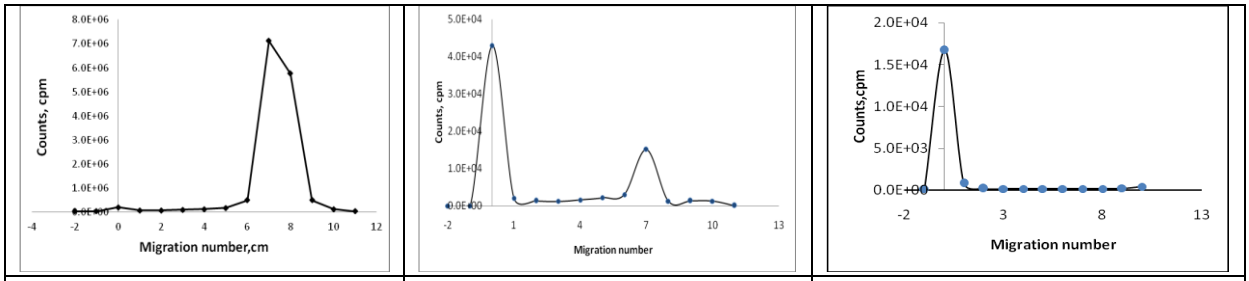


Figure 2. Radiochemical purity of (A) ^{131}I solution; (B) AuNPs-PAMAM G4 –(^{131}I) nimotuzumab before purification and ; (C) AuNPs-PAMAM G4 –(^{131}I) nimotuzumab after purification.

Radiochemical purity of the compound AuNPs-PAMAM G4 –(^{131}I) nimotuzumab before purification was also analysed the same way (Figure 2B), as well as radionuclidic ^{131}I as standard for determining the radiochemical impurity (Figure 2A). Radiochemical purity of the compound AuNPs-PAMAM G4 –(^{131}I) nimotuzumab before and after purification using sephadex G25 (PD 10) column were 88.22±0.55% and 96.06±0.6% respectively.

Biodistribution in normal mice was performed with the aim to evaluate the accumulation of AuNPs-PAMAM G4 –(^{131}I) nimotuzumab compound in organs/tissues. According to the biodistribution data of ^{198}AuNP-PAMAM G4-Nimotuzumab previously, significant radioactivity of ^{198}Au was also found in the liver and spleen [10]. The biodistribution pattern of AuNPs-PAMAM G4 –(^{131}I) nimotuzumab and ^{198}AuNP-PAMAM G4-Nimotuzumab (%ID/g) in normal mice are shown in Fig. 3.

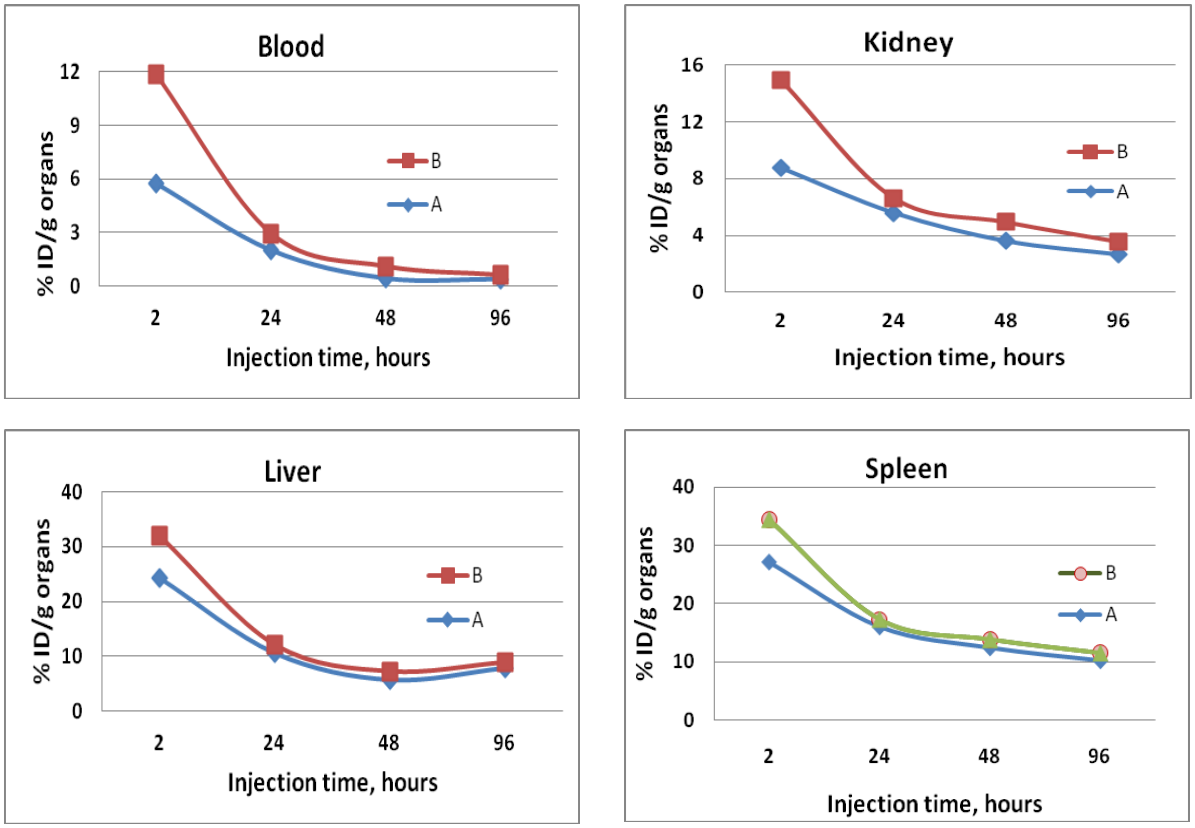
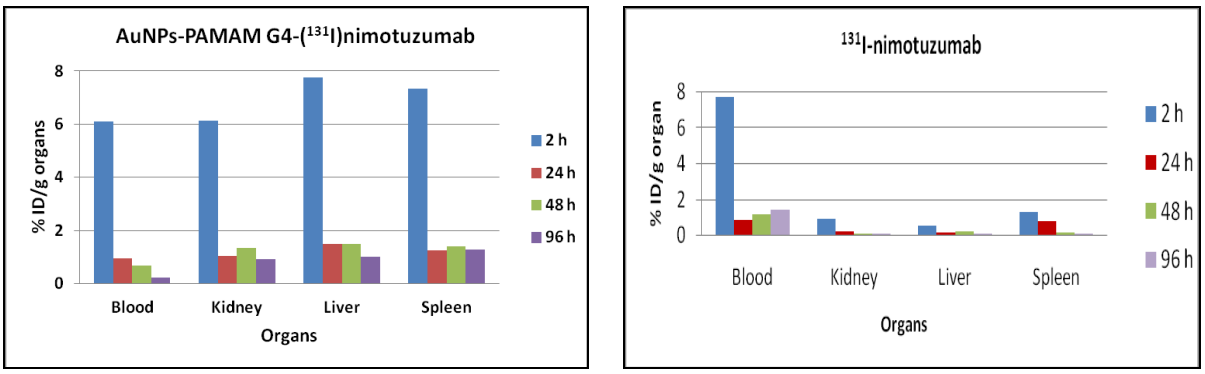


Figure 3. Biodistribution pattern of ^{198}AuNP-PAMAM G4-Nimotuzumab [10] (A) and AuNPs-PAMAM G4 –(^{131}I) nimotuzumab (B) in blood, kidney, liver and spleen at 2 h, 24 h, 48 h and 96 h after injection.

Observation of the biodistribution pattern of ^{198}AuNP-PAMAM G4-Nimotuzumab and AuNP-PAMAM G4-(^{131}I) Nimotuzumab in blood, kidney, liver and spleen has been done at 2 hours, 24 hours, 48 hours and 96 hours after injection. Accumulation of ^{198}AuNP-PAMAM G4-Nimotuzumab and AuNP-PAMAM G4-(^{131}I) Nimotuzumab from 2 hours till 96 hours post injection in blood were indicated similar pattern. Biodistribution pattern of both (% ID/gram organ) in kidney showed that in the kidneys 2 hours,24 hours and 96 hours after injection, radioactivity of the ^{198}AuNPs-PAMAM G4 –nimotuzumab compound was higher compared with AuNP-PAMAM G4-(^{131}I) Nimotuzumab; it means that excretion of AuNP-PAMAM G4-(^{131}I) Nimotuzumab was faster than that of ^{198}AuNPs-PAMAM G4 –nimotuzumab. Biodistribution pattern of both compounds in liver showed that radioactive accumulation of the compound ^{198}AuNP-PAMAM G4-Nimotuzumab in liver was higher than of the AuNP-PAMAM G4-(^{131}I) Nimotuzumab compound. It was concluded that significant uptake in the liver and spleen of ^{198}AuNP-PAMAM G4-Nimotuzumab [10] was not caused by the release of a AuNPs from cavity of dendrimer. The other possibility of the high radioactivity in a liver may be caused by the release of ^{131}I-nimotuzumab from complex AuNPs-PAMAM G4-nimotuzumab.

Figure 4 showed the results of the biodistribution test of AuNPs-PAMAM G4- (^{131}I) nimotuzumab at 2 hours, 24 hours, 48 hours and 96 hours after injection compared with ^{131}I-nimotuzumab.



a b

Figure 4. Biodistribution pattern of AuNPs-PAMAM G4 –(^{131}I) nimotuzumab (a) and ^{131}I- Nimotuzumab (b) in blood, kidney, liver and spleen at 2 h, 24 h, 48 h and 96 h after injection.

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From the biodistribution pattern of AuNPs-PAMAM G4 –(^{131}I) nimotuzumab and ^{131}I- Nimotuzumab, 2 hours after injection high radioactivities in blood, kidney, liver and spleen was found in both of compounds. On 24 h, 48 h and 96 h after injection radioactivity of all four organs in AuNPs-PAMAM G4-(^{131}I) nimotuzumab were greater than that of ^{131}-I-nimotuzumab. It conformed with previous studied, biodistribution test on PAMAM-based compounds was carried out by Opina, Wong, Griffiths et al. [11] by using the ^{153}Gd-DOTA-PAMAM G5 and the results showed that accumulation in the liver at 24 hours, 7 days and 30 days after injection were 32.3 ± 3.0%, 31.41 ± 0.91 and 20,10 ± 2,51% respectively from injected dose. Victoria Calzada et al., also reported that uptake of ^{177}Lu-DOTA-nimotuzumab in CD-1 mice showed high level radioactivity in liver and spleen at 1 h, 4 h and 24 h [15]. Significant radioactivity has been found in liver, this was due to the native of antibodies which is cleared through the reticuloendothelial organs and EGFR was also present in liver [14].

4. CONCLUSIONS

Biodistribution test result of AuNPs-PAMAM G4 –(^{131}I) nimotuzumab compared with ^{198}AuNPs-PAMAM G4-nimotuzumab and ^{131}I-Nimotuzumab indicated that a significant uptake in the liver and spleen was not caused by the release of AuNPs from the cavity of PAMAM G4 dendrimer or from the release of ^{131}I- Nimotuzumab but due to the native of antibodies and or PAMAM dendrimer which is cleared through the reticuloendothelial organs.

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Effect of a Turmeric (*Curcuma longa*) Decoctum on Vascular Endothelial Growth Factor (VEGF) and Vascular Endothelial Cadherin (VE-cadherin) Expression in the Chick Embryo

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Abstract – Turmeric (*Curcuma longa*) is a medicinal plant that is widely consumed by the public, including pregnant women. The absence of dose standardization and the regulation of turmeric consumption in pregnancy raises concerns, especially if taken in early pregnancy. Angiogenesis is the process of new blood vessel formation from previously existing blood vessels, and plays an important role in embryogenesis and placentation during pregnancy. Angiogenesis is regulated by angiogenic molecules such as vascular endothelial growth factor (VEGF) and vascular endothelial cadherin (VE-cadherin). Turmeric has antiangiogenic effects in which the crude extract is pharmacologically more potent compared to the pure curcumin form. This study aimed to determine whether a turmeric decoctum affects the expression of VEGF and VE-cadherin in chick embryos. Turmeric was extracted by the decoction and freeze drying methods to obtain a turmeric decoctum powder. This was tested on embryonated chicken eggs which were divided into four groups; control group (2% DMSO) and treatment groups receiving various doses of the turmeric decoctum (200 ppm, 300 ppm and 400 ppm). The eggs used were less than 7 days after oviposition and incubated for 16 hours prior to injection of the treatment solution *in ovo* to the center of the yolk, followed by reincubation for up to 56 hours. Intraembryonic VEGF and VE-cadherin expression was assessed by whole-mount immunohistochemistry and quantified using image analysis techniques. This study showed no significant difference in VEGF and VE-cadherin expression post turmeric decoctum administration. Administration of a turmeric decoctum up to 400 ppm had no effect on the expression of VEGF and VE-cadherin in chick embryos.

Keywords: Chick, embryo, Turmeric, VE-cadherin, VEGF

1. INTRODUCTION

Turmeric (*Curcuma longa*) is a medicinal plant with multiple uses [1]. Based on surveys, it is known that the users of herbal medicines are mostly women, and some of them are pregnant [2, 3, 4]. Limited information about dose standardization and regulations regarding the consumption of herbal medicines during pregnancy have gained significant attention in global health debates [5,6]. In Indonesia, traditional herbal medicines, known by the term "*jamu*", are made from many kinds of medicinal plants, including turmeric [7]. Turmeric preparations are usually made using the decoction method, in which the turmeric is thinly sliced, placed in boiling water and allowed to boil for a period of time, after which it is ready for consumption [8].

Turmeric is a plant that is belonging to the Zingiberaceae family [9]. There is quantitative variation in each component of turmeric compounds depending on the variety, origin, planting site, conditions during growth and the age at harvest [10]. Turmeric is known to have antiangiogenic effects through one of its active compounds, curcumin [9]. Several studies have shown significant effects of curcumin on vascularization and embryo development [11]. Other studies have shown that the crude extract of turmeric has a greater antiangiogenic effect compared to the pure curcumin form [12].

Angiogenesis is the process of blood vessel formation from pre-existing blood vessels [13]. Angiogenesis is regulated by angiogenic molecules such as vascular endothelial growth factor (VEGF) and vascular endothelial cadherin (VE-cadherin) [14]. The binding of VEGF to its receptor plays a critical role in vascular permeability, the migration and proliferation of endothelial cells, tube formation, and vascular lumen development [15]. VE-

cadherin as an adherens junction protein, plays a role in maintaining polarity between endothelial cells and stabilizing blood vessels [16]. Abnormalities in angiogenesis could potentially cause a disruption in vascularization during embryonic growth and placentation and could even cause embryonic death [14].

Angiogenesis in the embryo can be studied using chick embryos [14]. Chick embryos (Gallus gallus) are applicable as a model for studies of biological growth, embryology and teratology that precisely target specific developmental stages in the absence of maternal metabolism [17]. After 48-56 hours of incubation, the chick embryo is comparable to 24 days of human gestation[18,19]. This study aimed to determine whether a decoctum of turmeric affects VEGF and VE-cadherin expression in chick embryos.

2. MATERIALS AND METHODS

2.1 Animals

Fertile chicken eggs were purchased from the avian breeder group "Lestari Sejahtera" in Mojokerto, East Java, Indonesia. The eggs were stored in coolers set at approximately 15 to 20°C for fewer than 7 days after oviposition and incubated at 37.5°-39.5°C up to stage 3+ according to the criteria of Hamburger and Hamilton (1951), or 16 h of incubation [17, 20, 21, 22].

2.2 Herbal preparation

Fresh turmeric rhizomes were purchased from UPT Materia Medica Batu, Health Department of East Java, Indonesia. An aqueous extract was prepared using the decoction method. A total of 500 g of turmeric rhizomes were thinly sliced, then placed in 2,500 mL of boiling distilled water and left to simmer for 15 minutes [23] The solution was allowed to cool, filtered through sterile muslin cloth and stored at -20°C. A freeze-dried extract was obtained from the solution to produce a turmeric decoctum powder, which was stored at 4°C [24].

2.3 Phytochemical detection

Phytochemical detection was performed by a qualitative phytochemical test, thin layer chromatography (TLC) [25]. TLC was conducted to test for both curcuminoid and terpenoid compounds in the turmeric decoctum using a standard protocol from the Pharmaceutical Laboratory, Pharmacy Study Program, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia.

2.4 Treatment solutions

A stock solution (500 ppm) was made by dissolving 500 mg of turmeric decoctum powder into 2% DMSO. The stock solution was used to prepare treatment solutions for at various doses, i.e. 200 ppm (P1), 300 ppm (P2) and 400 ppm (P3). The control group received 2% DMSO without the turmeric decoctum powder.

2.5 Administration of treatment solutions on chick embryo

Treatment solutions (200 μL) were injected into the center of the egg yolks after 16 h of incubation. Eggs were placed horizontally with respect to the long axis, and a small hole was made in the blunt end of each egg with the use of a probe. A 1-mL disposable syringe (23 gauge) was inserted horizontally and treatment solutions were delivered into the center of each egg yolk. After injection, the hole was sealed with vinyl tape and the egg was turned 180° and reincubated until 56 h of incubation [17]

2.6 Collecting Data

Each egg was cracked into a petri dish. The square frame of thick filter paper (Whatman 3MM) with external dimensions of about 1.4 x 1.4 cm and with an internal window of about 1 cm^2 was laid down onto the surface of the egg yolk such that the embryo was in the center of the window, and the filter paper was allowed to become wet. The vitelline membrane outside of the frame was cut with spring scissors and lifted gently using forceps, then washed in a dish of 0.9% NaCl solution to remove adherent yolk. The embryo was fixed in 4% neutral paraformaldehyde solution and stored at 4°C [21]. The whole mount immunohistochemistry technique was used to detect VEGF expression and VE-cadherin expression in intraembryonic endothelial cells of the chick embryo using anti-VEGF antibody and anti-VE-cadherin antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Samples were observed under a stereomicroscope with a magnification of 20 x and images were captured by a Panasonic Lumix DMC-GH2 digital camera. The expression of VEGF and VE-cadherin was defined as mean density and was quantified using ImageJ software[26].

2.7 Ethical approval

All materials and methods for the experiments included in this study were approved by the Ethical Committee of the Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia (262/EC/KEPK-S2/06/2016).

2.8 Statistical analysis

Data are presented as mean±SD. Statistical analysis was performed using the IBM SPSS (version 24) statistical package. The relationships between turmeric decoctum and VEGF expression or VE-cadherin expression were assessed using Pearson correlations. The comparisons between VEGF expression or VE-cadherin expression in various treatment groups were analyzed using one-way analysis of variance (ANOVA). A p-value of less than 0.05 was considered to indicate statistical significance for the correlation and comparison.

3. RESULTS

3.1 Phytochemical detection

TLC identified curcuminoids in the turmeric decoctum, while terpenoids were not detected or were present in very small amounts.

3.2 Effect of the turmeric decoctum on VEGF and VE-cadherin expression

The results of the whole mount immunohistochemistry assay of VEGF expression are shown Figure 1 and those for VE-cadherin expression are depicted in Figure 2. Statistically, turmeric decoctum had no effect on VEGF or VE-cadherin expression in chick embryos (Table 1). Descriptively, our results show that all doses of the turmeric decoctum caused a decrease in VEGF or VE-cadherin expression. At the turmeric decoctum dose of 200 ppm, VEGF expression decreased, but VEGF expression tended to increase compared with the control group at the doses of 300 ppm and 400 ppm, as depicted in Figure 3. VE-cadherin expression increased with a turmeric decoctum dose of 200 ppm and decreased with turmeric decoctum doses of 300 and 400 ppm, as shown in Figure 3

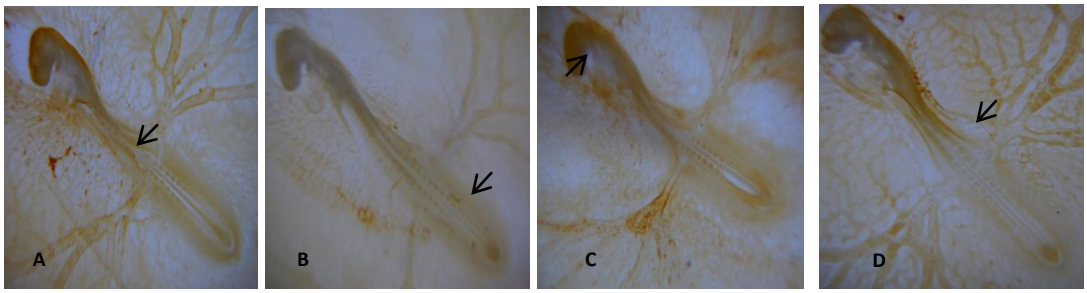

Figure 1. Whole mount immunohistochemistry using a specific antibody recognizing VEGF. VEGF expression in chick embryos (curved arrows) exposed to (A) 2% DMSO, (B) the turmeric decoctum at 200 ppm, (C) the turmeric decoctum at 300 ppm and (D) the turmeric decoctum at 400 ppm (whole mount IHC: A-D, 20x).

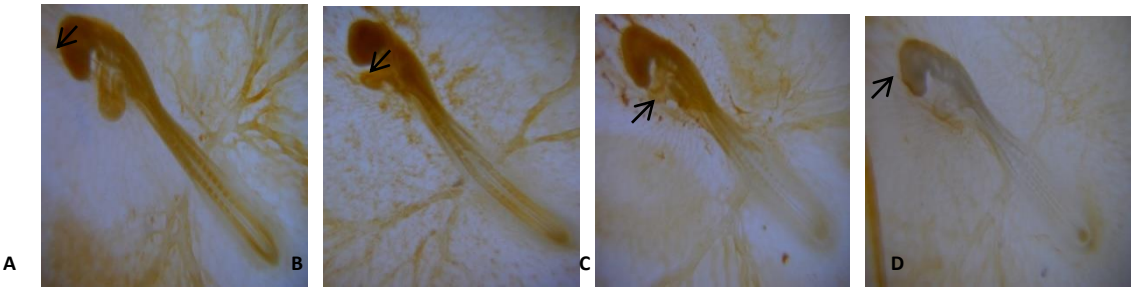

Figure 2. Whole mount immunohistochemistry using a specific antibody against VE-cadherin. VE-cadherin expression in chick embryos (curved arrows) exposed to (A) 2% DMSO, (B) the turmeric decoctum at 200 ppm, (C) the turmeric decoctum at 300 ppm and (D) the turmeric decoctum at 400 ppm (whole mount IHC: A-D, 20x).

Table 1. Number of VEGF and VE-cadherin expression of view of chick embryo specimens from the studied groups

Variable	Control 2% DMSO	Turmeric decoctum 200 ppm	Turmeric decoctum 300 ppm	Turmeric decoctum 400 ppm	*P-value*	
					Pearson Correlations	*ANOVA*
VEGF expression	161.6±8.7	151.3±9.6	153.8±5.3	155.9±10.9	0.403	0.254
VE-cadherin expression	161.3±9.5	172.7±21.8	164.5±13.1	150.5 ±13.8	0.177	0.120

Data is represented as mean ±SD. DMSO: Dimethyl sulfoxide; ppm: part per million. Significant $p<0.05$ for Pearson Correlation and ANOVA

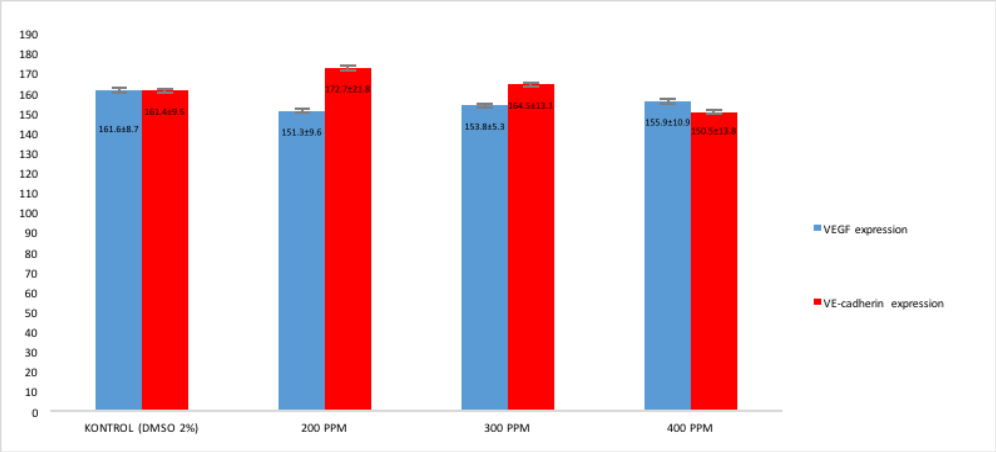

Figure 3. The trend of VEGF and VE-cadherin expression from the studied groups.

The graph represents the average number of VEGF and VE-cadherin expression in each group. At the treatment of turmeric decoctum dose of 200 ppm, VEGF expression decrease, but VEGF expression tends to rise toward the control group at the dose of 300 ppm and 400 ppm. VE-cadherin expression increase in turmeric decoctum dose of 200 ppm group and a decrease in 300 and 400 ppm.

4. DISCUSSION

Turmeric has antiangiogenic activity. The active curcuminoid compound, curcumin, is known to downregulate HIF-1α and interrupt VEGF gene transcription, resulting in a decrease in VEGF expression [27]. The results of this study show that VEGF expression was lower in all the turmeric decoctum treatment groups than in the control group. A decrease in VEGF expression is thought to restrain the migration and proliferation of endothelial cells, interrupt the formation of tubes and the vascular lumen, and disrupt vascular permeability, resulting in vascularization defects that interfere with embryo organogenesis [15]. However, the decreased expression of VEGF in the treatment groups was not statistically significant, so these results need to be strengthened with data on macroscopic extraembryonic vascularization (not done).

Figure 3 shows that, after treatment with the turmeric decoctum at a dose of 200 ppm, VEGF expression decreases, but expression tended to rise versus the control group at the doses of 300 ppm and 400 ppm. This finding indicates the existence of additional effects of curcumin, the predominant active compound in turmeric. The angiogenic effect of curcumin depends on the dose [28]. With low-dose exposure (200 ppm), curcumin showed an antiangiogenic effect through the decreased expression of VEGF. However, at higher doses (doses of 300 ppm and 400 ppm), curcumin appeared to have other effects through a different pathway, and may have indirectly affected the expression of VEGF. It is known that the active compound of turmeric (curcumin) can affect various pathways [9]. In addition to being controlled by its transcription factor, VEGF expression levels are also indirectly influenced by transforming growth factor-β1 (TGF-β1), which can induce the transcription and expression of VEGF [29]. Curcumin is known to induce the increased expression of TGF-β1 [30]. Increased VEGF expression at the doses of 300 ppm and 400 ppm may be associated with a mechanism of action of curcumin via pathways that induce the expression of TGF-β1.

This study also showed that VE-cadherin expression in various groups formed a non-monotonic curve, i.e. there was an increase in VE-cadherin expression with a turmeric decoctum dose of 200 ppm and a decrease at the doses of 300 ppm and 400 ppm, as depicted in Figure 5. These results indicate the existence of a hormetic response. A hormetic response in a dose-response interaction may occur as a result of exposure to a substance that has stressor or potentially toxic effects [31]. Some studies have demonstrated a relationship between angiogenesis and antiangiogenic agents with a hormetic response [31]. Curcumin is known to induce a hormetic response at the cellular and molecular levels [32].

VE-cadherin (cadherin-5, CD144) is the adhesion protein component of adherens junctions between endothelial cells, and plays an important role in regulating adhesion between endothelial cells, endothelial cell polarity and blood vessel stability [16,33]. VE-cadherin expression is regulated by a number of transcription factors, including NF-κB [9] Exposure to an external stressor can trigger stress in a cell, which is characterized by an increased level of NF-κB as a defense mechanism to help the cell survive.[34] Based on the expression levels of VE-cadherin in this study, a low dose of the turmeric decoctum (200 ppm) may induce stress in endothelial cells, resulting in a defense response to the external stressor by increasing the level of NF-κB. With an increasing dose of the turmeric decoctum, this defense mechanism may no longer be able to compensate for the external stressor, resulting in decreased expression of VE-cadherin in the of 300 ppm and 400 ppm treatment groups.

The decreased expression of VE-cadherin at the 300 ppm and 400 ppm doses is in line with the results of a study by Aggarwal et al. [35] showing that the antiangiogenic effects of turmeric, through the active curcuminoid component curcumin, result in decreased regulation and expression of adhesion molecules through the downregulation of NF-κB [35]. Interference in VE-cadherin expression will result in the disruption of adhesion between endothelial cells and endothelial cell polarity and thus destabilize blood vessels. If this condition continues, it will lead to vascular defects and potentially cause embryonic death.

The differences in VEGF and VE-cadherin expression after treatment with the turmeric decoctum were not statistically significant. These results are not in line with other studies that showed a significant antiangiogenic effect of turmeric. Especially when compared to the pure form of curcumin, the antiangiogenic effects of turmeric become pharmacologically more potent when given in a crude form of turmeric extract [12]. The non-significant effect of turmeric decoctum treatment on VEGF and VE-cadherin expression in chick embryos is thought to be influenced by several factors, including the extraction method used and the dose of turmeric decoctum given. The antiangiogenic effects of turmeric are caused by some components of the active compounds contained within it. Approximately 235 compounds, including phenolic and terpenoid compounds, have been identified in various parts of the turmeric plant [12]. There are quantitative variations in each component of turmeric, depending on the variety, origin, planting site, conditions during growth and the age at harvest [10]. In addition, the extraction method and the solvent type also influence the composition and quality of the active compounds in turmeric that can be extracted [12].

The antiangiogenic effects of turmeric are mainly mediated by active curcuminoid compounds. Curcuminoids have been identified as the most prevalent components of turmeric. Various extraction techniques and solvents can affect the amount and composition of curcuminoids extracted. According to Li et al. [12], Soxhlet extraction with ethanol as the solvent provides excellent curcuminoid extraction, i.e. up to 27% (w/w), while hydrodistillation can extract only 2.1% (w/w) curcuminoids [12]. Curcuminoids are poorly soluble in water, but soluble in ethanol, methanol, acetone and DMSO [9,12] The limit of curcumin solubility in water is 0.29% (w/w) [36].

Other active compounds in turmeric which are thought to have antiangiogenic effects are essential oils (terpenoid compounds). When essential oils, especially the sesquiterpenoid group, work together with curcuminoid, this produces synergistic effects in bioactivity [12]. Aromatic turmerone (ar-turmerone) shows antiangiogenic effects through the inhibition of endothelial cell proliferation and motility, suppression of new blood vessel formation, and disruption of vascular tube formation, either in vitro or in vivo. Ar-turmerone is also able to increase curcumin transport into intestinal cells, such that curcumin absorption in the body is significantly increased [11].

It is known that the curcuminoid group is difficult to dissolve in water, and the terpenoid class (monoterpenoids and sesquiterpenoids) have similarly limited solubility in water; however, these compounds are soluble in ethanol [37]. The poor solubility of the active compounds of turmeric in water may have influenced the results of this study, particularly the absence of significant effects on VEGF and VE-cadherin expression in chick embryos following treatment with the turmeric decoctum.

This conclusion is supported by the TLC results on the freeze dried turmeric decoctum. The identified active compounds belonged to the curcuminoid group, while the essential oil compounds of the terpenoid group (monoterpenoids and sesquiterpenoids) were not detected or present in very small amounts. In relation to the active compound in turmeric that exerts the antiangiogenic effect, if it was extracted in a small amount through the decoction method, as suspected in this study, it is likely that the amount of that active compound was too small to have a significant effect on VEGF and VE-cadherin expression in chick embryos.

Based on the results of this study, there was no effect of the turmeric decoctum on VEGF and VE-cadherin expression in chick embryos. However, it has not yet been determined whether turmeric decoctum are safe to take during pregnancy.

5. CONCLUSIONS

This study shows that the administration of turmeric decoctum up to a dose of 400 ppm had no effect on the expression of VEGF and VE-cadherin in chicken embryos.

6. CONFLICT OF INTEREST

Authors declare no conflict of interest.

7. ACKNOWLEDGMENTS

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Correlation Test Computerized Aid Diagnose (CAD) for Thorax Radiography Children Patient in RSAB Harapan Kita with Markov Random Field Method for Detecting Abnormalities with Infection Trends

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Abstract – This study developed a correlation test Computer Aided Diagnosis (CAD) radiographic of children pulmonary using segmentation Markov Random Field (MRF) method to detect lung abnormalities with infection trends. MRF method searched abnormalities by value of the image pixel. MRF method used four variations, namely MRF without a filter, median filter+MRF, wiener filter+ MRF, and adapthisteq+MRF. ROC segmentation results wiener filter is relatively higher than without a filter. ROC wiener filter results show the value of accuracy 81.4%, sensitivity 82.0%, specificity 80.0%, precision 91.1% and overall error of 18.6%. While the ROC for unfiltered and filter others show lower than the value of ROC wiener filter. However, differences in ROC for any kind of success rate is not more than 5%, meaning that all four methods MRF can still be implemented. Abnormal lung pixel value with MRF method without filter, median filter+MRF, and adapthisteq+MRF same namely 205-255. Abnormal lung pixel values by the method of wiener filter+MRF is 197-255. Radiographic of children pulmonary can not definitively determine lung infections in children.

1. INTRODUCTION

This study developed Computer Aided Diagnosis (CAD) radiographic of children pulmonary using segmentation Markov Random Field (MRF) method to detect lung abnormalities with infection trends. Lung nodules are expected to be directly visible, sometimes covered by other complex lung tissue which is normal tissues. The separation between normal tissue with nodules become interesting to study by computer-based digital image processing. The success of such a separation will be easier to read, so it can be an additional input for radiologists in the detection automatically with computer, it called CAD (Computer Aided Diagnosis)[1]. With CAD readings will be easier for reading image made of pixels per pixel.

2. METHOD

This research was conducted by CR image of pediatric lung, 1-15 years old, from Rumah Sakit Anak dan Bunda Harapan Kita. The format of image was DICOM image "for presentation" to the inspection standards PA, AP (anteriorposterior) and supine. CR image was equipped with a physician evaluation results, normal and abnormal. There was 136 images used in the study. It was taken from November 2014 until January 2016. It was 20 normal images, 40 tuberculosis images, and 76 pneumonia images. From 136 images, 66 images was used as the model data (20 tuberculosis images and 46 tuberculosis images) and 70 images was used as the test data (20 normal images, 20 tuberculosis images, and 30 pneumonia images).

CAD program developed with MatLab software 2013. Some commands are used in the program include the active contour, filter, Fuzzy Clustering Means (FCM) segmentation and Markov Random Field segmentation. CAD development of children CR lung is divided into three phases: preparation, image segmentation, and evaluation. The preparation phase is done by grouping samples of lung CR images of children by disease and then proceed with normalization, and image cropping. Image segmentation phase is done by four methods of segmentation MRF without a filter, the median filter+MRF segmentation, wiener filter+MRF segmentation and adapthisteq+ MRF segmentation. Phase evaluation is performed by calculating the success rate is expressed by the ROC (Receiver Operating Characteristics). The following chart of the steps of this research work.

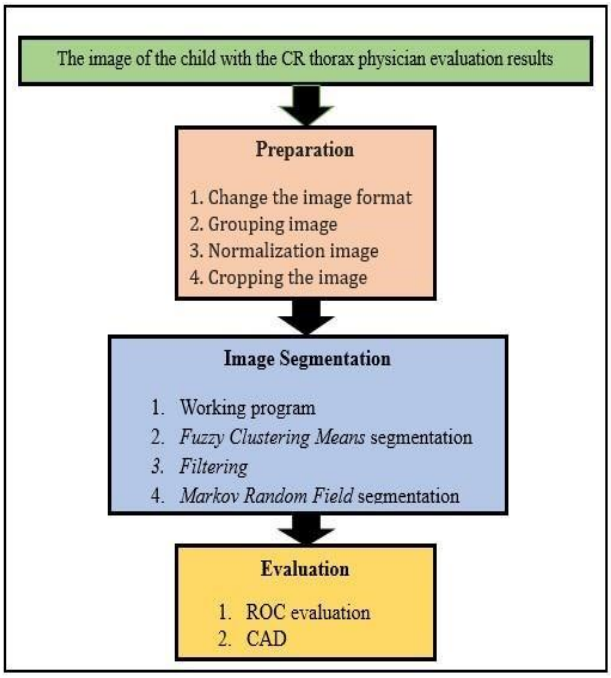


Figure 1. The chart of research steps

On the image segmentation phase, each image was processed by (1) MRF segmentation (without filter), (2) median filter+MRF segmentation, (3) wiener filter+MRF segmentation, and (4) adapthisteq+MRF segmentation. It was four outcomes to be compared. Furthermore, it was processed at the stage of initial (initial segmentation). Initial segmentation was done with the minimum point search method histogram and multilevel thresholding. The minimum point of the histogram used again to find the number of regions/clusters in the image and determine a threshold value, while multilevel thresholding aims to determine the initial cluster each pixel in the image. Thresholding process was the process of separating between the object and the background. In each image histogram is always contained two peaks (Lindquist, 1999). The first peak indicates an object, while the second peak indicates background. Therefore, to separate the two, the point of minimum (valley) histogram used as a threshold limit value (threshold).

Evaluation phase was done by calculating the ROC (Receiver Operating Characteristics) image segmentation methods against a reference image every expert evaluation. The reference image is physician evaluation results. ROC counts is the accuracy, sensitivity, specificity, precision, and overall error. ROC uncertainty calculation is repeated five times by crossing data/cross validation. Cross validation is useful to determine the stability of the method. If the program is already running as expected, the next program will be equipped with a GUI (Graphical User Interface) that will make the program more user friendly so that the program can be used easily by radiologists.

3. RESULTS AND DISCUSSION

The author was inspired by research Ralind Remarla to detect lung abnormalities image segmentation of adults using FCM and obtained good results. Furthermore, the use FCM segmentation is done with some variations clusters ie n = 4, n = 6, n = 8 and n = 10 (Remarla 2015a). In contrast to Ralind, in this study the number of clusters is done automatically, so there is no coercion number of clusters in the image. Determination of the number of clusters is done with the minimum point search method histogram automatically. The minimum point of the histogram is used to find the number of regions/clusters contained in the image and determine the threshold value. The difference results without image MRF and MRF with FCM FCM can be seen in Figure 3. MRF image without FCM clarify that it is difficult to distinguish bone and bone abnormalities. While the FCM bone image can be a bit blinded follow cluster around it.

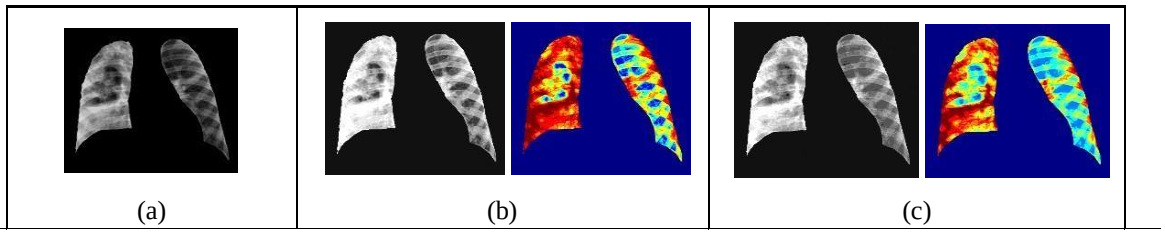


Figure 3. FCM image segmentation results (a) original image, (b)MRF without FCM, (c)FCM+MRF

Thoracic image has a lot of noise. Noise is most disturbing is the ribs. To reduce the influence of the ribs to the image of the lung treated with various filters including a gaussian filter, median filter, a standard deviation filter, wiener filter, histeq, histeq + wiener filter, adapthisteq and adaphisteq + wiener filter. Filter criteria used in this study is a filter that can reduce noise without creating a blurry image and can keep the details of the sharpness of the corners of the object. Turns filters that meet this criteria and is considered the most appropriate is the median filter, wiener filter and adapthisteq. Figure 4 is the result of the use of eight filter on the X-ray image of children aged 13 years with a diagnosis of tuberculosis.

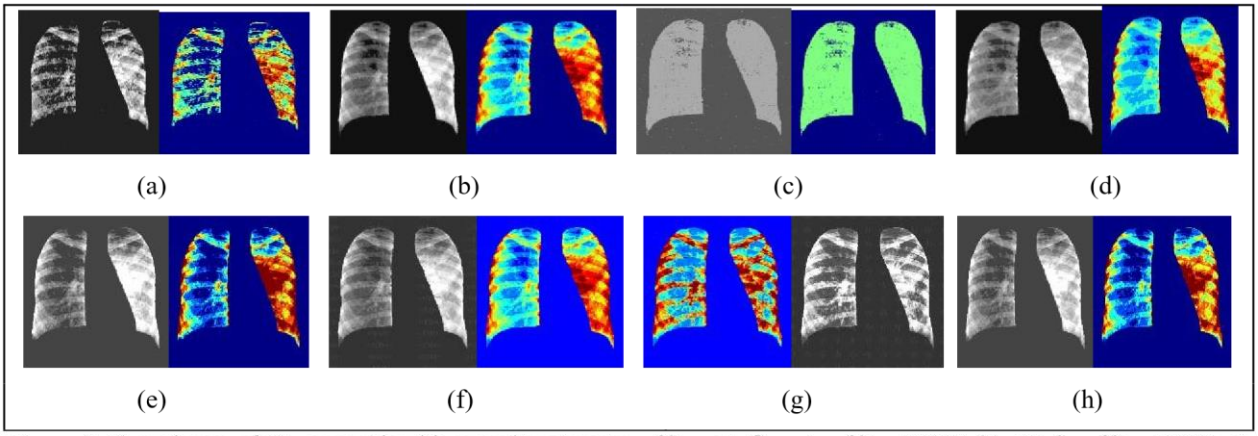


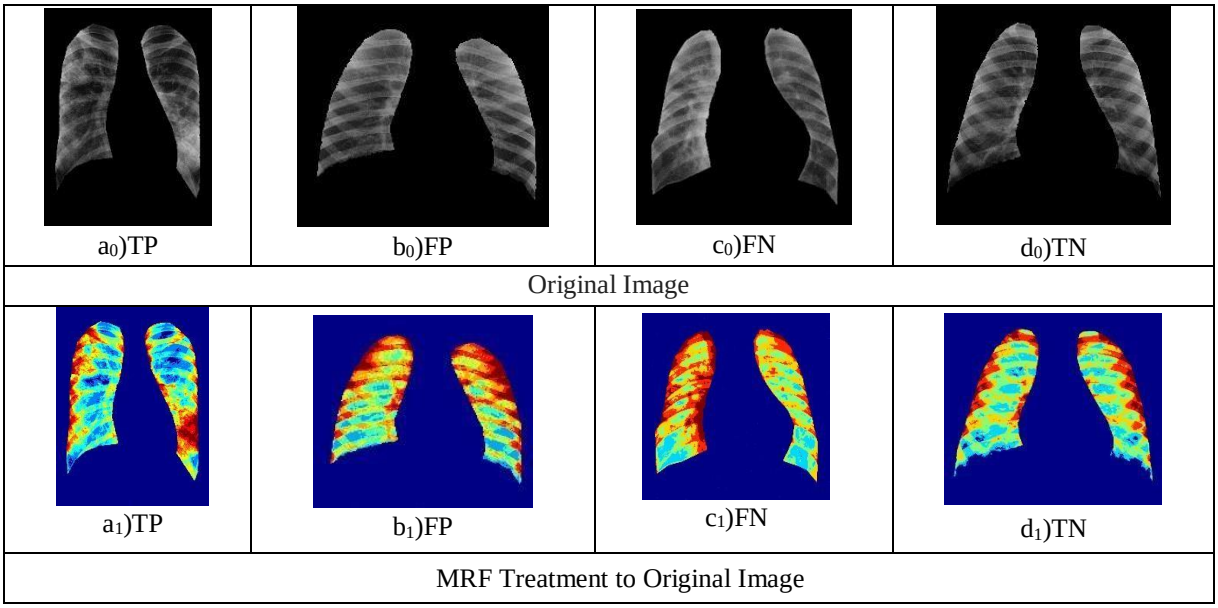
Figure 4. X-ray image of 13 -year olds with several treatments *filter*. a*). Gaussian filter +MRF,* b). *Median filter +MRF,* c). *Standart deviation filter +MRF,* d). *Wiener filter+MRF,* e).*Histeq+MRF,* f). *Adaphisteq+MRF* dan g). *Adaphisteq+wiener filter+MRF* and h). *Histeq+wiener filter+MRF.*

It can be seen that the filter that comes closest to the original image is wiener filter but for subsequent studies have median filter, wiener filter and adaphisteq for comparison. By looking at figure 4 above, have median filter, wiener filter and adaphisteq that selanjutrnya used for further research.

The first abnormal cluster search done by looking at the pixel values of the cluster. Through the observation of 66 abnormal image, the range of values obtained abnormal image pixel in the image that is subsequently used as the pixel value of data models. Table 1 is a cluster of range pixel values obtained by averaging the pixel values of the image.

Table 1. Range of pixel value *cluster*

Metode	***Range* of pixel value**
MRF	205-255
Median filter + MRF	205-255
Wiener filter + MRF	198-255
Adaphisteq + MRF	205-255



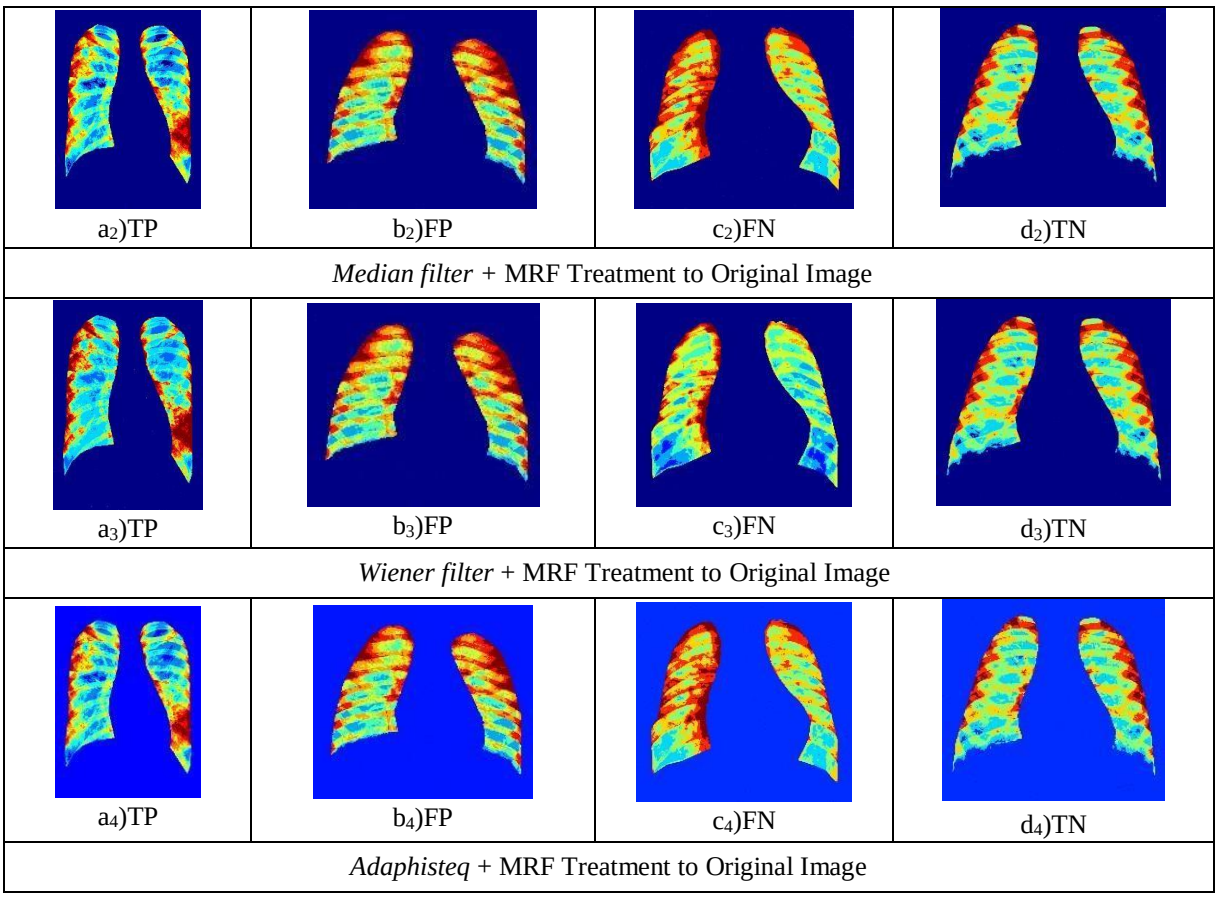


Figure 5. ROC result of abnormal image (tuberkulosis detection) and original image(a_0, b_0, c_0, d_0), MRF segmentation without *filter* (a_1, b_1, c_1, d_1), *median filter*+MRF (a_2, b_2, c_2, d_2), *wiener filter*+MRF (a_3, b_3, c_3, d_3), and *adaphiste*q+MRF (a_4, b_4, c_4, d_4).

ROC segmentation results wiener filter is relatively higher than without a filter. ROC wiener filter results show the value of accuracy 81.4%, sensitivity 82.0%, specificity 80.0%, precision 91.1% and overall error of 18.6%. The image of every process can see in figure 5. While the ROC for unfiltered and filter others show lower than the value of ROC wiener filter. However, differences in ROC for any kind of success rate is not more than 5%, meaning that all four methods MRF can still be implemented. Abnormal lung pixel value with MRF method without filter, median filter+MRF, and adapthisteq+MRF same namely 205-255. Abnormal lung pixel values by the method of wiener filter+MRF is 197-255. Radiographic of children pulmonary can not definitively determine lung infections in children.

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Development of Intertextual Learning Strategy with Model-Based Inquiry on Redox Reaction Concept to Improve the Student's Mastery of Concept

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Abstract - This qualitative study aims to develop the intertextual learning strategy with a model-based inquiry on the redox reaction to improve the student's mastery of a concept. This research uses the a qualitative descriptive method. The method used in this study was a research and development (Research and Development) to generated the intertextual learning strategy with a model-based inquiry. In particular, the object of study was the intertextual learning strategies with the model-based inquiry. The instruments used in the form of sheets validation was used to determine the congruence of learning activities by step model-based inquiry learning and mastery of concepts with aspects of learning activities that are validated by five validators. The result of the development of intertextual learning strategy with a model-based inquiry on the redox reaction concept to improve the student's mastery of concepts showed that were valid by five validators with some improvements based on suggestions of validators. The development of learning strategy was using intertextual relationships in the model-based inquiry learning steps to improve the student's mastery of concepts.

Keywords: Intertextual Learning Strategy, Model-Based Inquiry, Redox Reaction, and Mastery Concept

1. INTRODUCTION

Improving the quality of education is a continuing effort by the government. This effort aims to improve the quality of Human Resources (HR) in order to compensate for the advancement of Science and Technology (Science and Technology). Chemistry is one of the branches of Natural Sciences (IPA), playing a role in the development of science and technology, so it is important to learn. According to Brady (2012, p. 2), chemistry is a part of natural sciences studied the composition, properties, and transformations of matter and how the composition of a material affects its properties. Chemistry involves the understanding and application of chemical concepts (Li &Arshad, 2014). Chemical concepts or knowledge can be represented at different levels of representation, known as a chemical triplet (Talanquer 2011) or triplet relationship (Gilbert &Treagust, 2009). In other words, it takes a deep and thorough understanding of a concept of chemistry (Li &Arshad, 2014). However, Carlos (in Rosenthal & Sanger, 2012) states that students often have difficulty in studying chemistry concepts in the form of an inability to think about the chemical processes at three levels, namely the macroscopic level, the level of submicroscopic and symbolic level. Cho et al. (in Rosenthal & Sanger, 2012) argue that this difficulty can lead to misunderstanding or misconception on knowledge of students' concept which is not acceptable in the scientific realm. One topic of chemistry students has difficulties and misunderstandings (misconceptions). Misconceptions that occur to students within the redox (reduction-oxidation) topic is the difficulty in identifying students oxidation-reduction reaction. According to Rosenthal and Sanger (2012), one of the reasons for this difficulty is the use of textbooks and chemistry teachers who use more than one definition for the oxidation-reduction process.

According to Garnett &Treagust, 1992; Rosenthal & Sanger, 2012; Brandriet, &Bretz, 2014; misconceptions students on redox reactions can lead to mastery of concepts the student is not intact. Brandriet and Bretz, (2014) revealed that misconceptions experienced by students one reason is unqualified students in chemistry represents broadly. Tasker research results and Dalton (2006) explains that the misconceptions in chemistry due to the inability of students to visualize the structures and processes at the submicroscopic level or molecular chemistry learning while most involve only the macroscopic level and symbolic level. Also, Wu (2003) in his book reveals that involving chemistry learning relationship conceptual between macroscopic, microscopic, and representation symbolic. This is consistent with the results of a preliminary study that gathered researchers from several schools in the South Coastal District Padang that learning chemistry in materials reduction and oxidation reaction is considered one of the materials that are difficult to understand by the students because it is dominated by one level without attaching level macroscopic level sub-microscopic and symbolic level and also without connecting a real experience with events in the classroom students in learning chemistry. Regarding solve this

problem, it requires the appropriate learning strategies in understanding the chemistry learning in all three levels of chemical representation and attaches three levels and associate real experience of students and events in the classroom. This is particularly important in the teaching and learning of chemistry. According to Hosnan (2014, p. 183) "learning strategy is a learning activity that must be done by teachers and students so that learning objectives can be achieved effectively and efficiently". According to Wu (2003) Chemical representation relationships, real life experiences, and events in the classroom can be seen as an intertextual relationship. Science activities can be performed using inquiry approach (Wenning, 2005). The relationship between the three levels of chemical representation needs to be demonstrated explicitly in the learning environment, for example in the context of the inquiry so that students can learn significantly (Kozma, et al., Wu 2003). One chemical that implements learning strategies curriculum in 2013 the strategy of inquiry learning. One of the inquiry-based approaches is inquiry-based models (IBM) that are an approach to learning that derives from the view that science is centered on the development of the model (Clement in Xiang &Passmore, 2014) and students must build on this understanding through a process that resembles how scientists understand the science.

2. RESEARCH METHOD

The method used in this study is a research and development (Research and Development) and object of this study is the intertextual learning strategy with a model-based inquiry. The subject of this study is Senior High school students Padang West Sumatra, Indonesia in the first half of academic year 2016/2017. Data analysis are validity, the congruence of learning activities by step the model-based inquiry and the congruence of learning activities with student's mastery of concepts by Analyzed with qualitative descriptions.

3. RESULTS AND DISCUSSION

The results of the validation of conformity aspect of mastery concept of oxidation, redox reactions based on changes in oxidation number, oxidizing and reducing agents Showed aspect mastery of the concept of "applying (C3) (Determining the oxidation number of elements that are changing in a reaction), applying (C3) (Determining the oxidation state in the equation), find facts apply (C3), explain (C2) (make an explanation of redox reactions is based on the concept of change in the oxidation state), applying (C3) (applying the determination of the oxidation state to Determine a redox reaction), explain (C2) (explaining the concept of a reductant and oxidizer), concluded on the redox reaction is based on the concept of change in the oxidation state.

3.1 Students Concept Mastery

Table 4.7 Results Pretest and Posttest Control Concepts Students in Material Redox Reactions

Concept	Result		numbers matter	The number of subjects	maximum score	Percentage Control Concept	
	pretest	posttest				On average pretest	On average posttest
Redox reaction by electron transfer	0	5.9	6	23	20	0%	29.5%
The oxidation number	2.6	4.8	1		5	52%	96%
	0.1	7.3	3		15	0.67%	48.67%
Redox reactions based on changes in oxidation numbers	6.4	17.7	2		20	32%	88.5%
	0	6.1	4		20	0%	30.5%
	0	5.9	6		20	0%	29.5%
Oxidizing and reducing agents	0.7	20	5		20	3.5%	100%

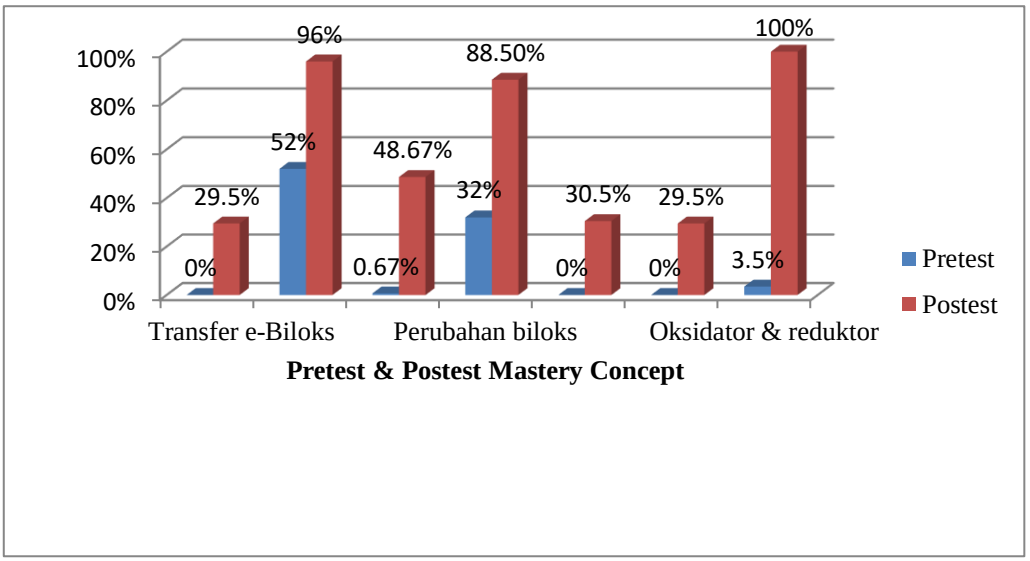

Figure 4.8 The results of pretest and Posttest Control Concepts

It can be seen that the most widely understood concept of students is the concept of an oxidizing and reducing agent. Furthermore, the concept of the oxidation state and redox reactions based on changes in oxidation number. The concept is based on electron transfer redox reactions revealed through about the number 6 which asks students to write the chemical equation of reaction formation of KCl based macroscopic picture and determine the redox reaction by electron transfer. In question number 6 students did not answer at the time of holding the pretest. This is presumably because they did not master the concept of the prerequisites of the formation of ionic compounds from the elements so that they had difficulty in determining which took off and which receives electrons so that the learning process can be improved on the concept precondition regarding the formation of ionic compounds from the elements. The concept of oxidation number of reactions revealed through Question 1 that asked students to explain the meaning of oxidation numbers, and Question 3 that asked students to determine the oxidation numbers of atoms of the elements in the compound. In question number 1 students were asked to recall the definition of oxidation states. It is used to answer question number 3 for determining the oxidation numbers of atoms of the elements in a compound. In Question 1, in general, students have answered correctly about the explanation of oxidation states. In question number 3 when the holding pretest no student answered, after learning and held posttest, some students are already answered correctly in determining the oxidation numbers of atoms of the elements in a compound. The concept of redox reactions based on changes in oxidation numbers revealed through Question 2, which asks students to write down the equation and determining the oxidation and reduction reactions is based on the concept of redox reactions that are known (redox reactions based on changes in oxidation number) seen from the picture and Question 4 which asked students to determine the oxidation-reduction reaction is based on the concept of change in the oxidation state of the equation, as well as about the number 6 that asked students to write down the equation and determining the redox reaction by electron transfer and redox reactions based on changes in oxidation number. In question number 2, 4, and 6 time of the pretest ability of students is still low. This is evidenced by the responses of the students was limited to be able to write the equation without determining the oxidation and reduction reactions is based on the concept of redox reactions known. Then do the posttest after learning activities, the ability of students increased. This is evidenced by the answers to students who are able to determine the redox reaction. That is the student's mastery of concepts increases at the rate applying. The concept of oxidizing and reducing agent revealed through Question 5 that asked students determine the oxidizing and reducing agent from the reaction is written in terms of the description. In question number 5, the initial ability of students is still lower than the pretest. However, after the post-test the ability of students to be increased as evidenced from the posttest. The average student is able to define the concept of the oxidizing and reducing agent in a redox reaction. That is the student's mastery of concepts increases at the rate applying.

3.2 Responses Teacher

According to teacher learning strategies intertextual with inquiry-based model is able to enhance the spirit of learning students because the students showed enthusiasm for the learning process because students get the experience to learn more in order to connect learning chemistry in three levels of representation of chemistry that is at the level of macroscopic level of sub-microscopic and level of symbolic

3.3 Student feedback

Statement items number 1, 3, 4, 6, 8, and 9 is a response to the students 'learning process and statement item numbers 2, 5, and 7 is the students' responses to the motivation to learn.

Table 4.8 Results of Student Responses to the Learning Strategy intertextual with Model-Based Inquiry

Responses of students	SS	S	TS	STS
Learning process (Item number 1, 3, 4, 6, 8, and 9)	(10, 1%)	(75, 4%)	(14, 5%)	(0%)
Motivation to learn (Item number 2, 5, and 7)	(7, 2%)	(75, 3%)	(17, 4%)	(0%)

4. CONCLUSION

Based on research that has been done, it can be concluded that it has generated suitability learning strategy between learning activities with model-based inquiry learning steps and learning activities with an aspect suitability mastery of concepts by some validators with some improvements based on feedback from the validator. Tested on one of the country's top schools with the results can improve students' mastery of concepts. As well as teachers and students give a positive response to the strategy developed.

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Intertextual Learning Strategy With Problem Solving on Buffer Solution Concept to Enchance Student's Concept Mastery

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Abstract – The aim of this study was to develop intertextual learning strategy with problem solving on buffer solution concept to enhance student's concept mastery. This study was conducted by consideration of some various studies which found that lack of student's mastery in chemistry concepts was caused by their inability to connect three levels of chemical representation. The method used in this research was R&D (Research and Development) method with steps are part of R&D cycle, they are: 1) research and data collection, 2) planning, 3) develop preliminary product, and 4) preliminary field testing. The object of this study was intertextual strategy with problem solving learning design on buffer solution concept. The subjects for the trial research were 12th grade science students in one of the MA in Bandung. Instruments used are conformity validation sheet of learning design, observation sheet with video record, and item test to calculate concepts mastery. The result of learning design validation include the conformance of learning activities and concept mastery. Learning design was validated by 5 validators with some suggestions for improvement. The trial result of the enforceability of learning activities showed that the developed strategy can improve student's understanding of buffer solution. The concept that students most understand was the nature of the buffer solution.

Keywords: intertextual learning strategy, problem solving, buffer solution, concept mastery.

1. INTRODUCTION

Chemistry's core topic is about matter and its properties, its changes, and the energy changes involved along with the processes (Whitten et al., 2014). Some phenomena in chemistry can be observed by naked eye, but need an explanation at the molecular level. This can lead to student's difficulty in learning chemistry that make their concept mastery is in low level (Okanlawon, 2010). The low of student's concept mastery is caused by misconceptions or alternative conceptions (Lin et al., 2010). According to Tasker & Dalton (2006), some of the misconceptions in chemistry are caused by student's inability to visualize structures and processes at submicroscopic or molecular level, due to chemistry learning usually involve only macroscopic and symbolic level. Johnstone (1991) suggested that in order to understand chemistry, student must be able to understand three levels of chemical representation (macroscopic, submicroscopic, and symbolic), and integrate all those levels. Jansoon, et al. (2009) said that those three levels are characteristic of chemistry representations. Madden (2011) stated that understand the three chemical representation levels can fasilitate students to build their understanding in chemistry more easier. However, in reality many students do not understand three representation levels and do not use them in explaining chemistry phenomenas (Talanquer, 2011).

Intertextual learning strategy using problem solving learning steps is a very useful tool in helping student to understand the representation of chemistry. The involvement of chemical representation, interaction in classroom, and everyday experience can help student to build their knowledge to understand the chemistry phenomena. Also learning environment including teachers, chemical materials, and the infrastructure can influence student's learning (Wu, 2003).

Problem solving is an important part of learning because problems are part of student's daily life. Alicigüzel (in Dogru, 2008) stated the problem based learning will help student to overcome their own difficulties in learning and at the end they will be able to solve problems in their daily life. In learning, problem can be used to stimulate student's interest, improve their discussion skills, and understand the chemical representation (Johnstone, 2006). Sartika, et al. (2014) research suggest that problem solving learning's step such as analyze the problem, plan how to solve the problem, and solve it in detail, allow students to maximize their understanding of three levels chemical representation.

Concept mastery can not be separated with learning process because it is the main goal of the process (Dahar, 2011). Concept mastery is defined as the degree to which a student is not just knowing the concepts, but really

understand it well, demonstrated by their ability in solving of various problems both related to the concept itself and its application in new situations (Anderson & Krathwohl, 2010). Concept mastery is crucial to build interconnection between concepts. Students need to be able to use the concepts to solve various problems.

The aim of this study is investigate effectiveness of intertextual learning strategy with problem solving to enhance students' mastery of buffer solution. Based on this aim, the research question stated "How can student's mastery on buffer solution concept studied by using intertextual learning strategies with problem solving?"

2. METHODOLOGY

The method used in this research is R & D with the stages are adopted from Research & Development cycle, there are: 1) research and data collection, 2) planning, 3) develop preliminary product, and 4) preliminary field testing. The object of this study was intertextual learning strategy with problem solving on buffer solution concept. Subjects in this study were 32 students of 12th grade in one of MA in Bandung.

The instruments used in this study were conformity validation sheet of lesson plan of the developed theory, observation sheet with video recording to supporting data, 5 questions to investigate concepts mastery. The lesson plan has been validated by five lecturers with doctoral degree in Departement of Chemistry Education and declared valid with some suggestions for improvement. The implementation of learning activity was recorded on observation sheet, video record, and sound record.

The test items of concept mastery was prepared based on C1 – C4 cognitive level. Indicator for C1 (remember) was identification of buffer solution components; for C2 (understand) was explaining properties of buffer solution; for C3 (apply) was calculating pH of buffer solution with addition of slightly acidic or basic solution; and for C4 (analyze) was analyzing the process of how buffer solution can maintain the solution pH with addition of slightly acidic or basic solution and analyzing the rule of buffer solutions in living things.

3. RESULTS AND DISCUSSION

3.1 Validation of Intertextual Learning Strategy with Problem Solving on Buffer Solution Concept

Validation sheet of intertextual learning strategies with problem solving to enchance the mastery of concepts in each column contains of problem solving learning steps and sub-steps, learning activities -consist of teachers and students' learning experience, aspect of concepts mastery, suitability of learning activities and problem solving learning steps, compliance of learning steps to gain concept mastery, and suggestions for improvement. The validation sheet was then validated by team of experts consist of three lecturers in chemical education and 2 lectures with doctoral degree of chemistry. The results showed that the learning design need to be improved more. Overall, the learning design declared invalid by the whole validators but there are some suggestions from the validators, which are used to revise the learning design.

3.2 Conformity Learning Activities by Validation Sheet

Conformity learning is the initial trial of developed learning design. Generally the conformity learning that done went well. Conformity learning were observed based on five steps of problem solving learning developed by Brandsford & Stein (1998) combined with the troubleshooting steps according Nitko & Brokhart (2011) includes the following steps: (1) Identify the problems, (2) Define and represent the problem, (3) Explore possible strategies, (4) Act on strategies, dan (5) Looking back and evaluate the effect of your activities. Conformity learning of developed strategy was achieved by 83% in both categories. Conformity learning assessment was based on the suitability of problem solving learning step with teacher and students's activities, time allocation and concept mastery achievement.

3.3 Concept Mastery on Buffer Solution

Student's concept mastery was measured by concept mastery instruments that consists of 5 test items in which each item represent every indicator of specific concept mastery. The instrument used in pretest and posttest. Intertextual learning strategy with problem solving is able to improve student's understanding. This enhancement is due to the learning strategy which is student centered. Learning activity used also help students to associate all three chemical representation. Student can also connect their prior knowledge with their new knowledge. Group discussion is mostly used in the process, it can facilitate students to work together to solve problem, students also have opportunity to express their opinions and communicate during learning process. Problem solving learning is based on constructivism, where students are required to be to build their own understanding. Along with Rosbiono (2007) who stated that student's activities in problem solving learning is to findi solution for problems in science concepts.

The average of student's concept mastery is in moderate category. This was presumably because of time limitation, each learning steps didn't conveyed properly. According to Gestalt (Graham, 2008), one of the

principles of learning is based on principle of the whole. In the learning process, students try to connect to as much as possible concepts. Therefore, learning have to done at each step in order to make students understand it easyly. The results of the average percentage score in pretest, posttest, and % N-gain on buffer solution concept by using intertextual learning strategy with problem solving was shown in Table 1.

Table 1. The Average Score In Pretest, Posttest, and % N-Gain on Buffer Solution Concept

No	Learning indicator	Pretest (%)	Posttest (%)	%N-*Gain*	Category
1	Describing properties of the buffer solution	65,00	90,63	73	High
2	Identifying components of the buffer solution	10,00	46,88	40	Moderate
3	Explaining the process of how buffer solution can maintain the solution pH with addition of slightly acidic or basic solution	20,00	60,00	50	Moderate
4	Calculating pH buffer solution in the addition of slightly acidic or basic solution.	20,00	46,25	33	Moderate
5	Analyzing the role of buffer solution in living things.	6,25	53,13	50	Moderate
	Average	**24,25**	**59,38**	**49**	**Moderate**

Tabel 1 shows concept mastery improvement on five indicators. The highest achievement is indicator of explaining properties of buffer solution with N-gain 73%. The lowest is indicator of calculating pH buffer solution in the addition of slightly acidic or basic solution with N-gain 33%. The highest N-gain achievement is because student's learning process in this indicator was presented as macroscopic level by doing experiment so that more students master in this concept.

The lowest N-gain can be related with student's understanding at symbolic level, because in calculating pH buffer solution students were more involved in symbolic representation which acquire student's mathematical abilities. According to Borner (2000), problem solving learning that involve calculation or algorithmic have to be linked to other representations (submicroscopic or macroscopic) so that students can understand the symbolic representation well. In the intertextual learning strategy with problem solving, students have been taught before on submicroscopic level representation on how buffer solution work to make them easily understand the concept of pH calculation. However, after doing the learning activity, some students still didn't show significant improvement. It was because students just memorized the formulas that have been given by the teacher on their previous learning instead of understanding the basic concepts that was provided in this learning activity.

In addition, the mathematical ability of students also affects of their learning outcomes. Students still have difficulty in understanding the power of negative or decimal number, simplifying the comparison, and in calculations involving formulas or notations. The lack of mathematical ability in students to solve the science problems was consistent with Ryland and Coady (in Faridhan, 2013) who expressed a lack of mathematical ability as a prerequisite that has been a problem since the 1970s, and still became a problem even in developed countries.

Mastery on first indicator, describing properties of the buffer solution, was represented through first question that asked students to explain the properties of buffer solution. Almost all students were able to explain it correctly. This mean that student has reached the cognitive level of understanding (C2). This was because students were able to understand the properties of buffer solution on macroscopic level by did the problem solving that presented in their worksheet. Every students did experiment so that they can easily understand the concept through macroscopic representation. In accordance with the results of the study by Coll (2007) who stated that in general students understand the macroscopic level well.

Mastery on second indicators, identify components of buffer solution, was represented through second question that asked students to identify the components of buffer solution of weak acid with its conjugate base. In the pretest, students could not answer this question correctly. In the post test, their abilities in this indicator increased but only slightly. That was because during the implementation of the lesson, teacher did not explore the submicroscopic level quite deep. Even so, there was some questions on student's worksheet to guide them understand the submicroscopic level on buffer solution components. Teachers tend to put emphasis more on the buffer solution reaction which is including symbolic level. For future study, improvement is needed especially on showing students the image of each components of buffer solution in submicroscopic level. That can help students to explore their ability to determine the components of buffer solution such as the salt and its conjugate acid or base.

However, there're some students who were able to write the components of buffer solution correctly. Those student has reached the cognitive domains level of remember (C1). That was because learning process emphasis more on understanding chemical concepts. In student worksheets there were questions that guide them to understand the concept of buffer solution components. In accordance with the students' responses to the chemistry subject interest has an average value of 3,83 that is classified of both criteria. Responses of students' interest in chemistry subject, especially on buffer solution can be seen in Figure 1.

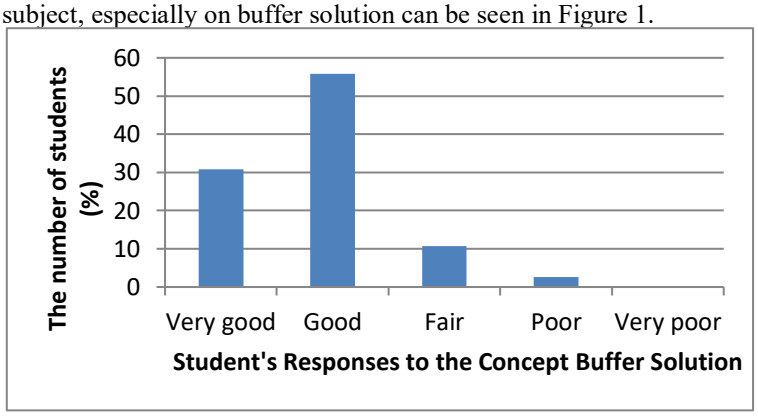

Figure 1. Graph of the average percentage of student's interest responses to the buffer solution concept

Based on Figure 1, we can see that 58% of students respond on good criteria, 13% on very good criteria, 27% on fair criteria, and 2% on poor criteria. It can be concluded that intertextual learning strategy on buffer solution concept can make students become more interested in learning buffer solution concept. The good response can be caused by the learning process that begins with phenomena relating to daily life, then student was demanded to conduct experiment so they can directly observe. Those processes make students active in build their own concepts and also put their attention in learning process.

Mastery on third indicators, analyze how buffer solution works in addition of acid or base solution, was measured by third question. The students were asked to analyze how buffer solution works by a diagram and description of buffer solution particles visualization. Some students were able to understand the concept and can correctly answer the question a, b and c with equilibrium reaction and molecular images. Those students has reached the forth cognitive domains level, analyze. Students were able to provide proper explanation about submicroscopic level of the how buffer solution works by drawing all particles in buffer solution. That was because during the learning process, students were taught to understand how buffer solution works by integrating all three representation levels in their worksheet. In general, students respond well to the learning process. The average students response is 4,9 which includes in good categories. Student's response about the worksheets used in the learning process can be seen from Figure 2 below.

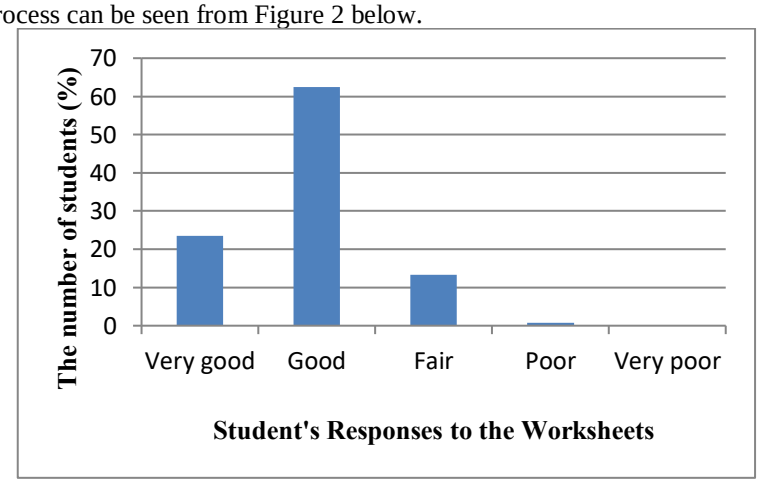

Figure 2. Graph of student response to the worksheet

Figure 2 shows that 63% students gave good response, 23% gave very well response, 13% gave fair response, and 1% gave poor response. It can be seen from these data that worksheet is highly needed as student's guidance in intertextual with problem solving learning process. On the third question of the worksheet, there are images of buffer solution particles formed from a mixture of a weak base (NH_3) with its conjugate acid (NH_4^+). Ionization reactions that occur in the mixture:

$$NH_3\,(aq) + H_3O^+\,(aq) \rightarrow NH_4^+\,(aq) + H_2O\,(l)$$

Before the addition of the H_3O^+ species that exist in the buffer components is NH_3 and NH_4^+ are in the same amount (see Figure 3).

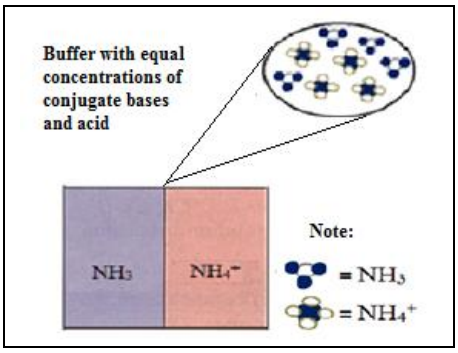

Figure 3. Representation of Molecular Components of Buffer Solution (NH_3 dan NH_4^+)

On adding a slightly of acid, H_3O^+ ion reacts with NH_3 to form NH_4^+ so that the amount of NH_4^+ increased and the number of NH_3 will be reduced in proportion to the addition of acid (see Figure 4).

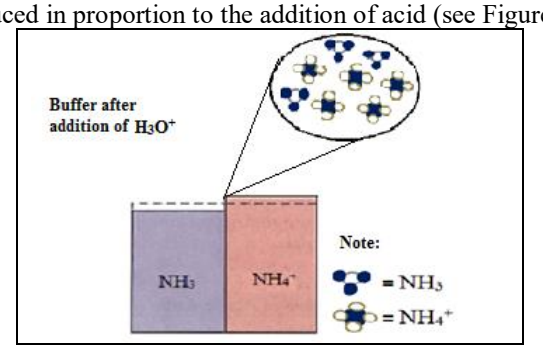

Figure 4. Representation of Molecular Components Buffer Solution (NH_3 and NH_4^+) in Acid Addition

On the addition of a slightly base, OH^- ions will react with NH_4^+ to form NH_3 and water molecule, so that the amount of NH_4^+ is reduced and the amount of NH_3 increased (see Figure 5).

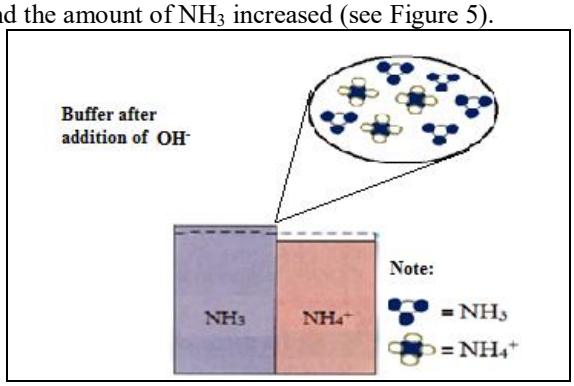

Figure 5. Representation of Molecular Components Buffer Solution (NH_3 and NH_4^+) in Base Addition

In general, most students weren't able to write a complete path of how buffer solution works, only some of the keywords. It shows they only partially understand the concept, so that the cognitive level of analyze (C4) can't be achieved. This was because students didn't able to associate submicroscopic and symbolic level. They are more dominant on the submicroscopic level so that students have difficulties in writing the symbol of particles involved. Students also didn't realize that buffer solution can maintain the solution pH because they have components that can handle the addition of acid or base. Further study needs to show representation of a particles in media such as video and animation along with explanation of the reaction equilibrium, so that students can connect the submicroscopic and symbolic level.

Mastery of fourth indicator, calculating buffer solution pH on addition of acid or base, was measured by fourth question. Students were asked to calculate the pH of the buffer solution in the addition of slightly acidic or basic solution. There are only few students who were able to answer correctly. This is because the pH calculation was dominated the symbolic level. Students need to understand how buffer solution works, instead they just memorize the pH formula for buffer solution. Most of the students wrote the formula $[H^+] = Ka.\frac{weak\ acid}{conjugate\ base}$ (students didn't clearly write "weak acid" and "conjugate base", instead they wrote "acid mole" and "conjugate base mole"). Students memorize buffer solution formula using equilibrium concept that has been studied in

implementation learning. For future learning, it needs to emphasize analyze path to getting pH formulas, and also use many cases in their daily life in buffer solution pH calculation.

Mastery of last indicator, identify the role of buffer solution in living thing's body, was the moderate category. In posttest, students can answer questions correctly. They write down the H_2CO_3/HCO_3^- buffer equilibrium reaction when acid or base added. There is increasing concept mastery in students from pretest to post test for this indicator. The student has reached of the cognitive domains of analyze (C4). Students were able to understand the role of buffer solution in everyday life. That was because in learning process, students can associate buffer solution concept with other chemistry concepts and apply them to dialy life problem. When students learn about the role of a buffer solution, they must be able to explain its properties, components, and how buffer solution works. Problem solving learning is a form of learning based on constructivism, where students are required to take an active role in building their own understanding of the knowledge. This is consistent with Rosbiono (2007), who stated that in problem solving learning, student activities are centered on the problem and its solution which is based on basic science concepts.

The learning implementation received positive response from students. The average responses is 4,15 which include good category. The students' response about the implementation of intertextual learning strategy with problem solving can be seen from Figure 6 below.

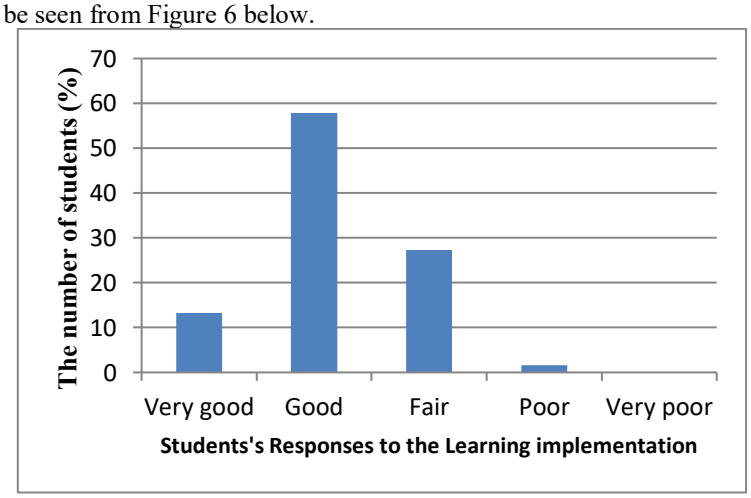

Figure 6. Graph of students' responses about the learning implementation

From Figure 6 it can be seen that 56% students gave good response, 31% gave very good response, 11% gave fair response, and 3% gave poor responses about the implementation of intertextual learning strategy with problem solving.

From the data, it can be said that the intertextual learning strategy with problem solving help students learn buffer solution concept easier. It makes students participate actively on building their concept.

4. CONCLUSION

Student's concept mastery has increased from pretest to posttest. The most understood concept is properties of buffer solution. That was because the learning process on this indicator is emphasize on macroscopic level by conducting experiment. This improvement also because the learning process was student-centered. Problem solving learning can applied mostly in group discussion to facilitate students to work together in solving problems. Students also got opportunity to express their opinions and communicate during learning process.

5. ACKNOWLEDGMENTS

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Soil Vulnerability Analysis Using Microtremor Data In Tengger Caldera, Bromo Tengger Semeru National Park

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Abstract – Indonesia is a country with a complex geology, and it leads to the formation of many volcanoes. One of active volcano famous for tourist attraction is Mount Bromo, which belongs to Tengger Mountains and located in Bromo Tengger Semeru National Park area. Tengger caldera is in the form of a broad sandy area formed from the past eruption and is the center of tourist activity of Mount Bromo. The thickness of the sand sedimentation needs to be investigated in order to identify the soil vulnerability so that the risk of earthquakes due to tectonic and volcanic activity can be reduced. Microtremor is the most suitable method to determine the soil vulnerability based on the value of natural frequency. From the research conducted, the value of natural frequencies is 1.5 to 13.5 Hz. To determine the thickness of sediment then seismic refraction and VES approach performed to get the value of Vs30, then the result obtained is sediment thickness value which is 5-85 meters. When the sediment medium is passed through by wave, amplification will have a high value, with the character of soft sedimentary rocks would be more destructive than the rock with more compact character. The results of this research recommend the location of assembly points and evacuation routes for disaster tackling when an earthquake happens on the east side of the caldera sand.

1. INTRODUCTION

Indonesia is an archipelago country which has very complex tectonic. This tectonic background caused Indonesia has a lot of active volcanoes. One of the volcanoes are still active and became a tourist area is Mount Bromo located in Tengger Mountains, Bromo Tengger Semeru National Park. Tengger caldera mountains is a vast sea of sand (Figure 1). The composition of the volcanic deposits Mount Bromo is an interesting phenomenon, exotic, on a specific type of volcano that forms the cylindrical cone inside the caldera. From the results of the deposition material is known that Bromo eruption occurred many times, evidenced by sorting sand is not uniform.

Figure 1. Satellite image from Google Earth at 2015.

HVSR (Horizontal to Vertical Spectral Ratio) method was first introduced by Nakamura (1989), used to estimate the natural frequency and amplification of the local geology. The natural frequencies generated from the microtremor data. HVSR method can estimate the vulnerability of the survey area. HVSR method is based on the trapping of the shear wave in the sediment medium above the bedrock. If Vs is the shear wave velocity and 4h represent the depth of the bedrock, so the equation of natural frequency is;

$$f = \frac{V_s}{4h} \tag{1}$$

So the natural frequency value can represent the value of the thickness of the sediments in the Tengger Caldera

The value of shear velocity (Vs) obtained by Vs30 value approach of seismic refraction and resistivity (VES) with the equation discovered by Morelli, 2013;

$$Vs(z) = Vo(1+z)^{x} \tag{2}$$

$$m = \left[\frac{Vo(1-x)}{4f} + 1\right]^{1/(1-x)} - 1 \tag{3}$$

Where,
Vs (Z) = shear wave velocity at z depth
V0 = shear wave velocity on surface (m/s)
Z = depth
X = exponential factor
M = layer thickness (m)
Fr = natural frequency (Hz)

2. METHODS

This study uses microtremor MAE type. From the data processing obtained Horizontal to Vertical (H / V) graph, which is the result of the Fast Fourier Transform (FFT) (Figure 2).

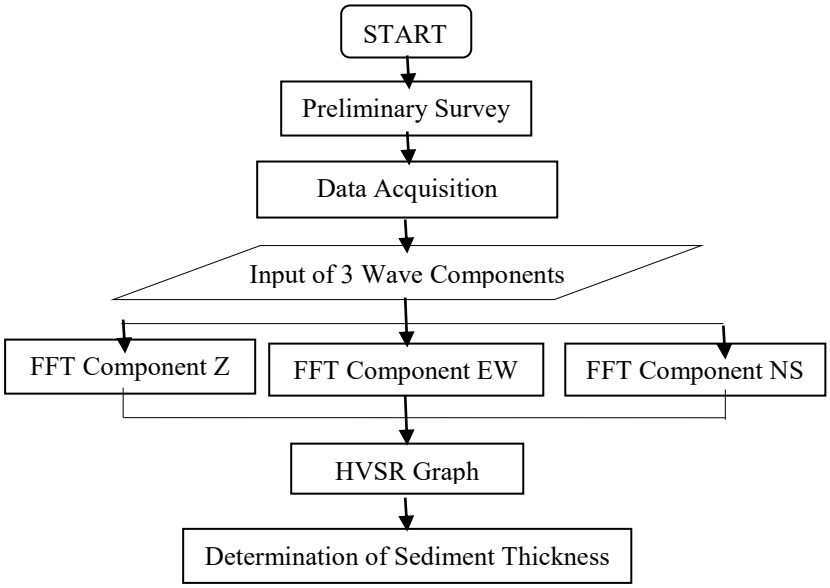

Figure 2. Diagram showing the data processing to obtain the HVSR graph

3. RESULTS AND DISCUSSION

After the FFT applied to the data, then obtained a graph Horizontal to Vertical (H/V). Graph H/V contain information about dominant frequency and amplification (Figure 3).

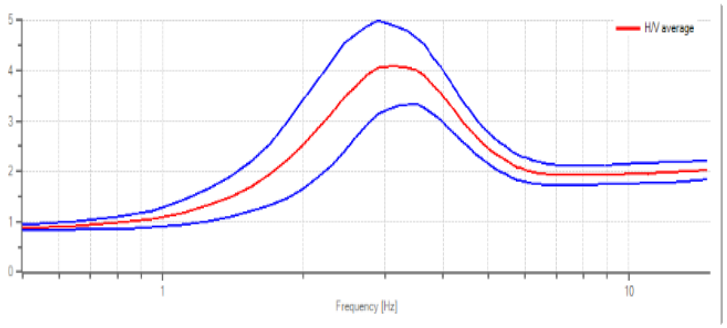

Figure 3. Graph H/V, the X axis is frequency, and the Y-axis value is the value of H/V (amplification). Red line represents the H/V average.

After the dominant frequency value obtained from the data processing then the value of the dominant frequency of several points plotted on a contour map of the dominant frequency and the thickness of sediments. The results of data processing obtained the value of natural frequencies from 1.5 Hz to 13.5 Hz (Figure 4). A small natural frequency 0.5-5 Hz showed thick sediment layer, and the natural frequency 5.5 to 13.5 Hz show a thin layer of sediment.

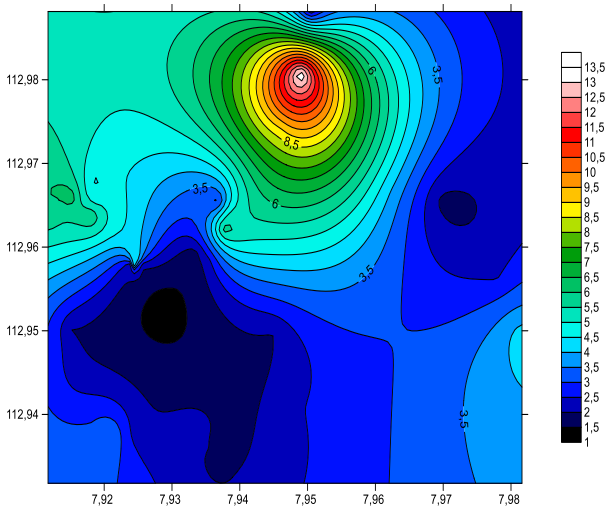

Figure 4. Natural frequency map. Values of X and Y are the coordinates of the point of measurement and color scale represents the natural frequency.

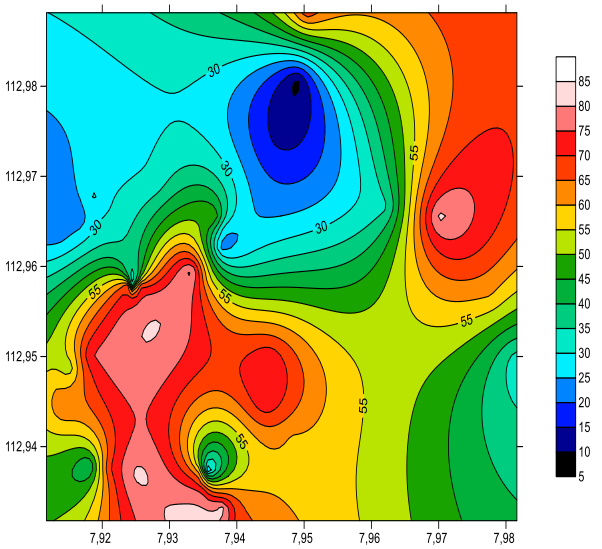

Figure 5. Sediment thickness map. X and Y are the coordinates and color scale indicates the depth distribution of sedimentation.

In figure 5 shows the caldera sediment thickness is 5-85 meters. When the wave passed sediment layers, amplification would be a great value, with soft sedimentary rock characteristics will be more destructive than the more compact rocks.

4. CONCLUSIONS

The results were obtained dominant frequency values from 1.5 to 13.5 Hz. To determine the thickness of sediment carried out seismic refraction and VES approach to determining the value Vs30, and obtained sediment thickness 5-85 meters. When the wave passed sediment layers, amplification would be a great value, with soft sedimentary rock characteristics will be more destructive than the more compact rocks.

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The Development of Worksheet and Problem Sheets Based on Problem-Solving Skill Using Multimode Representation for Physics Learning in Senior High School

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Abstract – This research aimed to develop problem-solving skill based on the worksheet and problem sheets using multimode representation on the heat and temperature material for physics learning in grade X of senior high school. The method used was the Research and Development (R & D) by Borg and Gall using Representational development Approach Learning to Write. Stages of problem-solving skill used in this study referred to the framework by Crebert and David Rosengrant. The instruments used in this study were quality test questionnaire, main idea comprehension test, and problem-solving skill test. The data analysis technique used to analyze the quality test questionnaire and main idea comprehension test were descriptive analysis. Meanwhile, problem-solving skill tests were analyzed by calculating the N-gain, statistical tests, and effect size test. In addition, based on the results of the trial, the developed worksheet and problem sheets could improve the problem-solving skill of students with an N-Gain score of 0.71, which was in the high category. Based on the calculation of effect size, it was known that the effectiveness of using the developed worksheet and problem sheets in improving problem-solving skill worth of 1.54 with big impact category. The conclusion of this study was the worksheet and problem sheets in based on problem-solving skill using representation multimode effective to improve the problem-solving skill of students.

Keywords: worksheet, problem sheets, multimode representation, problem-solving skill, heat and temperature

1. INTRODUCTION

Physics is not only a collection of knowledge about inanimate objects and living beings, but also concerns the way of working, thinking, and problem-solving. It is in line with the needed skill on the 21st century. The learning paradigm of the 21st century emphasizes the students' ability in problem-solving, the students' capability of connecting science to the real world, mastering information technology, communicating and collaborating. Instructional materials have important role in the learning process. This research aimed to develop problem-solving skill based on the worksheet and problem sheets using multimode representation on the heat and temperature material for physics learning in senior high school.

Natural science (IPA) is a science that the main subject is nature and all its contents. Things that is learned in IPA is a causal relationship of events that occur in nature. In accordance with the fact that the activities in IPA are always associated with experiments that require skill and diligence. Thus IPA is not only a collection of knowledge about inanimate objects and living beings, but also concerns the way of working, thinking, and problem-solving. Physics is one of the main pillars of science and technology that provides a better understanding of natural phenomena as well as the possibility of its application in improving the welfare of humankind. The principle of the learning process is learning. Meanwhile, learning itself is a relatively fixed process as a result of the experiences.

There are four functions of teaching material for educators. Among them are they may change the role of educators from a teacher to a facilitator, improve the learning process to become more effective and interactive, as a guide for educators who will direct all of their activities in the learning process and a substance of competence which should be taught to students. Teaching materials are an important part of the implementation of education.

Problem-solving skill is important because it is in accordance with the demand of the existing curriculum. It is

supported by field study result in one senior high school in Bandung (2014) which stated that the problem-solving skill of students was still low; the result was obtained by giving problem-solving skill tests to students in the form of an essay question about Newton's laws. The result of the tests showed that the problem-solving skill of students was in the very low category.

From the above explanation, teaching materials in the form of worksheets and problem sheets that are designed to improve problem-solving skill are needed. Teaching a subject should be adjusted to the characteristic of the concept/topic and the development of students' thinking, so that hopefully there will be suitability between the learning that emphasizes on understanding concepts and the learning that emphasizes on problem-solving skill.

2. METHODS

The method used was the Research and Development by Borg and Gall using Representational development Approach Learning to Write. The instruments used in this study were quality test questionnaire, main idea comprehension test, and problem-solving skill test.

2.1 Instrument

The instruments used in this study were quality test questionnaire, main idea comprehension test, and problem-solving skill test. The data analysis technique used to analyze the quality test questionnaire and main idea comprehension test was descriptive analysis. Meanwhile, problem-solving skill tests were analyzed by calculating the N-gain, statistical tests, and effect size test. The instruments used in a whole series of research activities consisting of a set of the developed worksheet and problem sheets in based on problem-solving skill using multimode representation. Worksheet and problem sheets contained tasks that must be done by the students in based on problem-solving skill along with practice questions. Worksheet and problem sheets were made according to the learning objectives and learning topics. Learning topics used in this study were temperature and heat for senior high school level. Checklist sheet reviewed from various quality aspects of the created worksheet that must fulfill the assessment aspect was used to assess the worksheet contents quality. Meanwhile, the instrument of discourse main idea test with scoring rubric was used to assess the main idea comprehension. The subjects in this study were students of grade X at one of senior high school in Bandung.

2.2. Research method

The method used was the Research & Development by Borg and Gall using Representational development Approach Learning to Write. Stages of problem-solving skill used in this study referred to the framework by Crebert and David Rosengrant. The research design was pretest and posttest control group. This research steps consisted of seven stages, among them were the stage of research and information gathering, the stage of planning, the stage of development, the stage of the initial test, the stage of product revision, the stage of the field trial, and the stage of product revision.

The numbers of respondents for data validation of worksheet and problem sheets quality in based on problem-solving skill using multimode representation were 16 experts consisted of 3 physics lecturers and 13 physics teachers. Meanwhile, the numbers of respondents for the data of main idea comprehension were 27 students at one of senior high school in Bandung.

3. RESULTS AND DISCUSSION

The presented study results consisted of the validation results of worksheet and problem sheets in based on problem-solving skill using multimode representation from the experts and the results of main idea comprehension from the students. The feasibility of worksheet and problem sheets was viewed from two aspects, namely the quality validation aspects by the experts and main idea comprehension aspects by the students. There were 3 components to review the quality validation of worksheet and problem sheets. They were suitability component, a content component, and activity component of worksheet and problem sheets. The average percentage of worksheet and problem sheets quality amounted to 82.9%. The data processing was done by way of the rating scale. The quality validation result of the worksheet and problem sheets in based on problem-solving skill using multimode representation in each component can be seen in Table 1 below:

Table 1 Quality Component of Worksheet and Problem Sheets

No	Quality component of worksheet and problem sheets	Percentage (%)
1	Correspondence between indicator and basic competencies	83
2	Correspondence between indicator and description of content	80.7
3	Correspondence between basic competencies with breadth and depth of content	85.2

The result of main idea comprehension test from the students amounted to 60.67% with a largely readable category. The comprehension percentage category was adapted from Koentjaraningrat, 1997. Main idea comprehension test consisted of 27 respondents with 8 sub-topics, namely heat, black principle, length expansion, width expansion, volume expansion, heat transfer by conduction, heat transfer by convection, and heat transfer by radiation. Table 2 below was the main idea comprehension test result.

Table 2 Score of Main Idea Comprehension Test

Content	Percentage (%)
Heat	70.83
Black Principle	62.5
Length expansion	83.3
Width expansion	62.5
Volume expansion	75
Conduction	56.25
Convection	50
Radiation	25
Average Percentage	60.67

The data processing of quality validation result and main idea comprehension test result of worksheets and problem sheets was used to assess the feasibility of worksheet and problem sheets. Afterward, quantitative data obtained in this study were analyzed. Based on the percentage data of quality test and main idea comprehension test, it could be said that the worksheet and problem sheets in based on problem-solving skill using multimode representations were in a quite decent category.

The improvement of students' problem-solving skill was calculated based on the data results of pretest and posttest by giving worksheet and problem sheets in based on problem-solving skill using multimode representation to the students. The gain score was calculated by using the pretest and posttest scores to see the overall improvement of students' problem-solving skill. After processing the normalized gain data, there was a difference result of pretest and posttest in problem-solving skill between these two classes. The normalized gain score in the controlled class was 0.45 and in the medium category, while the normalized gain score in the experimental class was 0.71 and in the high category. Table 3 below was the improvement of problem-solving skill.

Table 3 Scores Pretest, Posttest, and N-Gain Problem-solving Skill

Class	*Pretest Score*	*Posttest Score*	*N-Gain Score*
Control	10,83	47,71	0,45
Experiment	14,27	70,83	0,71

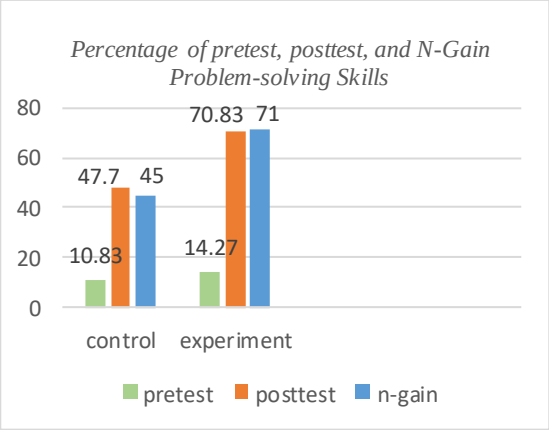

Figure 1 Percentage of Pretest, Posttest, N-Gain Problem-solving Skill

The purpose of these materials effectiveness was to see how much the use of worksheet and problem sheets in based on problem-solving skill using multimode representation influenced the student learning. The effectiveness was reviewed from the increasing difference test and the effect size test. In addition to process the normalized gain data, the effect size of using worksheet and problem sheets in based on problem-solving skill using multimode representation was calculated afterward. Table 4 and Table 5 below were the data processing normality test and effect size.

Table 4 Data Processing Normality Test

t-test	Z_h	Sig.	H_0
N-gain	0.6187	0,000	Ditolak

Table 5 Effect Size Score

m_c	m_E	SD_c	SD_E	SD_σ	d (Cohen)
39,7	60,42	0,02	0,05	13,45	1,54

4. CONCLUSIONS

The worksheet and problem sheets in based on problem-solving skill using multimode representation were in a quite decent category. The N-Gain score of the students' problem-solving skill that was given the developed worksheet and problem sheets in based on problem-solving skill using multimode representation increased by 0.71 and in the high category. Meanwhile, the N-Gain score of the students' problem-solving skill that was given the commonly used worksheet and problem sheets increased by 0.45 and in the medium category. The developed worksheet and problem sheets in based on problem-solving skill using multimode representation were more effective to improve the problem-solving skill of the students compared with the commonly used worksheet and problem sheets.

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Development of Instructional Material Used Multimodus Representative for Learning Physic Oriented on Cognitive Abilities and Science Process Skills for High School Students on Topic Optical Devices

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Abstract – This research aims to develop instructional material using a multi-mode for learning the physics-oriented representation of cognitive ability and students' science process skills sma on the topic of optical devices. The method used in this research is the Research and Development (R & D) by developing methods Representational Approach Learning to Write. The instrument used in this study is to test the main idea of test, questionnaire quality testing, cognitive ability test questions and test questions science process skills. Based on the percentage of legibility test and the quality test showed 87.6%, so the instructional material is categorized feasible. In addition, the increase in cognitive ability has N-gain of 0.61 with the moderate category and improvement of science process skills have N-gain of 0.44 with the medium category. Hypothesis test results indicate that there are significant differences between the improved cognitive ability and science process skills of students using the instructional material with multiple modes of representation and students who use instructional material used in the schools. The impact result of cognitive abilities is 0.60 with moderate category and science process skills 1.13 with the high category. The conclusion of this study is the instructional material representation using multimode effective in improving cognitive ability and science process skills students'.

Keywords: instructional material, multi-mode of representation, cognitive ability, science process skills

1. INTRODUCTION

The 21st century is an era of globalization where in this century there is a change in all aspects of life, including science and technology. One of the government's efforts to create quality human resources is that students have some skills, including critical thinking skills and problem-solving, creative thinking skills, collaboration skills, and communication skills. Skills began to be trained in the science process skills for science process skills is a basic skill experiment as supporting scientific method. Aktamis and Omer (2008) states that the science process skills needed for the manufacture and use of scientific information, conduct scientific research, and to solve problems. Facts on the field based on the observation on a preliminary study researchers showed (1) learning only teach factual knowledge, concepts and principles only; (2) The RPP is composed of teachers already visible indicators of science process skills, but the implementation is not applying the RPP; (3) instructional material used yet teach science process skills.

Ideally instructional material must be in accordance with the curriculum, but the fact that existing instructional material teaches cognitive science process skills are not taught to expect by the curriculum. Based on the analysis instructional material, researchers found that the instructional material A already includes the 45% indicator of science process skills, instructional material B and already includes 44% of the indicators of science process skills, and instructional material C already includes the 55% indicator of science process skills. Results of analysis of the three textbooks do not teach indicators science process skills prediction, hypotheses, and planned experiments. The indicators of science process skills of interpretation, communication, applying concepts and asking questions about the 50% who apply these indicators to an instructional material. To achieve the goal of education is a need for the development of instructional material in accordance with the curriculum.

Based on these problems is suspected that the low causes science process skills are not yet using the scientific approach, in addition to the textbooks used in schools do not teach science process skills. Thus the need for efforts to teach science process skills such as through the development of instructional material in accordance with the curriculum.

2. METHODS

2.1 Method and Design

The method used is research and development (R & D). Borg and Gall (1983) defined that research development as a systematic process to develop, refine, and assess programs and educational products. This research will develop instructional material that can be used by students in the learning process. It can be realized in the form of technical planning objectives and types of activities that will be done at each stage. But in this study, the research and development will only be conducted until main field testing and product revision. Instructional material development was performed using Representational Design Approach Learning to Write (Sinaga et al. 2015). Stage test trials conducted through the early trials instructional material in one school. Stages of product trials conducted at the stage of field trials. Testing is done with experiments, comparing the effectiveness of instructional material developed with the existing instructional material.

2.2 Research Instruments

The instrument used in this study are a readability test, the test quality of instructional material, cognitive test, science process skills test and questionnaire responses of students. The following is a description of each instrument.

a. Readability test
   This instrument to see the students' understanding of instructional material developed.
b. The test quality of instructional material
   These instruments are used to see the quality of instructional material physics using multi-mode representation. The instructional material quality instrument is an instrument rating on the National Education Standards book, consists of four aspects, namely the content of the content, presentation of the material aspects, aspects of language and graphic aspects. then modified with quality instruments developed instructional material Sinaga (2014), which includes the presentation, mode of representation used, the conceptual hierarchy and organization of writing, the key idea of writing, writing rules, and compliance with the indicators of science process skills.
c. Cognitive test
   These instruments are used to seeing an increase in students' cognitive abilities after learning using an instructional material developed.
d. Science process skills test
   These instruments are used to seeing an increase in students' science process skills after learning using an instructional material developed.
e. Questionnaire responses of students
   Questionnaires given to students to determine students' perceptions of instructional material developed.

3. RESULTS AND DISCUSSION

Based on the test results of the main ideas developed in the instructional material is 81.3% level of legibility criteria higher. The main idea of test results can be seen in Table 1.

Table 1 Result of the main ideas test

Sub-Topics	Criteria Readability		
	High	**Medium**	**Low**
Eye	4	3	-
Lup	3	-	-
Microscope	6	2	-
Cameras	9	2	-
Binoculars	8	-	-
Total	30	7	-

Based on the percentage of legibility test and the quality test showed 87.6%, so the instructional material is categorized feasible. In addition, the increase in cognitive ability has N-gain of 0.61 with the moderate category and improvement of science process skills have N-gain of 0.44 with the medium category. Hypothesis test results indicate that there are significant differences between the improved cognitive ability and science process skills of students using the instructional material with multiple modes of representation and students who use

instructional material used in the schools. The impact result of cognitive abilities and science process skills can be seen in the table below (table 2).

Table 2 The impact result of cognitive abilities and science process skills

Aspect	Effect Size	Criteria
Cognitive abilities	0,60	moderate
science process skills	1,13	high

Based on the results of the assessment of the quality of instructional material by teachers and lecturers are 83% stated accordingly. The instructional material has an important role in the learning process so that it affects student learning outcomes in this case cognitive ability. Sinaga et al. (2014) suggest that a good instructional material should include: 1) a description of the concept to be correct and clear. 2) The discussion is arranged in a deductive or inductive, 3) subject matter in accordance with the level of development of learners, 4) using verbal and visual representation, 5) the use of appropriate representation so as to clarify the concept, 6) use of punctuation in accordance with the norms of Indonesian.

Instructional material developed can increase students' cognitive abilities because the instructional material was developed using a multi-mode representation that helps students understand the concepts described. So students who have difficulty in understanding a concept with verbal explanation can be helped with a visual representation so that cognitive ability can be increased. This is consistent with the statement Ainsworth (1999) that multi-representation has three functions are complementary, limiting the interpretation and understanding of the builder. Besides instructional material developed this using simple language that students easily understand explanation of the concept.

Based on data in Table 2 shows that the effect size of science process skills students is 1.13 with high criteria. This finding means that the instructional material developed a major impact on the improvement of students' science process skills. This is because the instructional material was developed covering all aspects of the science process skills. In this instructional material also given examples of exercise science process skills that will help students to have science process skills. The use of multiple modes of representation also supports students to practice some aspects of the science process skills.The findings in this study that the science process skills of students have increased significantly more than the instructional material in schools. This increase is due to the use of multiple modes of representation. The use of multi-mode in the development of physics instructional material developed in addition to improving students 'cognitive abilities may actually enhance students' science process skills. In addition to instructional material developed students are trained to have aspects of science process skills through various activities in accordance with aspects of science process skills.

4. CONCLUSIONS

Based on the findings and discussion, be concluded that quality instructional material using a multi-mode for learning the physics-oriented representation of cognitive ability and science process skills including excellent category. Instructional material using multi-mode representations for learning physics more effectively enhance cognitive abilities compared to using an instructional material in schools. Instructional material using multi-mode representations for learning physics can be significantly more effective in improving students' science process skills compared to using an instructional material in schools.The response of students to the instructional material using a multi-mode for learning the physics-oriented representation of cognitive ability and science process skills to respond positively, they find instructional material developed has advantages compared with instructional material in school.

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Herbs Diversity In Hiking Trail of Mount Andong Valley, Sawit Village, Magelang, Central Java

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1. INTRODUCTION

Indonesia is one of 17 mega biodiversity countries that has 25,000 species or more than 10% of world's flora. One of the many Indonesian plants is the herbs. Herbs is a kind of plants that has phytochemical compound that can be used for medical purpose. Because of the diversities of its herbs species, an information development regarding to the benefit of the herbs is needed. Therefore, a research on herbs diversity and its potential benefit is need to be conducted. The research was conducted along the Andong Mountain hiking trail. The location was chosen because of its high potential of herbs diversity and the lack of information about it on site.

2. METHODS

The research was conducted using an exploration method along the Andong Mountain hiking trail. Exploration method is an observation method through exploring an area to discover a thing or a particular organism that will be elaborate in a form of a description to generate a systematic, factual and accurate picture of researched objects. The steps of the research process:

1. Collecting data and specimens
   - Field data collection which includes temperature, pH, humidity, geographic coordinate
   - Specimens collection and herbarium
2. Morphology description applying *Morfologi Tumbuhan* (Tjitrosoepomo, 1988) while identification based on plant morphology applying *Flora of Java* (Backer, 1985)
3. Analysis of Data

Data collected include in qualitative data, the classified is form of species names and descriptions of morphology in processed whit tabulation. Then do the study of literature related to the benefits and the phytochemical compounds based on the book *Tumbuhan Obat dan Khasiatnya* (Hariana, 2004), *Atlas Tumbuhan Obat Indonesia* (Dalimartha, 2000), 100 *Plus Herbal Indonesia* (Trubus, 2010), *Buku Pintar Tanaman Obat* (Agromedia, 2008) dan *Tanaman Obat Tradisional* (Thomas, 1989).

3. RESULTS AND DISCUSSION

As much as 40 species covering 26 families of herbs such as *Asteraceae, Apiaceae, Portulacaceae, Brassicaceae, Balsaminaceae, Melastomataceae, Caryophyllaceae, Euphorbiaceae, Acanthaceae, Oleraceae, Amaranthaceae, Campanulaceae, Labilatae, Rubiaceae, Gramineae, Liliaceae, Malvaceae, Theaceae, Verbenaceae, Mrytaceae, Phyllanthaceae, Lauraceae, Mimosaceae, Moraceae, Plantaginaceae* and *Camelliaceae*. The indicated herbs were classified according to its anatomy because it was more accesible on the field. The total of the herbs discovered are 24 species of bush, 7 species of shrubs, and 8 species of trees.

1) Bushes
   Ageratum conyzoides L.; *Hydrocotyle sibthorpioides* Lam.; *Portulaca oleracea* L.; *Foeniculum vulgare* Mill.; *Vernonia cinerea* (L.) Less.; *Nasturtium montanum* Wall.; *Centella asiatica,* (L.) Urb.; *Impatiens balsamina* L.; *Melastoma candidum* D. Don.; *Taraxacum officinale* Wiggers.; *Gynura aurantiaca* D.C.; *Eclipta alba* (L.) Hassk.; *Artemisia vulgaris* L.; *Drymaria cordata* (L.) Willd. *ex* J.; *Plantago mayor* L.; *Phylanthus urinaria,* L.; *Justicia gendarussa* Burm.F.; *Jasminum sambac* (L.) Ait.; *Acalypha australis* L.; *Amaranthus spinousus,* L.; *Isotoma longiflora* (L.) C.Presl.; *Coleus scutellarioides,* (L.) Benth.; *Achyranthes aspera* L.; *Hedyotis corymbosa* (L.) Lamk.; *Imperata cylindrical* Beauv.
2) Shrubs
   Cordyline fruticosa (L.) A.Chev.; *Sida rhombifolia* L.; *Cammelia sinensis* (L.) Kuntze; *Lantana camara* L.; *Psidium guajava,* L.; *Sauropus adrogynus* (L.) Merr.; *Heliotropium indicum* L.; *Cinnamomum verum* J.S. Presl.; *Leucaena leucocephala* Lmk. De Wit.

3) Trees
Cinnamomum cassia Nees. *ex* Bl.; *Cinchona ledgeriana* Moens; *Coffea arabica* L.; *Ficus septic* Burm.F.; *Persea gratissima* Gaertn; *Schima wallichii* Choisy.

One of the herbs that has long been known by the local society and has been used as a traditional medicine is Alang-Alang. Alang-Alang is believed by the local society to have the ability to cure heatiness, dehydration, lowering blood pressure and cough. The locals prepare Alang-Alang as a remedy by using the root that has been dried before and the boiled it. The potion resulted from the process will be used continuously until the disease is cured. The phytochemical compounds contained in Alang-Alang are aurondin, fernenol, isoarborinol, silindrin, simiarenol, kampesterol, stigmasterol, β-sitosterol, skopolin, p-hidroksibenzaladehida, catechol, chlorogenic acid, isokloregenat acid, p-kuramat, neoklorogenat acid, acetic acid, oxalic acid, d-malic acid, citric acid, potassium, calcium, imperanere, cylindol-A, graminone-B and 5-hydroxytryptamine. The phytochemical compounds that can affect health are *imperanene, cylindol-A* dan *graminone-B*. *Imperanene* has the effect to hamper platelet aggression. The hampering effect has the same effect as the asetosal used to stop bleeding on heart infark patient. Cylindol-A has the effect to hamper 5-lipoksigenase enzym influenced in the construction of prostaglandin to lesser the pain or myalgia (muscle aches). Other substance contained in Alang-Alang root is cylendrene which has the activity to hamper blood vessel contraction on smooth muscle so that the blood circulation remain fluid. Gramionone-b is useful in hampering the aorta.

4. CONCLUSION

It is concluded that:

1. Herbs have been discovered is as much as 40 species of Spermatophyta as well as 24 species of bushes, 7 species of shrubs and 8 species of trees. The herbs have been discovered covering 26 families.
2. Based on the literature review, the herbs have been discovered contain varied phytochemical compounds which have varied pharmacological functions in accordance with the herbs's ability to cure diseases. The above-mentioned herbs have been used as traditional medicines by the locals of Mount Andong to cure some diseases that commonly occurred such as cough, diarrhea, fever, dehydration as well as an anti-oxidants. The herbs is used traditionally by dehydrated it and or directly boiling it in order to extract it.

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The Difference of Student Scientific Reasoning Development Between The KTSP Curriculum School and the 2013 Curriculum Based

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Abstract – The scientific reasoning in the current research identified as the student arguments based on the *Toulmin Argument Pattern* (TAP). The five components of TAP are *claim, data, warrant, backing*, dan *rebuttal*. This study aimed to find the difference of student scientific reasoning development in two different senior high schools in a biology lesson. According to TAP components, the analysis process of student argument consist of two aspects is the completeness and the coherence of student argument.The research methodology used was the descriptive approach. The participant consists of 85 students (Class X, Class XI, and Class XII) from the KTSP Curriculum based, and 84 students from the 2013 curriculum based school. The data collected through several instruments are scientific reasoning test, the interview, questionnaire analysis, the teaching scenario (RPP) analysis, and the direct observation.The two schools are SMA 1 Tangggulun and SMA Atawazun, located in Subang regency, West Java, Indonesia.The most of the student scientific score indicate that 80 % of KTSP based curriculum student and 81 % of 2013 based curriculum student are arguing scientifically. According to the Toulmin Argument Pattern (TAP), the data score reach level 2 which consist of three aspects *are claim, data, and warrant*,without backing, qualifier, and rebuttal aspect. While, the result of argument coherence analysis, most of the student KTSP based arguments are not coherent (47 %) and the 2013 based curriculum of arguments were coherent (49 %).

1. INTRODUCTION

The curriculum has played an important role in realizing the effective education. It implemented in teaching and learning process. Therefore, the curriculum and learning process are undivided and influenceable each other. The KTSP curriculum had been implemented since 2006. The KTSP implementation is one of innovation in education and its necessary for Indonesia education system. When the innovation happens intentionally, the education will get better. The Innovation is a brand new *tool* to find the answer and to solve a problem. The main idea of KTSP implementation is a process of idea construction, the concept construction, and the process of curriculum policy. These processes which occur in teaching and learning activity aimed to reach a set of student competence as a result of student-environment interaction[6].

The KTSP implementation had begun in 2006. It was one innovation in education. The innovation is not only the system change but also it happens intentionally. The innovation output will affect positively to the education environment. It was a brand new thing in certain situation used to solve a problem. The *brand new thing* could be an idea, an argument, an object or an action. The innovation in education closes meaning to an education conception from the elite to democratic or the equal education, also from the conception which only develop one aspect (knowledge, attitude, or skill) to develope multiple aspects comprehensively. One of the new curriculum advantage (2013) is the acknowledgment of problem-solving, scientific reasoning, communication, and science use as the goal of teaching and learning activity in elementary school (SD), junior high school (SMP), senior high school (SMA), and vocational school (SMK).

The 2013 curriculum was the further development of competence base curriculum (Kurikulum Berbasis Kompetensi) which had been initiated in 2004.and the KTSP in 2006.These fundamental aspects are the attitude competence, knowledge competence, and integrated skill[7]. The science awaking as a learning process outcome comprises the student comprehension of the science essence and the student scientific reasoning [8]. The student ability to reason the evidence and to participate in the scientific argument now to be considered as a reform of science education[9].

2. METHODS

2.1 Research Design

The research methodology used was the descriptive which describes the origin condition comprehensively and specifically. The researcher didn't treat or manipulate, neither change its independent variables. But the researcher tasks are to measure, to analyze, and to describe the experienced condition [10]. The teaching preparation (RPP) and learning activities carried out by the teacher in the current school without any intervention.

2.2 Sample and Population

The populations were all student of SMAN 1 Kalijati and SMA At-Tawazun from class X, XI, and XII. And the sample proportionated to 169 students. In the notice, the SMAN 1 Kalijati used 2013 curriculum, and SMA At-Tawazun was KTSP curriculum based.

2.3 Data analysis

The data analysis had been done by following several procedures. The scientific reasoning identified from the student argument which differentiated by two aspects are the completeness and the coherence of an argument. The argument component identification based on Toulmin Argument Pattern (TAP) which consist of *claim, data, warrant, backing, qualifier, and rebuttal*. The student arguments which had been analyzed are the written answer (during scientific reasoning test) and the interview answer.

3. RESULTS AND DISCUSSION

The information about student scientific reasoning (KTSP school and 2013 curriculum school) was identified from the student arguments which analyzed by two aspects (the component completeness and the argument coherence). The argument components referred to Toulmin Argument Patterns (TAP). The patterns are a *claim, data, warrant, backing, qualifier, and rebuttal*. According to student arguments completeness, it can be categorized into five levels are level 1, level 2, level 3, level 4, and level 5. The result of student arguments described in the chart below :

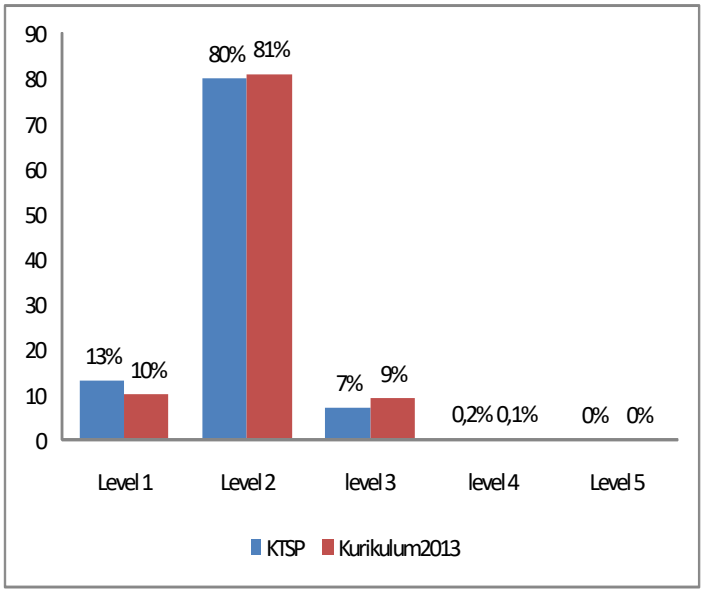

Figure 1. The Graph of student argument component according to TAP

From the Figure 1 above concluded that most of the student argument reach 80 % (KTSP School based) and 81 % (2013 curriculum based), both of them at the level 2. This means the student can only submit the claim completed with *data or warrant* without *backing, qualifier,* and *rebuttal*. And from the figure 1, no one among student argument reach level 5 in the two schools. it caused no student arguments attain the phase of *backing, rebuttal* and even *qualifier.*

The teacher must tell the student scientific approach steps which will be implemented in the teaching activity (2013 curriculum). It must be appropriated with the lesson characteristic because the scientific approach is different for every single lesson. Such as the biology which a part of natural sciences, will associate the scientific approach to the science. Thus, the biology teacher should apply the teaching based on science process

skill. In the other hand, the KTSP teaching ruled out the 5 steps of the scientific process (to observe, to ask the question, to make the experiment, to associate, and to communicate).

According to the teaching activity observation, both of school held the discussion in the main teaching activity, but as the explanation before, although the 2013 curriculum used a scientific approach, but the real fact it's rare, or none teacher used it in the classroom learning (5 steps of scientific approach). If there was use it, the teacher just applies one of 5 steps. For this reason, the student arguments coherence were low from both of school.

The next step is to analyze the student arguments coherence between two different school curriculum based. The quality of an argument is not only decided by the component completeness but also decided by its coherence and relevance. The result of coherence analysis from KTSP school and 2013 curriculum school described by the chart below :

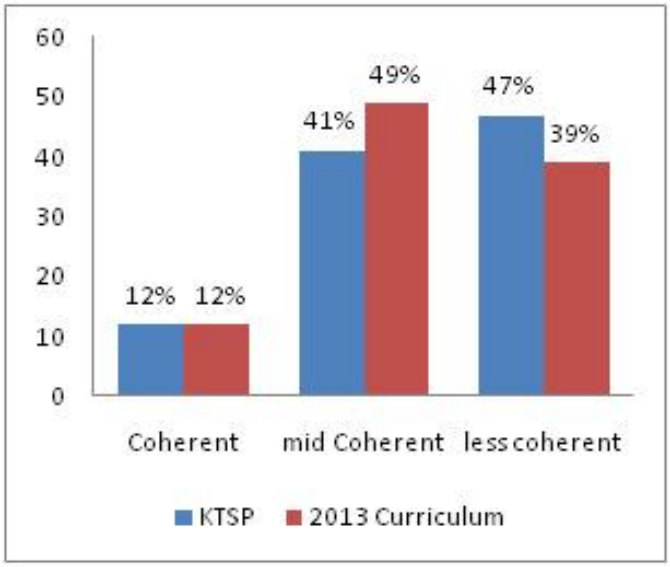

Figure 2. The graph of student argument coherence - KTSP School and 2013 Curriculum based

The graph above describes that most of the submitted arguments are less coherence and coherent enough. The data indicate that the student argument from these school rank TAP component of claim and the basic claim *(data, warrant, and backing*). These arguments are invalid and illogical and irrelevant until couldn't support claim level. For the illogical argument example: (1) "may be under the banyar tree (*beringin*) its no care about its cleanliness" (level:claim, illogical). (2) "that square may be maintained" (level:warrant, it's not true). These facts indicate that most of the student are unskillful arguing. The cause possibly by the teachers who unusual to ask their student for explaining an argumentation (claim) without asking them for reason and evidence.

The result of learning observation in classroom indicates that most of the teachers only gave the comment : "your answer was right or wrong " after asking the student question. They didn't ask the reason and evidence of the answer. Sometimes the teacher asks for evidence to the student, but they answers were inaccurate, so didn't answer the question. the fact similar with the assumption which asserts that ineffective student argument caused by the teacher domination[11].

The observation found that the discussion held in the main learning activity in these school. But, as explained previously, although the 2013 curriculum used the scientific approach teaching, but real activity the teacher didn't. If there were teacher used it, they just apply one of the five steps from scientific teaching activities *(observing, questioning, experimenting, associating or reasoning, and communicating)*. For this reason, we conclude that the argument coherence both of school in the same level (low coherence).

The previous researches about the classroom discussion model indicate that the discussion activity (experiment group) had the higher scientific reasoning score than nondiscussion class (control group). For this reason, the classroom discussion is one activity which could enhance student scientific reasoning. The students will explain their argument each other in class discussion, and they will strengthen it to be accepted by another student.

4. CONCLUSIONS

The argument had an important role to construct the explanation, the model, theory as the scientist who used the argument to connect the evidence to the claim through warrant and backing component [12]. Nevertheless, it is necessary for the senior high school students to habit arguing. In fact, the TAP level of students from both school (Class X, XI, and XII) was low level (level 3,4, and 5). It was caused by unaccustomed submitting the *backing, qualifier, and rebuttal* support *claim* component. This condition indicates that the teacher didn't

elaborate the evidence of student answer. In addition, the arguing difficulties caused also by teacher-centered learning [13]

The creative teaching is needed to get the coherence student argument. It must be done by the teachers because they had the important role in the learning process. Thus, they play an important role in learning activity which could develop the student reasoning skill [14]. In fact, the student had been given the problems stimulated submitting a claim, justifying it, and constructing the rebuttal (disagreement), but the teacher didn't ask the student to do it in daily teaching.

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Implementation Modified Free Inquiry Model for Learning Zoology Avertebrate to Increase Skills of Scientific Work and Creative Character of Biology Student Teachers

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Abstract – Pre-service biology teacher students are demanded to have skills of being a good facilitator in the learning process. However, the student's dependent on information that delivered by the lecturer of zoology avertebrate is still dominated. The learning process can be done with student-centered one so they can achieve their self-autonomy and responsibility to the task that given. Besides, laboratory activities are still verified, so that the character of the creative and scientific work of students is less explored. One of the alternatives learning models that can be applied to foster the skills of scientific work and creative character of student teachers are the model of modified free inquiry. The purpose of this study was to determine the effectiveness of the implementation of Modified Free Inquiry (MFI) models, to know the increase of creative character and skills of scientific work as well as responses biology student teachers after the implementation of MFI models. The method used is the method of quasi-experimental research design with one group pretest-posttest design. Sampling techniques using cluster random sampling from the 3rd-semester student population of 160 people was taken a sample of 40 people. Techniques of data collections using tests, observation, and questionnaire. Data analysis techniques to the analysis item (validity, reliability, the level of difficulty), N-Gain, hypothesis tests, scoring the percentage of creativity and skills of scientific work. The results showed that the average value of the N-gain of 0.65 at medieval criteria. Based on the results obtained t-test p-value less than 0.05 was so effective MFI models to be applied. Creativity of students included in the criteria for an excellent 25%, good criteria 57.5% and 17.5% enough and no student is low creativity. The average percentage of students' scientific work skills by 85.55% including the criteria very well. The percentage of student responses by 54% stronger and 46% criteria very strong criterion, the students gave positive responses to the implementation of MFI learning model.

Keywords: Modified Free Inquiry, Character Creativity, Scientific Working Skill

1. INTRODUCTION

Biology students as teacher candidates are required to have the ability to be a good facilitator in the learning process. Because, in fact, the learning is a process of change students behavior through interaction with the environment (Hamalik, 2007). Students should be able to find their own knowledge and to transform the complex information to solve problems and find all things to their self (Trianto, 2007). Therefore, students should be able to find and develop their own knowledge with the responsibility to plan and driven the learning activities.

The course of zoology of avertebrate is one of the course which integrated with learning theory that face to face in the classroom and lab activities. During this time, students in doing lab activities are always provided guidebooks of lab work by lecturer who are verified, so that, students are less able to develop their creativity and skills of scientific work in designing and doing lab activities. Lab activities that have been done is still verbalism because the students have not been able to find and implement the concept of an inquiry to the optimum, but there are many types of lab work that can be explored by students to support the understanding of the concept.

One of the alternative learning model that can be applied to improve the character of the creative and scientific work skills, namely the model of Modified Free Inquiry. This model is a modification of the model of free inquiry and open-ended inquiry. Learning activities can be done either individually or in groups. In the process of learning by using a modified model of free inquiry, students are able freely to plan and to design lab activities, which involves setting goals, the selection of supported the theory, the selection of tools and materials, analyze the results of observation and report the investigation. Lecturer only acts as a consultant to provide assistance if needed by students during the investigation.

Through the applying of model of *modified free Inquiry*, students are expected to understand the concepts being studied but also gain a learning experience as well as to foster a creative character and skills of scientific work. The character development of creative and scientific work skills through the implementation of the modified free inquiry is also expected to enhance the competence students as teacher candidates of biology, so that, someday when be a teacher can be to learn not just a concept or material but also be able to develop of laboratory skills for learners.

The purpose of this research, are: To determine the effectiveness of the implementation of the Modified Free Inquiry on learning avertebrate of zoology, to know how much Modified Free Inquiry can enhance the character of creative students as teacher candidates of biology, find out how big the model of Modified free Inquiry can improve the skills of scientific work students as teacher candidates, student responses after the application of the model Modified Free Inquiry on learning Zoology avertebrate.

2. METHODS

The type of this research is descriptive quantitative research with quasi-experimental research methods. The research design using one group pretest-posttest design. The research subjects were students of the third semester of Biology Science Education who is currently studying zoology avertebrate courses and lab work. The samples were taken by cluster random sampling as many as 40 people. The data collection technique using research instruments, tests and observation sheet to measure the character of creative students as teacher candidates, observation sheets to measure the skills of scientific work students as teacher candidates, observation sheet product assessment the draft of lab work and practical reports students as teacher candidates, a questionnaire to determine the responses of students as teacher candidates on the model of applied in the learning process. The data were analyzed using normality test, homogeneity test, N-gain, hypothesis testing with T-test and descriptive analysis to scoring percentage of the creativity and skill of scientific work.

3. RESULT AND DISCUSSION

The research retrieval of data begins with a pretest to determine the condition of the initial creative thinking skills of students as teacher candidates of biology, later after the learning model application, then the posttest. The data from pretest, posttest, and N-gain can be seen in Table 1.

Table 1. The value of Pretest, Postest, and N-gain

Numb.	Value	Highest	Lowest	Average
1	*Pretest*	78	39	42,35
2	*Postest*	95	54	76,30
3	N-gain	0,86	0,27	0,65

Based on the table 1 shows that there was an increase in the value of pretest and posttest. N-gain calculation results obtained by the N-gain average of 0.65 including the criteria for medium. The result of the calculation of the percentage of N-gain criteria in classical can be seen in Table 2.

Table 2. The result of criteria in percentage of N-gain

Numb.	Range of value	Students	Percentage (%) Gain	Criteria
1	g>70	5	12,50	High
2	30<g<70	33	82,50	Medium
3	g<30	2	5,00	Low

Based on table 2, it can be seen that the percentage of students who qualify as high gain as much as 12.50%, 82.50% gain medium and low gain as much 5%. With the increase in the value pretest and posttest, then the application of the model has been effective. To examine the effectiveness of the learning model that has been applied to the test which mean to examine the hypothesis by using t-test (t-test) T-test results can be seen in Table 3.

Table. 3. The result of *t-test* which Increase the Character of Creativity

Numb.	Data	Mean	n	t- count	p-value	Criteria
1.	*Pretest*	42,35	40	5,596	0,000	Significant
2.	*Postest*	76,30	40			

Based on Table 3, it can be seen that there is an increase in the value pretest to post-test and the results of t test obtained the p-value of 0.000, with significant criteria. If the P value <0.005 it can be concluded that the modified free inquiry learning model effectively applied to the learning of avertebrate zoology.

The assessment creativity of biology students as teacher candidates include assessment of processes and the products, then calculate the scoring of the observation sheet indicators of creativity, the percentage of results obtained in the classical criteria of creativity that can be seen in Table 3.

Table 4. The Percentage of Creativity Criteria of Students

Numb.	Range of Value	The Number of Students	Percentage (%)	Criteria
1	80 - 100	10 people	25	Excellent
2	61 - 80	23 people	57,50	Good
3	41 - 60	7 people	17,50	Enough
4	21 - 40	-	-	Low

Based on the table 4. It can be seen that the creativity of the students were included in the criteria of an excellent 25%, good criteria 57.50% and 17.50% Enough and no student is creativity that qualify is low.

Based on the observation skills of scientific work biology students as teacher candidates during practical activities and learning activities, from 10 indicators of scientific work skills, observer observing each indicator done by the students. Based on the results showed that the percentage of each indicator of scientific work skills mostly shows the percentage over 80%. From 10 indicators of scientific work skills, only two indicators that have good criteria that compose hypotheses accuracy and quality of design lab inquiry, while 8 other indicators have a very good criteria. The average percentage of Students Scientific work skills by 85.55% including the very good criteria.

The response of Biology students as teacher candidates on the implementation of the modified free inquiry in learning model in the course of avertebrate zoology can be seen in Figure 1.

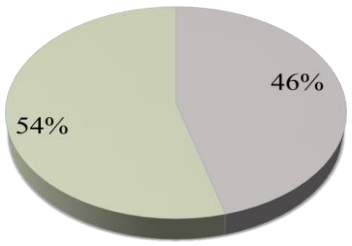

Figure 1. The percentage response diagram of students to implemented the learning model of *modified free inquiry*

Based on Figure 1 can be seen that the results of percentage from student's feedback to the questionnaire have been calculation, it obtained strong criteria as much 54% and a very strong 46%. This shows that students give positive response to the modified free inquiry learning model that has been applied.

The learning model of modified of free inquiry is a collaboration or a modification from previous inquiry two approaches, namely: guided inquiry approach and the approach of free inquiry. Even the problems that be the topic for investigation still provided by the lecturer. However, if there are any students who are unable to resolve the problem, then the guidance can be given indirectly by providing examples that are relevant to the problems faced, or through discussion with students in other groups.

The learning zoology avertebrate with a modified model of free inquiry requires students to solve the problems that raised by validating their hypothesis through laboratory tests in the lab. Investigation or inquiry which is accompanied by laboratory experiments can improve the skills of scientific work response of Biology students as teacher candidates. Through practical activities modified free inquiry, students can work together in a group to produce the best solution. The student group will work effectively in practical activities if the number of group members are little. Students can cooperate in the investigation work, actively participate and share the role with a group of their peers. In addition, in doing lab activities, students are free to meet and find information, the environment and the atmosphere is responsive, clear direction and focus problems can be solved by the students so that the students can think creatively.

4. CONCLUSION

The implementation of the Modified Free Inquiry learning model effectively implemented in the course of zoology avertebrate. The implementation of learning model of Modified Free Inquiry in zoology avertebrate can foster the skill of scientific work and creative character as Biology student teacher. Students responded positively to the implementation of the Modified Free Inquiry learning model, these shown from the results obtained by calculating the percentage of responses 54% in strong criteria and 46% very strong criteria.

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The Experimentation of Number Head Together (NHT) with *Make A Match* Technique Model based on Creativity on The Subject Line and Angle at 7th Grade Students Junior High School in Sukoharjo

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Abstract – Math requires creativity in exploring the issue in order not to replicate the work of others. The higher level of creativity of the students the better ability to solve problems. *Numbered Head Together* (NHT) is a learning model that emphasizes student's creativity to process, and report information from various sources that finally presented in front of the class. *Make A Match* learning model can improve student learning activities, both cognitively and physically, and also can improve student's understanding of the material being studied. This paper will compare the learning model *Numbered Head Together* (NHT) combined with *Make A Match* with Direct learning model . The sample were junior high school students in Sukoharjo on 7th grade. Researchers used three schools for the study, which is a school with a category of high, medium, and low. Each school was taken one class as experiment class, and one control class. Results showed that students in *Numbered Head Together* (NHT) combined with *Make A Match* learning have better achievement than a student in Direct learning model. A student with high creativity has better achievement than a student with medium creativity and low creativity. A student with medium creativity has the same achievement with a student with low creativity.

1. INTRODUCTION

The learning process is an activity that involves teacher and students. Success or failure of learning is influenced by several factors including environmental places and learning process, how teachers deliver material, the condition of students. Each student has different in many ways, one of which is creativity in learning. Creativity is one of the factors that affect student achievement. Mann (2006) in his study claimed that mathematics requires creativity in exploring the issue so as not to replicate the work of others. The higher the level of creativity of students, then the better the ability to solve problems.

In addition to the factors of students, teachers also have to master the material being taught and be able to convey the material well so that students are able to understand it well. To create a learning environment that can encourage learners, teachers can create new innovations in the learning fun. One model of learning that can improve student achievement is learning model *Number Head Together* (NHT). According to Munawaroh (2015) in his study claimed that the learning model *Numbered Heads Together* (NHT) is better than learning cooperative learning model of STAD, although the value of both is equally good. According to Ishabu (2013) in his research, cooperative learning *Numbered Heads Together* (NHT) can improve learning outcomes and creativity of the students to count the number of subjects operating in Class IV SD 63 District Ambon. Fakhrudin (2014) in his research stating that the mathematical skills of students subjected to the PBL learning model approach *Make A Match* higher than those subject to PBL learning model. Rusman (2011) one of the advantages of this technique is the way learners looking for a partner while learning about a concept or topic, in a pleasant atmosphere.

This paper about research learning model type *Number Head Together* (NHT) with *Make A Match* technique. This research is compare learning model type *Number Head Together* (NHT) with *Make A Match* technique with direct learning in mathematics learning based on creativity in line and angle subject.

2. METHODS

This research done in Junior High School in Sukoharjo in Central Java province with the research subjects were students of class VII SMP second semester of academic year 2016/2017. Researchers took this sample of three junior high schools in Sukoharjo, the junior high school who belongs to the category of high achievement, medium achievement, and low achievement based on the mathematics score of National Test last year.

The research is a quasi-experimental research (Quasi-experimental research). Quasi-experimental research is research that aims to get the results expected to be obtained from actual experiments. The situation is not possible to control or manipulate all relevant variables so that experiments can not actually perform.

In this study, intended to provide treatment samples taken from a population. Treatment in this study is learning by using learning model type Head Together (NHT) with Make A Match as an experimental class, and direct learning model as a control.

Instruments used in this research is to test students' mathematics achievement and mathematics learning creativity questionnaire. Mathematics learning achievement test in the form of multiple choice questions of 30 questions about the material lines and angles. While the students' creativity questionnaire of 30 questions.

Analysis of the data used in this study are:

1. Test prerequisites, which consists of a test for normality and homogeneity test
2. Test the balance
3. Test the hypothesis, the ANOVA test two different cell and mean comparison test between column

3. RESULTS AND DISCUSSION

Before the hypothesis test, there are prerequisites test and balance test. The prerequisite test showed that all samples derived from normally distributed population and the population have the same variance. Based on the test result that the balance of the sample in the experimental group and the control group in a state of balance. Further research hypothesis test. Table 1 below shows on average each cell and the mean marginal shown in.

Table 1. Average of Students' score and Marginal Average

Learning Model (A)	Creativity(B)			Marginal Average
	High (b_1)	Medium (b_2)	Low (b_3)	
NHT with Make A Match (a_1)	85.6000	79.8750	78.5714	81.3590
Direct (a_2)	80.4286	75.9655	74.0000	76.7042
Marginal Average	81.9773	78.0000	76.7750	

Summary of two-way analysis of variance with different cells are shown in Table 2 below:

Table 2. Two-Way ANOVA with Different Cell

Source	JK	dk	RK	F_{obs}	F_{tabel}	Conclusion
A	749.7746	1	749.7746	5.0087	3.9073	H_{0A} rejected
B	1189.0189	2	594.5094	3.9715	3.0594	H_{0B} rejected
A*B	9.6170	2	4.8085	0.0321	2.0594	H_{0AB} approved
Error	21406.2200	143	149.6938			
Total	23354.6304	148				

Based on the results of two-way ANOVA in Table 2, it can be concluded that:

1. learning model influence on mathematics achievement students.
2. students' creativity effect on mathematics achievement students,
3. there is no interaction between learning models and creativity of student learning.

Based on the calculation results obtained H_{oA} ANOVA rejected. Means learning model provides the same influence on mathematics achievement of students. Students are given the learning model type *Number Head Together* (NHT) with *Make A Match* have mathematics learning achievement better than students who are given direct learning model.

4. CONCLUSIONS

Based on the results of hypothesis testing in the previous section, can be concluded that:

1. The learning model influence on mathematics learning achievement student. The student that are given *Number Head Together* (NHT) with *Make A Match* technique learning model have mathematics learning achievement better than students who are given direct learning model.
2. The students' creativity gives effect on mathematics achievement students, that is:
   a. mathematics achievement of students with high mathematics learning creativity is better than mathematics achievement of students with medium mathematics learning creativity,
   b. mathematics achievement of students with high mathematics learning creativity is better than mathematics achievement of students with low mathematics learning creativity,

c. mathematics achievement of students with medium mathematics learning creativity was as good as the students with low mathematics learning creativity.

3. there is no interaction between learning models and creativity of student learning.

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Design Datawarehouse For Disaster Mitigation By Star Flake Method

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Abstract – The responsibility to dissemination of knowledge about disaster mitigation can not be charged to center and local disaster management only, but should be assisted by all levels of society. To create a learning media disaster mitigation needs to be a great repository named is datawarehouse and knowledge management applications to be able to accommodate historical data. The research methodology in the development of knowledge management for datawarehouse design uses a nine-step methodology. As a first step needs to be determined what information and knowledge needed in disaster mitigation to be answered in the final step in the methodology. Results of design datawarehouse designed starflake design chosen because it requires changes in the dimensions of time and area by category for mitigation strategies and mitigation action.

Keywords : Disaster Mitigation, Star Flake, Design, Datawarehouse, Knowledge Management

1. INTRODUCTION

Based on the survey that was conducted by our team earlier in Yogjakarta and Kediri in 2014-2016, the disaster management by local government agencies are still too focused to the prevention of post-disaster than before the disaster. Whereas natural disasters in Indonesia came and went almost in all regions of Indonesia. Resulting in the quality of service agencies that are still far from the expectations of society.

"Mitigasi Bencana Setengah Hati" as the title is written in the print media Kompas dated December 26, 2016 [1] in commemorate 12 years of the tragedy Aceh tsunami. That title appears when observed many people have not to learn disasters in Indonesia, whereas through disaster mitigation, knowledge of the community will be increased to be able to know the risks before the disaster that material losses and casualties can be minimized and life society became organized and better. The responsibility of learning on disaster mitigation is the duty of all society elements because of the vast territory of Indonesia and also in a slab of earth disaster-prone. On this basis, moved to create a website disaster mitigation knowledge management in order to improve public knowledge in the face of disaster. Problems arise when designing a database structure that will be effective in disseminating knowledge to the public.

2. METHODS

This study uses Nine Step Methodology of Kimball [2] which has nine steps as follows: [a] Choosing the process, [b]Choosing the grain, [c] Identifying and conforming the dimensions, [d] Choosing the facts, [e] Storing pre-calculations in the fact table, [f] Rounding out the dimension tables, [g] Choosing the duration of the database, [h]Tracking slowly changing dimensions, [i] Deciding the query priorities and the query modes.

3. RESULTS AND DISCUSSION

3.1. Choosing the Process

The process refers to the subject matter of the piece of data marts. The data mart to be built should fit the needs and can address issues that are important. Election process is conducted to clarify the limits of the data warehouse that will be created.

Subject matter:

a) What type of disasters occur frequently in Indonesia?
b) How much material damage and casualties caused by natural disasters?
c) Which is the province affected?
d) What causes each kind of disaster?

e) How to reduce the risk of various types of disasters?
f) What Local Wisdom is relevant to disaster mitigation?
g) How effectively spread knowledge of disaster mitigation?

3.2. Choosing the Grain

Selection of grain means determining exactly what is presented by the record in the fact table.

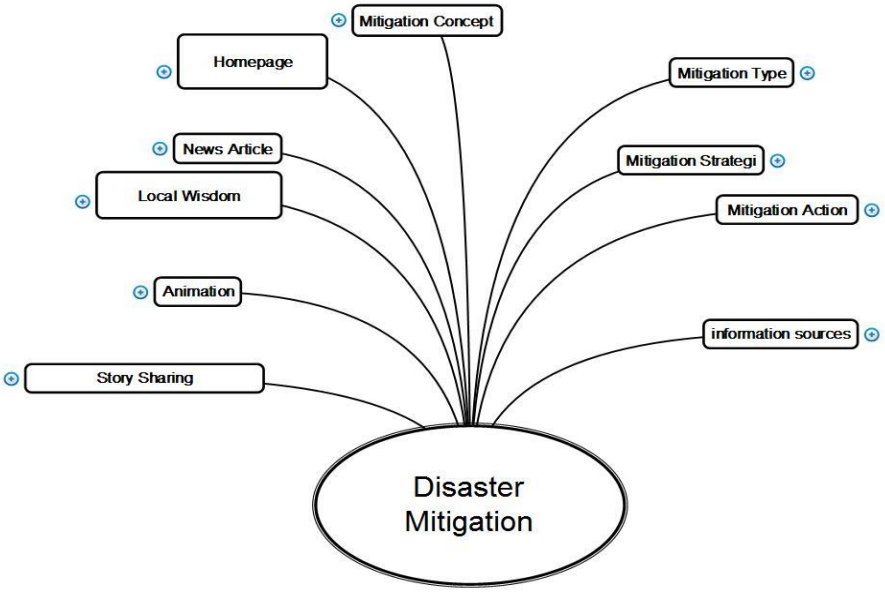

Figure 1. Primary Grain

3.3. Identifying and conforming the dimensions

Dimension to define the context for asking questions about the facts in the fact table. Set of dimensions built by either creating a data mart can be understood and easy to use. Dimension to identified in sufficient detail to describe data such as client and properties of grain right.

If there is a dimension which appeared in two data marts, then both the data mart must have the same dimensions, or one of the data mart is a subset of the mathematical Data Mart others. Only in this way the two data marts share one or more dimensions in the same application. When a dimension is used by more than one data mart and these dimensions are not synchronized between data mart then the entire data warehouse will fail, because the two data marts can not be used at the same time. At this stage, adjusting the dimensions and grain.

Table 1. Dimension and Grain

Grain\ Dimension	Type of Disaster	Mitigation Concept	Mitigation Strategy	Mitigation Action	News Articles	Local Wisdom	Animation	Story Sharing	Disaster Information	Sources Information
Categories	✓	-	✓	✓	✓	✓	✓	✓	✓	✓
Time	-	-	✓	✓	✓	-	-	✓	✓	✓
Region	-	-	-	-	✓	✓	-	✓	✓	-

3.4. Choosing the Facts

Grain from the fact table determines which facts that can be used in the data mart. All facts should be stated based on the level implied by the grain. Facts can be added into the fact table at any time to record these facts are consistent with the grain of the table. Selection of facts seen in the fact constellation schema below:

3.5. Storing Pre-Calculations in the Fact Table

Once the facts have been selected, then performed the review to determine whether there are facts that can be applied to preliminary calculations. The results of this initial calculation is stored in a fact table. Calculate fact share stories, animations, news articles, mitigation strategies, mitigation action and local wisdom are a number of records which is the amount of data for each process that is worth 1 (one) for each record in the fact table the type of disaster. While the other table is not connected in the unity of the fact table.

3.6. Rounding out the dimension tables

In this stage, back to the dimension table and add a text description of the dimensions are possible. Picture of the text should be easy to use and understandable by the user.

Table 2. Dimension Table

Dimension	Field	Description
Time	Date	— There is the fact table Mitigation Strategy, Mitigation Action, Article News, Story Sharing, Disaster Information, Sources Information — To determine the development of the disasters that can be traced patterns of knowledge evolved.
Region	Region	— There on Article News, Local Wisdom, Story Sharing and Disaster Information — To find out which regions in Indonesia are often affected.
Category	Category	— All facts table has a category, except table Mitigation Concept

Star Flake selected as the scheme because not all fact table to do the process of de-normalize, as well as efficient and flexible for further development

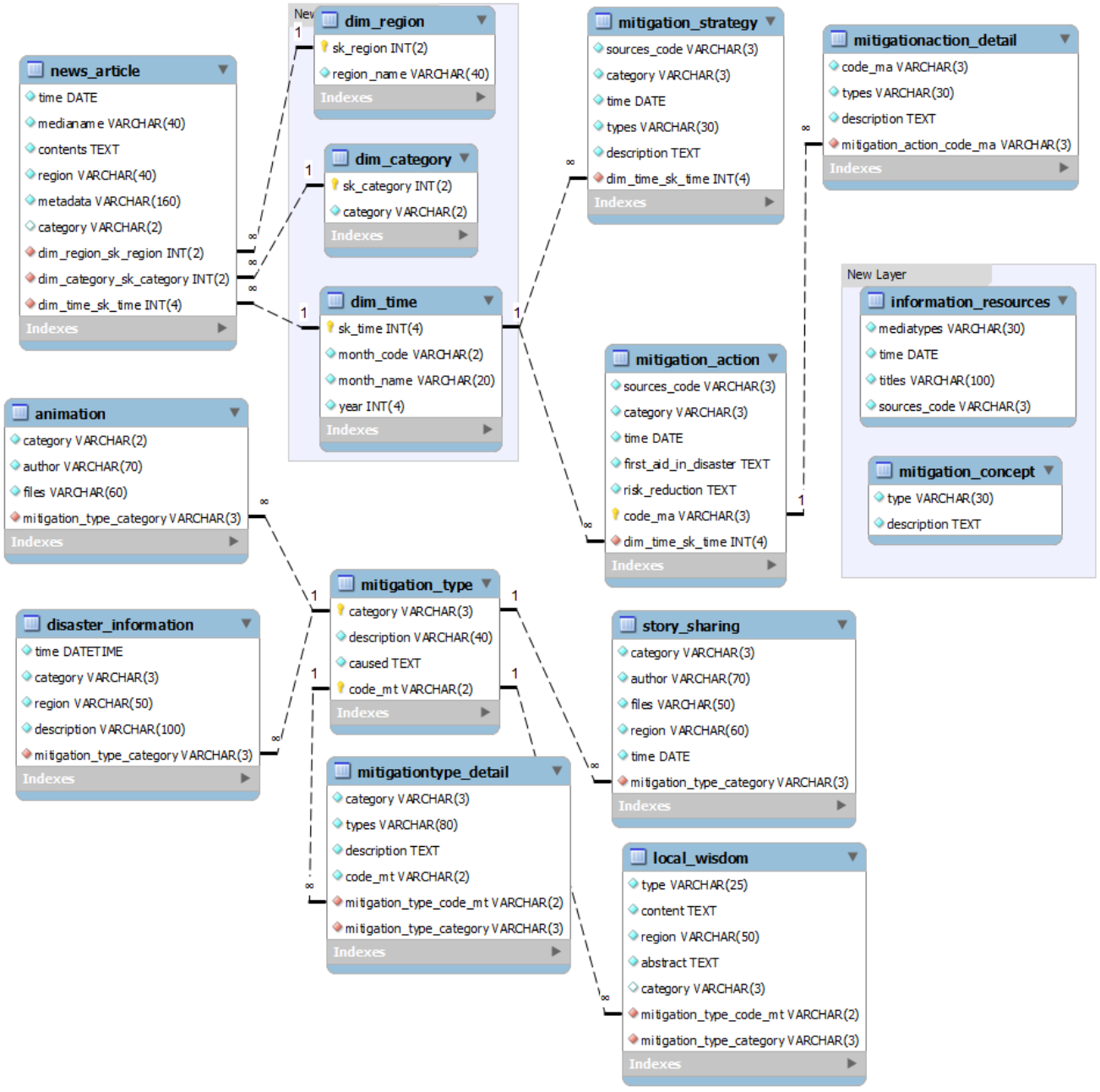

Figure 2. StarFlake Schema

3.7. Choosing the duration of the database

To obtain a change of concepts and knowledge which occur so as to find a different pattern.

Table 3. Duration of The Database

Application Name	Database	Databases have been there since Year	Time Data Log Into Datawarehouse	Period of the data is inside Datawarehouse
Disaster Mitigation	Table News Article	2012	2012-2015	3 years
	Table Mitigation Concept, Mitigation Strategy, Mitigation Action	1994	2015	21 years

3.8. Tracking Slowly Changing Dimensions

Viewing the change of dimension in the dimension tables can be done in three ways, namely directly replace the dimension table, forming a new record for any new changes or changes in the data that make up the new column is different. In a knowledge management system is used to add a new record so that the data / information long stay there so it can be distinguished from the pattern of knowledge that occurs.

Table 4. Changing Dimension Knowledge

Dimension Name	Table Can Be Changed
Time	Mitigation Strategy, Mitigation Action

3.9. Deciding the Query Priorities and The Query Modes

In the design of data warehouse, storage capacity is one aspect that needs to be considered in addition to the sorting order fact table on the disk. The storage capacity of concern in the design of this system is the fact table animation, need to be regulated pixel efficient but still convenient to be shown.

Table 5. The Query Modes

What type of disasters occur frequently in Indonesia?	MDX Query in Dim_Category and News_Articles
How much material damage and casualties caused by natural disasters?	MDX Query in Dim_Time and News_Articles
Which is the province affected?	MDX Query in Dim_Region and News_Articles
What causes each kind of disaster?	SQL Query in Mitigation_Type and Dim_Category
How to reduce the risk of various types of disasters?	SQL Query in Mitigation_Action and Dim_Category
What Local Wisdom is relevant to disaster mitigation?	SQL Query in Local_Wisdom and Dim_Category
How effectively spread knowledge of disaster mitigation?	Website is equipped with popular social media such as twitter, facebook or instagram to disseminate information and knowledge of disaster mitigation

4. CONCLUSIONS

Results of system requirements analysis using nine methodology, data warehousing scheme used is Star Flake is a hybrid structure that contains a combination of Star schemes that denormalized and Snowflake that normalized. Structure Star Flake of disaster mitigation requires historical data that exist in the data store "news_article" containing disaster events that have happened to be learning the present and the future. Table dimension of "dim_category" used as sorting various types of hazard, table dimension of "dim_provence" is used to determine which regions in Indonesia are often affected. While the dimension table of "dim_time" is used to determine the development of the disasters that can be traced patterns of knowledge evolved.

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Effect of Fertilization of Takakura with Addition EM4 to Growth Seedling Dates (*Phoenix dactilivera*)

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Abstract - Date Palm is desert plants that have many trouble for seedling and germing in Indonesia. Actually, this seedling can be successfully cultivated by the fertilization. The aim of this research is to know the effect of takakura organic fertilization with EM4 and liquid fertilizer in addition for the growth of Date Palm. The research was done at Laboratory Biology and Structure of Plant Function, Faculty of Science and Mathematics Diponegoro University. Research design that used for this research was the Complete Random Design just with single factor, witch the factor was from the fertilizer that were used. The treatment was Po (without fertilizer), P1 (fertilized with takakura organic fertilizer and EM4), and P2 (fertilized with liquid fertilizer). Then the data was analyzed using Analisis of Variance (ANOVA) then continued with Duncan Multiple Range Test (DMRT) if there was show some significant rate at 95% to find the real difference. The parameters used were the plant height, number of leaves, wet weight, and root length. The result shows organic fertilizer treatment takakura with the addition EM4 and liquid fertilizer had no significant effect but tends to increase plant height (P1) is 30.03 cm, leaf number (P1) 4 strands, plant fresh weight (P1) is 21.16 grams, and root length (P2) is 48.66 grams. Fertilizing with organic fertilizer with the addition of EM4 takakura better growth compared to liquid organic fertilizer and control.

Keywords: takakura, liquid fertilizer, growth, seed

1. INTRODUCTION

Dates are a tough desert plants cultivated in Indonesia because normally live in warmer temperatures, but the attempt to cultivate them still do. The first attempt to do is hard seeds germinate. This is done by breaking the dormancy of hard skin by soaking them in strong acids such as HCl, H_2SO_4 or chromic acid.

Application of chemical fertilizers on an ongoing basis with a dose increase each year can cause the soil to be loud and disturbed soil nutrient balance. Biological properties of soil will decrease as well as the activity of microorganisms in the soil is disturbed so that the decomposition of soil organic matter is inhibited and reduced soil fertility [1]. In contrast, application of organic fertilizer is highly recommended as an organic fertilizer has many advantages compared to chemical fertilizers, which are more environmentally friendly, able to maintain a balance of land, increasing land productivity as well as reducing the environmental impact of land.

Some of the roles of organic fertilizers in the soil, among others: (1) improve soil structure with the aim of processing the soil becomes easier because the soil becomes lighter and crumbly, (2) Organic fertilizers contain elements of macro and micro nutrients required by plants, (3) microbial-microbial contained in the organic manure helps improve soil fertility through the binding of nitrogen, and also helps in the mineralization process chemical compounds in the soil, (4) organic fertilizers also contain hormones and antibiotic substances essential for plant growth [2]. Organic fertilizers must have a C/N ratio which is the ratio of carbon content (C) and nitrogen (N) in the ingredients. The C/N soil is 10-20 so that the material has a value of C/N approaching the C/N soil, can be directly used [3].

Takakura is a household scale composting methods and scale of the region, does not require a large area and capacity to match the volume of domestic waste that is disposed of by households daily. With this method, household organic waste can be managed easily, odorless, does not take much time in processing and results are directly used[4]. There are two types of liquid organic fertilizer made through the composting process. The first is a liquid organic fertilizer which is prepared by dissolving the organic fertilizer that has been finished or semi-finished into the water. This type of suspension liquid fertilizer solution is less stable and easy to settle. Nutrients contained in the liquid fertilizer solution of this type is really liquid. So the solution is more stable and if left to settle. Therefore, the nature and characteristics were different with liquid fertilizer made from solid fertilizer dissolved in water. Leaves and stems can absorb fertilizers applied directly through the stomata or pores that exist on its surface. Theoretically, the only plant capable of absorbing the nutrients available in the soil no more

than 2% per day. On the leaves can be estimated to number no more than 2%. Therefore, liquid organic fertilizer on the leaves should be diluted first [5].

Growth is one aspect of the development of plants in addition to differentiation, both at the cellular, tissue, organ or individual as a whole. Growth is a quantitative aspect of development is irreversible. Their division and cell expansion caused by the synthesis of organic compounds on the absorption of nutrients and photosynthesis, at the cellular level. Research by Syafrina [6] showed that administration of some types of organic materials and liquid organic fertilizers provide results that organic materials significantly influenced plant height, number of branches, shoot dry weight, number of pods, root dry weight and the weight of 100 seeds of green beans.

2. METHODS

The study was conducted in the Gardens of Biology and Lab. BSF Plant Biology Department, FSM UNDIP. The research was conducted from January s.d July 2016

a. Ingredients: Seeds of date palm (*Phoenix dactaylifera*), takakura organic fertilizer and liquid organic fertilizer, water, and soil.

b. Tools: Tools used in this research that label, stationery, cameras, digital scales Ohaus, oven and newsprint, sprayer, pot.

2.1 Ways of Working

Polybag size 25 cm x 25 cm 12 units prepared in advance. Then each polybags filled with soil. Prepared by 20-soaked palm seeds role in hydrochloric acid (HCl) 50% for 30 minutes, then wash water for a minute and then planted in the polybag. Planting is done by making a hole in the ground in a polybag then put the seeds in a hole approximately 1 cm deep. After 3 months of age have 9 seed dates are homogeneous in the polybag. Watering is done using water as much as 50 mL per seedling every day aiming seeds get a water supply that will be used for photosynthesis so that growth is not hampered.

Table 1. Treatment

No	Treatment	Treatment	g/plant
1	Control 1 (P0)	Without fertilizer	
2	Treatment 1 (P1)	Organic fertilizers takakura with EM4	200 g/plant
3	Treatment 2 (P2)	Liquid organic fertilizer (PO)	5 mL/plant at 7 o'clock and 16 o'clock

Fertilization is carried out according to treatment that uses organic fertilizer takakura with addition of EM4 at a dose of 200 grams at any time polybag seedlings age of 4 months and 5 months, being a liquid organic fertilizer (PO) at a dose of 5 mL / polybag plants every morning and evening. PO Spraying is done for 3 months starting after the plant was 3 months to 6 months. As for fertilization treatment do not control at all.

Growth parameters: (1) High-Seed: palm plant height measurements made after 6 months old plants design way measured from the stem to the tip. (2) Number of Leaf Seed: Counting the number of leaves is done by counting the number of leaves of date palm plants in each polybag. (3) Seed Wet Weight: The weight of the wet plant age 6 months is done by then revoked plants in fresh condition weighed. (4) The length of the roots of seedlings: The length of the roots of plants is measured by how measured from the stem to the tip of the root.

2.2 Research design and data analysis

This study was conducted using a completely randomized design (RAL) of the factors, the type of fertilizer with 3 treatments and each treatment three replications. Data were analyzed by Analysis of Variance (ANOVA), as shown significant results then continued by Duncan's Multiple Test (DMRT) at the level of 95%.

3. RESULT AND DISCUSSION

Table 1. Average seedling height (cm), number of leaves, seeds wet weight (g) and root length (cm) seeds of date palm (Phoenix dactylifera L) after treatment.

Treatment	Plant height	Number of leaves	Gross weight	Long roots
Po	28,66	4,66	13,66	19,66
P1	30,03	4,00	21,16	26,66
P2	30,00	5,33	13,86	48,66

Description: Po = control, P1 = fertilized with compost takakura, P2 = fertilized with liquid organic fertilizer (po)

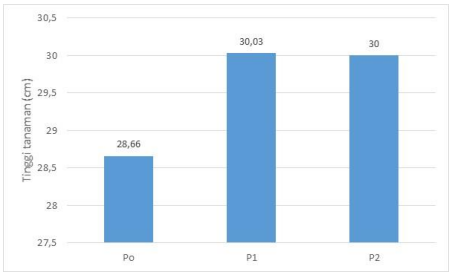

Figure 3. Histogram palm plant height after treatment

Based on the test C/N ratio with addition of EM4 fertilizer takakura shows the value of C/N ratio of 20.61. This is in accordance with the opinion of Damanhuri and Tri (2007) which states that the value of C/N soil 10-20, so the fertilizer takakura C/N it approached the ground and can be directly used for treatment of high mean The results of observation of plants / seedlings of palm age 6 moon presented in Table 1 which shows that the average height growth 6 months old palm plant is fertilized with organic fertilizer takakura followed by underneath fertilized with a liquid organic fertilizer and control. Results of ANOVA analysis at a level of 95% indicates that fertilization treatment did not significantly affect plant height. But fertilization treatment plants tend to show a high increase when compared with the control without fertilization. According to Guadalupe (2000), one of the advantages of organic fertilizer is that it can improve soil structure with the aim of tillage easier because the soil becomes lighter and crumbly. Furthermore, the absorption of nutrients from the soil to become more optimal. Nutrients will be used in the metabolic process and will produce energy in the growth meristematic tissue. Hormone auxin plays to spur cell division in the apical meristem so that the stems be growing longer. Higher plants are given a lower liquid organic fertilizer because the liquid organic fertilizer nutrient content of less than takakura fertilizer. Liquid organic fertilizer digestible nature leaves but volatile and unstable and only contain micro-nutrients, so it must be absorbed by plants.

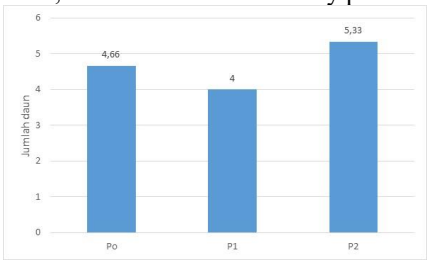

Figure 4. Histogram amount palm plant leaves after treatment

Results of ANOVA analysis of fertilization on the number of leaves showed no significant or not significant (Table 1), however, shows that there is a tendency to increase the number of leaves. Takakura fertilizing with manure treatment showed that the leaves wider and wider. This suggests that organic fertilizers can improve the structure of the media and provide nutrients that are used for vegetative growth such as leaves. According to Gardner et al. [7] the number and size of leaves is affected by genotype are internal factors of the plant. It is also in accordance with the opinion of Pranata [8] that N is the nutrient that most influence on the growth and development of the leaves. Levels of N which generally produces leaves many more and larger.

Results of ANOVA analysis of fertilization on plant fresh weight showed no significant or not significant (Table 1), however, shows that there is a tendency to increase the plant fresh weight. It shows that the availability of essential elements such as nitrogen, phosphorus, and potassium contained in the organic fertilizer takakura more balanced and sufficient for the growth of plants, so plants / seedlings of palm has a higher wet weight. In quality organic fertilizer takakura superior to liquid organic fertilizer. In accordance Poerwowidodo [9] that the nitrogen

contained in organic fertilizers act as building blocks of protein, while phosphorus and calcium play a role in promoting cell division in the meristem tissue and stimulates the growth of roots and leaves. As a result, the level of absorption of nutrients and water by plants to optimum limit will be used for the extension, division, and cell differentiation.

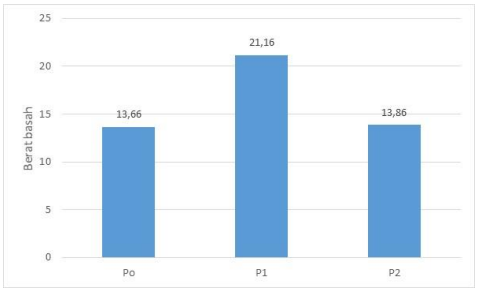

Figure 5. Histograms palm plant fresh weight after treatment

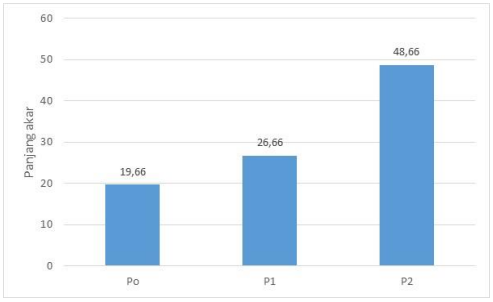

Figure 6. Histogram palm plant root length after treatment

Results of ANOVA analysis of fertilization on root length showed no significant or not significant (Table 1), however, shows that organic fertilization spurred a tendency increase in length of plant roots. Fertilization treatment with a liquid organic fertilizer showed the longest roots. After morphogenesis, the roots will grow by interfering with certain hormones work actively in the cell wall to stretch.

4. CONCLUSION

Organic Fertilizing no real effect on the growth of seedlings but tends to increase plant height, leaf number, fresh weight and root length. Optimal growth achieved by seed plant seed plants with organic fertilizer treatment takakura.

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Application System of Mamdani Fuzzy Inference to Determine Teacher Achievement in Vocational High School

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Abstract – In the process of learning, teachers have a crucial role as they are required to manage or organize the learning process to run in a good condition. Selection of outstanding teachers is meant to encourage motivation, dedication, loyalty and professionalism of teachers, which is expected to be a positive influence on performance improvement. In this paper, the authors use matlab program to develop a Fuzzy System Inference (FIS) that determine the outstanding teachers. The variables that are used in this system are teacher performance, creative and innovative work, and mentoring students. Designing a system to obtain the output is done in several stages: fuzzyfication, inference rule, and discernment (defuzzyfication). This system is expected to assist schools in making an informed decision to determine the outstanding teachers in their schools. The calculation in determining the application of outstanding teachers that developed using mathlab program shows that the calculation on mathlab applications have an average difference of 0.51 compared with manual calculations

1. INTRODUCTION

Teachers are professional educators with the primary task of educating teaching, guiding, directing, assessing, and evaluating students in early childhood education, formal education, primary education and secondary education [1]. In the process of learning, teachers have a crucial role as a teacher is required to manage or organize for the learning process to run in a good condition. Due to continued development now, teachers should continue to improve their performance to produce qualified human resources, skilled, productive, and competitive.

Global era requires high-quality human resources and ready to compete, both at the level of national, regional, and international levels. Selection of outstanding teachers is meant to encourage motivation, dedication, loyalty and professionalism of teachers, which is expected to be a positive influence on performance improvement. The performance improvement can be seen from the quality of graduate as qualified human resources, productive, and competitive.

In determining the outstanding teachers, there is a need for a solution in the developing and designing a computerized system with the Aritficial Intelligence (AI) ability to optimize the system. Application of fuzzy logic in dealing with the problem is based on reasons, namely: (1) the mathematical concept of fuzzy is easy to understand; (2) The fuzzy logic is very flexible; (3) The fuzzy logic can tolerate data that is not appropriate; (4) The fuzzy logic is able to model nonlinear functions are very complex; (5) The fuzzy logic can develop and apply the experiences of experts directly without having to go through the training process; (6) The fuzzy logic can be cooperated with the control of conventional technique; and (7) the fuzzy logic is based on natural language [2].

The development and design to be applied here is outstanding teachers determination system using fuzzy logic methods *mamdani*. Mamdani FIS is the most known or used in developing fuzzy models. The output of the system is generally defuzzified resulting fuzzy sets are combined using aggregation operator from the consequent of each rule of the input [3]. This development can impact on the ease in determining the outstanding teachers at the school.

Based on the problems background described above, the purpose of writing this article is used to: (1) determine the category of outstanding teachers at Vocational High School that are applying mamdani fuzzy logic method; and (2) generate mathlab program developed by mamdani method to determine the outstanding teachers at Vocational High School.

2. METHODS

In the process of calculating the fuzzy logic there are three steps that must be passed, namely: (a) fuzzyfication, (b) inference engine, and (c) deffuzyfication [4].

2.1 Fuzzyfication

Fuzzyfication is the process of mapping the input values (crisp input) coming from controlled systems (massive non fuzzy) into a fuzzy set by its membership function. The fuzzy set is a fuzzy input which be processed in fuzzy on the next process. For changing the crisp inputs into fuzzy input must be determined by the crisp membership function for each input, then the process will take crisp fuzzyfication input and compare it with the existing membership function to generate the value of fuzzy input. In this case, the crisp input variable is the teacher performance, work creative or innovative, and mentoring students. In fuzzyfication, range is determined by the opinion from education expert. The description of each variable can be seen in Table 1.

Table 1. The Association of Fuzzy

Type	Linguistic Variable	Characteristic	Range
Input	Teacher Performance	Very Low	0-40
		Low	30-60
		High	50-80
		Very High	70-100
	The Work of Creative or Innovative	Very Low	0-40
		Low	30-60
		High	50-80
		Very High	70-100
	Mentoring Students	Very Low	0-40
		Low	30-60
		High	50-80
		Very High	70-100
Output	Teacher Achievement	Poor	0-60
		Fair	60-80
		Good	80-100

2.2 Inference Engine

The inference engine is one of main components of expert system that influences the performance of expert system [5]. Inference engine is applied to determine the category of outstanding teachers using the input variables of teacher performance, the work creative or innovative, and mentoring students. In this program, there are 64 rules that can be used in Inference engine. Below are the examples of rules that used in program.

[R1] IF teacher performance is very low AND the works of creative and innovative is very low AND mentoring students is very low THEN teacher achievement is poor

[R12] IF teacher performance is very low AND the works of creative and innovative is high AND mentoring students is very high THEN teacher achievement is fair

[R27] IF teacher performance is low AND the works of creative and innovative is high AND mentoring students is low THEN teacher achievement is poor

[R32] IF teacher performance is low AND the works of creative and innovative is very high AND mentoring students is very high THEN teacher achievement is good

[R46] IF teacher performance is high AND the works of creative and innovative is very high AND mentoring students is low THEN teacher achievement is good

[R59] IF teacher performance is very high AND the works of creative and innovative is high AND mentoring students is high THEN teacher achievement is good

[R64] IF teacher performance is very high AND the works of creative and innovative is very high AND mentoring students is very high THEN teacher achievement is good

2.3 Deffuzyfication

Defuzzyfication is a process that maps a fuzzy set to a crisp set [6]. The input of defuzzification process is a fuzzy set that obtained from the composition rules of fuzzy, while the resulting output is a fuzzy set of numbers in the domain. So that, if the value that is given a fuzzy set within a certain range, it must be take a certain crisp values as its output. In this report, the deffuzification using centroid method with the formula:

$$y* = \frac{\sum y\mu_{R(y)}}{\sum \mu_{R(y)}}$$

3. RESULTS AND DISCUSSION

In this case, the proposed program is used to make decisions on the determination of teacher's achievement at school. Sample data were examined and randomly selected for the present study. The development of the program is conducted with the assistance of Matlab. Figure 1 and Figure 2 shows snapshots of the results of work done in Matlab. Meanwhile, the results for determination of teacher achievement by some examples of input can be seen in Table 2.

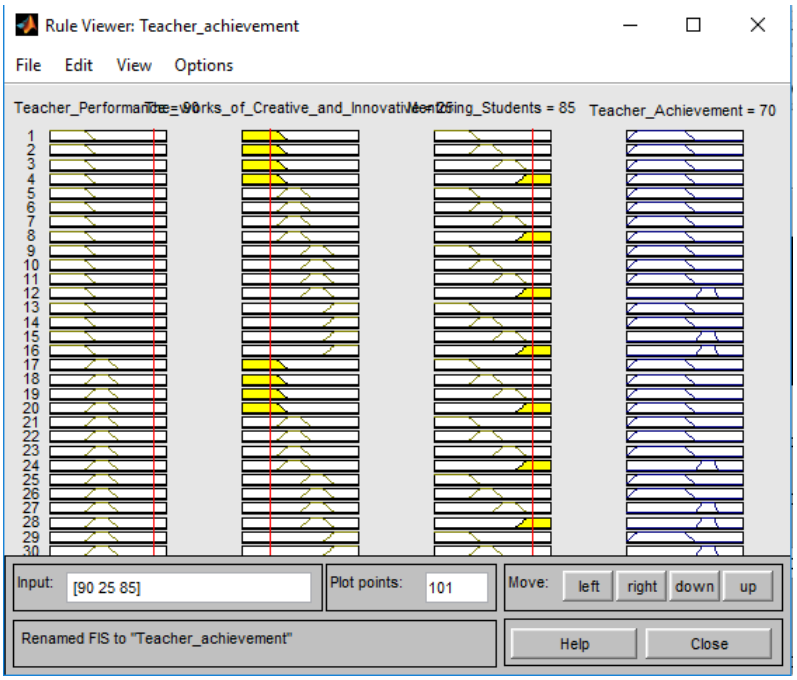

Figure 1. Rule viewer for inputs and output parameter

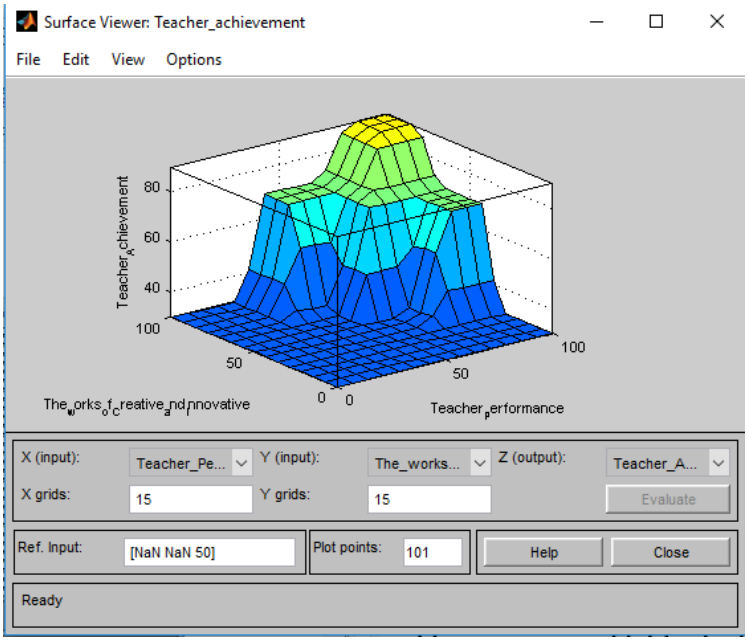

Figure 2. Surface Viewer of Input and Output Parameters

Table 2. Inputs, Outputs, and Results for selected case

Teacher	Input Variable			Output Variable		Difference
No.	Teacher Perform (0-100)	Works Creative (0-100)	Mentor-ing Students (0-100)	Teacher Achievement (0-100) Matlab Calculation	Teacher Achievement (0-100) Manual Calculation	Difference
1	5	10	15	30	29.8	0.2
2	35	55	60	39.6	39.9	0.3
3	70	55	30	30	29.8	0.2
4	70	80	30	70	70.3	0.3
5	85	85	10	70	70.2	0.2
6	90	25	85	90	90.8	0.8
7	90	90	95	90	90.7	0.7
8	75	75	75	80	81.2	1.2
9	60	60	50	70	70.2	0.2
10	55	65	95	80	81	1

4. CONCLUSIONS

In the process of learning, teachers have a crucial role managing or organizing the learning process so that it can run in a good condition. Therefore, Selection of outstanding teachers is meant to encourage motivation, dedication, loyalty and professionalism of teachers, which is expected to have a positive influence on performance improvement. This paper presents how fuzzy inference system can be used to determine teacher achievement. From the results of research conducted from the early stages to the implementation test of FIS Mamdani for the determination of outstanding teachers, it was concluded that the determination of outstanding teachers with FIS Mamdani was more accurate and objective than the usual methods that were used (by meeting and deliberation) hence the application of FIS Mamdani can help in the accurate determination of outstanding teachers. The calculation in determining the application of outstanding teachers that developed using mathlab program shows that the calculation on mathlab applications have an average difference of 0.51 compared with manual calculations.

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The Experimentation of TAPPS Learning Model Based on Emotional Intelligence on the Circle Subject XI Grade Public High Schools in State District Sukoharjo in the Academic Year of 2016/2017

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Abstract – In TAPPS, students work in pairs to solve the mathematics problems. Students who participated in TAPPS groups were assigned a listening partner and verbalized their thought processes in troubleshooting tasks. The purpose of this research is to know the difference: the influence of each learning model, emotional intelligence, and their interactions toward academic achievement of circle subject. This research is a quasi experimental research with a 2 × 3 factorial design. The population of this research is the whole XI grade odd semester of public high schools in Sukoharjo in the academic year of 2016/2017 with used KTSP curriculum. Sampling was conducted with stratified cluster random sampling techniques. The technique of data collection in this research is a documentation method, test, and questionnaire then Test requirement analysis Lilliefors method, Bartlett method, ANAVA, and the multiply comparison test with Scheffe' method. Conclusion of the results of this study are: (1) TAPPS provides better academic achievement than direct learning model. (2) High emotional intelligence students have better academic achievement than average emotional intelligence students and low emotional intelligence students, and average emotional intelligence students have academic achievement better than low emotional intelligence students. (3a) by TAPPS model, high emotional intelligence students have better academic achievement than low emotional intelligence students, high emotional intelligence students have same academic achievement with average emotional intelligence students, and high emotional intelligence and average emotional intelligence students have better academic achievement than low emotional intelligence students. (3b) by direct learning model, high emotional intelligence student have better academic achievement than average emotional intelligence and low emotional intelligence, and average emotional intelligence are students have same academic achievement with low emotional intelligence students. (4a) to high emotional intelligence students, TAPPS provides better academic achievement than direct learning model. (4b) to average emotional intelligence students, TAPPS provides better learning achievement than direct learning model. (4c) to low emotional intelligence, TAPPS provides same academic achievement with models direct learning.

1. INTRODUCTION

The development of science and technology is currently growing rapidly. Indonesia as a developing country, has a global challenge to compete with the developed countries. Efforts to confront these challenges by improving the quality of human resources through quality education. Therefore, education at all levels to constantly improve.

Mathematics has an important role in the development of science and technology. Mathematics as one of the basic sciences, both aspects of the application as well as aspects of thought, have a crucial role in the mastery of science and technology. Therefore, the matmatic comprehension and mastery at every level of education in Indonesia needs to be fixed.

Although mathematics has been studied at every level of education does not mean students master math well. According to Trianto [1] the main issue in the study of formal education (schools) nowadays is still the low achievement of student learning. This looks at the average value of national examination subjects mathematics school year 2014/2015 HIGH SCHOOL in Sukoharjo Regency based on data SHOWING OFF 2015 was 52.70. In addition, the low achievements of learning math relate directly seen from the low absorbance at the level of mastery of the material of the circle based on PAMER 2015 is 42,97% .

Based on that data needs to be followed up on related factors that cause the low achievement of

student learning in Sukoharjo Regency. Successful or whether someone in learning due to several factors that affect the achievement of learning. Slameto [2] States that the factors that affect learning, divided into two factors, namely the internal factors and external factors. These two factors have a translation in a number of other factors. Internal factors are factors that exist in the individual includes physical factors, psychological factors, and exhaustion. External factors are factors that are outside of the individual factors include family, school factors, and environmental factors.

Based on observational learning of mathematics in Sukoharjo Regency, most schools as long as it uses the direct learning model. This led to more focused on teachers so that teachers are more dominating class and the students are less active in the learning process. The liveliness of the students in the learning process is highly influence the success of student learning. This problem is encountered in the process of teaching and learning activities in the classroom. Therefore, it needs to be an effort to increase the liveliness of students and help students understand the material's learning through innovative learning models.

Handayani [3] revealed, students are in need of new innovations in the learning process which is able to facilitate in digging the potential abilities, namely the ability of reasoning, problem solving, communication and mathematics as output the most important mathematical skills for the student. One of the interesting innovations to accompany a change of paradigm and as solutions to the problems of those observations was applied to model learning cooperative. Researchers interested in applying cooperative learning model of the type of Thinking Aloud Pairs Problem Solving (TAPPS). Research results Pate and Miler [4] indicating that the use of model learning TAPPS gives better results compared to classes that are not applied to model learning TAPPS.

Johnson, et al. [5] revealed that this type of cooperative learning opportunities to the TAPPS students to discuss with other students in the process of resolving the issues each group consisting of two children become problem solver and two children into a listener. TAPPS Division has the role of listener and problem solver who performed alternately so that each student has the ability on problem solving. In addition, Pate & Miler [4] stated that, "learning strategies such as TAPPS, Metacognition can grow students in organizing and regulation in information processing to improve problem solving." Conceptually, this study's focus on improving student performance on complex problem-solving activities to develop the thinking of metacognition.

Learning activities of students are often exposed to psychological barriers inclu desaturation, despair, stress and others. The underlying thing of it all is how can one understand emotional use of intelligently through emotional intelligence. Being able to control the emotional intelligence, coping, and emotional life of discipline so that he can run a better life with activity in a balance. Therefore, the intellectual intelligence alone does not provide preparation for individuals to deal with the turmoil, opportunities or difficulty in life.

Goleman [6] States that emotional intelligence also has a significant influence in the success of one's life. So one of the internal factors that affect student learning achievements is the level of emotional intelligence. Each student has a different emotional intelligence. These differences allow distinction achievement learning as well. Following the opinion of the experts regarding the primacy of emotional intelligence.

According to Goleman [6], the skills of emotional intelligence work in synergy with the cognitive skills. Those high achievers have both. Without emotional intelligence, a person cannot use the capabilities cognitive them according to its maximum potential. It is in line with Nwadinigwe & Obieke [7] in his research that the results, there is a positive relationship between emotional intelligence and academic performance so that the development of emotional intelligence will increase academic achievement. Emotional intelligence can help students overcome the psychological obstacles that lay in the study. Therefore, emotional intelligence, owned the students very influential towards the results of the study because the emotions of someone's actions against fishing what it faces.

In the process of learning math, so that learning can be implemented either in accordance with the difference in emotional intelligence and to improve learning achievements of students, especially at the subject of circles of learning model is required. Based on the above explanation, then researchers interested in conducting research on the use of cooperative learning model of the type of Thinking Aloud Pairs Problem Solving (TAPPS). In addition to model learning, emotional intelligence is also a diverse students want to be seen by the author. The author would like to see the effectiveness of the use of cooperative learning model of the type of Thinking Aloud Pairs Problem Solving (TAPPS) and direct learning model on a circle of subjects with attention to students ' emotional intelligence.

2. METHODS

This research is quasi experimental research design with a factorial 2 x 3 which aims to find out the difference of influence of each model of learning, emotional intelligence, and the interactions between the learning achievements of students against both of them. In this study, the population is a whole grade xi odd semester academic year 2016/2017 SMA Negeri se-Sukoharjo regency KTSP curriculum. Sampling done by the technique

of stratified cluster random sampling. in this school are grouped into categories of high, medium, and low, based on the average value of national examination (UN) school year 2014/2015 on mathematical subjects.

the category height if the value of the average UN skills over 59.65 and categories are if the average value of UN skills more than or equal to 49.56 and less than or equal to 59.65 while low category if the average value of UN skills less of 49.56. Then from each category chosen one random, so the school was elected to the three schools, with each school represents one category. as for the school selected from the categories high is ALSO KNOWN AS SMU 3 medium category is Sukoharjo, SMAN 1 Kartasura, and low categories is SMAN 1 Nguter. From each school were then taken two classes to serve as random samples from each school. One class is used as class experiments with models of learning TAPPS and the other as a class controls with direct learning model.

The technique of data collection conducted in this study are: 1) the documentation used to define a class in a State of balance or no, 2) test which is used to obtain data on student learning achievements, 3) now used to learn emotional intelligent exposed by students.

Instruments in this research are mathematical learning achievement tests on the material of the circle. Test instruments in this research in the form of a multiple choice question consists of 25 rounds, but tried out as many as 40 grains. Before it used to take research data, the instrument is tested with test validity and reliability. As for the test instrument use grain power test criterion and difficulty level. In addition, the study also use the question form emotional intelligence consists of 50 grainsstatement, but now are tested as much as 68 grains in anticipation of grain. Before it used to take research data, instrument the now tested with test validity and reliability. As for the test instrument use grain internal consistency test.

Budiyono [8] data analysis techniques used in this research were: 1) test prerequisites include test and Lilliefors test using a normality its homogeneity using method 2) Bartlett, balance test using variance analysis of one road with the same cells, 3) test the hypothesis using variance analysis of two paths with the same cells, and 4) Test Method uses Anava Scheffe's post '.

3. RESULTS AND DISCUSSION

After all the population declared Gaussian and variance homogeneous student population, then proceeded to test the hypothesis with the analysis of variance two paths with the same cells and the level of significance of 5% so that the retrieved results as shown in the following table:

Table 1. Hipothesis Resume Results

Source	DK	JK	RK	F_{obs}	F_{tabel}	Decission test
Learning Model (A)	1	1098,3727	1098,3927	7,7368	3,84	H_{0A} rejected
Emotional Intelegence(B)	2	4148,6520	2092,3260	14,7378	3	H_{0B} rejected
Interaction (AB)	2	3391,4946	1695,7473	11,9444	3	H_{0AB} rejected
Error	174	24702,8214	141,9702	-	-	-
Total	179	33377,3606	-	-	-	-

Based on the test results, then it can be concluded: (1) HoA rejected, this means there is a difference between math learning achievements of students who are direct and TAPPS models. Based on its margin, the model TAPPS average achievement learning better than direct learning model. TAPPS supports students convey all the problem-solving ideas, mutual understanding, and understanding the correct step problem resolution. Learning brings more meaningful so that learning learning achievements of TAPPS providing better than direct learning model. This has been in accordance with previous research Benham [9] that the TAPPS achievement learn math better than direct learning model.

H0B rejected, this means there is a difference between math learning achievements of students with high emotional intelligence, low emotional intelligence, and to know that there is a difference between math study acchievement students with high emotional intelligence, low emotional intelligence or to comparee of test doubles between columns. Based on the calculation method of Scheffe' with its average margin obtained, students have high emotional intelligence is better than learning achievements of students emotional intelligence, and low student achievement has learned are better than students emotional intelligence is low. Students who have high emotional intelligence is actively involved in the discussion, the task group with full responsibility so that it is capable of constructing their own knowledge. Students who have emotional intelligence is being actively involved in the discussion, the task group with the reliance on students who have high emotional intelligence because of the confidence which belonged to low. Students who have a low emotional intelligence tend to be stubborn, easily frustrated in the face of the task group, and quickly despair when stress. The existence of a difference category student’s emotional intelligence, then the students learn math achievement will be different. This has been in accordance with previous research Nwadinigwe & Obieke [7] that, there is a positive

relationship between emotional intelligence and academic performance so that the development of emotional intelligence will increase academic achievement.

H0AB rejected, then to know there is a difference between math learning achievements of students with high emotional intelligence, emotional intelligence, or low emotional intelligence, each learning model need double comparisons of test done between cells on the same line. Based on the calculation method of the Scheffe ' by seeing its average margin obtained, (3a) on the TAPPS, students have high emotional intelligence is better than learning achievements of students emotional intelligence is low, high emotional intelligence as well as students are learning achievement has the same, and high emotional intelligence as well as students are learning achievements have better than students emotional intelligence is low. (3b) on the direct learning model, students have high emotional intelligence is better than learning achievements of students are both low emotional intelligence and emotional intelligence are students or low learning achievement has the same.

H0AB rejected, then to know there is a difference between math learning achievements of students who are on learning model or direct learning model TAPPS on each of the emotional intelligence test double comparisons need to be made between cells in the same column. Based on the calculation method of the Scheffe ' by seeing its average margin obtained, (4a) on students with high emotional intelligence model of learning achievements of TAPPS had better than direct learning model. (4b) in students emotional intelligence are with learning achievements of TAPPS model has better than direct learning model. (4 c) at low emotional intelligence students with learning achievements of TAPPS model has the same with models direct learning.

4. CONCLUSIONS

Based on the study of the theory and supported by variance analysis as well as referring to the formulation of the problem, for the students of class XI SMAN Sukoharjo to be drawn the conclusion: (1) TAPPS provides better academic achievement than direct learning model. (2) High emotional intelligence students have better academic achievement than average emotional intelligence students and low emotional intelligence students, and average emotional intelligence students have academic achievement better than low emotional intelligence students. (3a) by TAPPS model, high emotional intelligence students have better academic achievement than low emotional intelligence students, high emotional intelligence students have same academic achievement with average emotional intelligence students, and high emotional intelligence and average emotional intelligence students have better academic achievement than low emotional intelligence students. (3b) by direct learning model, high emotional intelligence student have better academic achievement than average emotional intelligence and low emotional intelligence, and average emotional intelligence are students have same academic achievement with low emotional intelligence students. (4a) to high emotional intelligence students, TAPPS provides better academic achievement than direct learning model. (4b) to average emotional intelligence students, TAPPS provides better learning achievement than direct learning model. (4c) to low emotional intelligence, TAPPS provides same academic achievement with models direct learning.

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Descriptive Study of Teachers' Asking Skill Components on Mathemathics Leraning Process Based on Teaching Experience

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Abstract – This study aimed to describe: 1) the components of questioning skill based on teaching experience; 2) the obstacles of asking question. The sampling technique is purposive sampling. The subjects of this study are 4 mathematics teacher whom were grouped into expert teacher and novice teacher. The data were collected by passive participation observation and semi-structured interview. The validity of data were obtained through the triangulation of method, triangulation of time and member check. The data analysis technique use in this study is descriptive analysis. The result of this study showed that 1) The expert teacher understand the components of basic asking skill better than the novice teacher do. 2) The expert teacher obstacles in asking question came from student characters and the novice teacher ones student characters and time allocation..

1. INTRODUCTION

A teacher not only required to have the ability of knowledge, but also teaching skills as support to improve the capabilities and achievements in teaching, one of the skill is asking. Asking skills is a response stimulus from the students. Brown argued that through these skills teachers can create a meaningful learning environment. [1].

The question that asked by teacher basically used to develop students' creative mindset. Critelli said that there are several variations of methods to increase student participation in the classroom, the most important method is asking, teacher need to consider the right way to asking the question so that questions can be effective in helping to develop the mindset of students in the learning process [2]. The observation result of teacher with teaching experience approximately 5 months in one of SMA in Probolinggo showed that how teacher asking questions is always asked to the overall students in the class. Barizi said that educational background and teaching experience are two aspects that affect the professionalism of teacher in education and teaching. The expert teacher have a minimum four years in teaching [3]. Kunandar argued that education and training affect teaching experience [4]. Some research suggests that there are differences in asking question between expert teacher and novice teacher. Wahyu said that novice teachers only do the distribution of the appointment, giving the time to think and switch the student's turn to answer [5]. McAnnich concluded that novice teacher have less experience in teaching so that the skills in asking questions does not correctly [6].

Based on these explanation above, this study is to describe the asking question skill components of teacher in mathematics learning process in SMA Taman Madya Probolinggo based on teaching experience and the obstacles in asking questions. This research is helpful as astudy materials and competent authorities in education to improve the quality of education.

2. METHODS

The study was conducted in SMA Taman Madya Probolinggo and SMA Taman Madya Kraksaan on Academic Year 2016/2017. The time on first semester of 2016/2017 in September-December, 2016. The main subject consist of two mathematics teachers of SMA Taman Madya Probolinggo which is expert teacher and novice teacher and two mathematics teachers of Taman Madya Kraksaan with the same criteria.

This study is a descriptive qualitative research which describes the kinds of questions that asked of teacher, how teacher ask questions to the students and the obstacles in asking question. The data were collected by passive participation observation and semi-structured interview.

The data analysis is an inductive analysis based on the data which is obtained, then developed into hypotheses. The fourth selected results of observations of each teacher will be triangulated. The triangulation results will be validated back to the interviews result then obtained the temporary results of research which is will be member check to the related subjects to improve the validity of the data so that the conclusion in corresponding to the data source.

3. RESULTS AND DISCUSSION

Primary data is the data observations relating to components that teachers questioning skills, both basic and advanced skills. Secondary data such as the results of semi- structured interviews statements related to teacher. The data regarding the constraints experienced teachers obtained by direct interviews related to teacher as much as 2 times the interview.

1. Teacher Asking Question Skill Components

   a. Basic Components

      Based on the survey results revealed that experienced teachers asking questions clearly and concisely. Teachers appear to offer informations which is understandable language by student when asking questions. The question that asked by teacher is a general question then focused it on the core questions that will be asked. When the teacher asked a question, the teacher switch the student's turn to participate in answering. Teacher ask the questions to all students and teachers on several occasions appealed to certain students. Teachers give students the opportunity to think about the answers. If the teacher feels the student difficult to answer, the teacher directs students to answer questions by asking questions is simpler. Based Hasibuan seen that in asking a question, an expert teacher has good understanding of basic skills components [7].

      Meanwhile, novice teachers asking questions with words that correspond student's understanding clearly and concisely. In addition, teachers appear to provide information that easily understandable language when asking questions. Teachers focused the questions. When the teacher asked a question, the teacher does not refer interchangeably students to participate in answering. Questions posed to all students and in some occasions the teacher did not ask to specific students. Teachers give students the opportunity to think about the answers. If the teacher feels the student trouble answering, the teacher looks directing students answer the questions by asking questions is simpler. Based Hasibuan seen that in asking a question, the teacher understand the basic skills, but teachers do not always switch the student's turn in answer [7].

   b. Futher Components

      Based on the research results are often teachers start with understanding the question, then teachers will improve the cognitive level if the student is able to answer the previous question that can develop students' thinking skills. The questions posed any teachers in accordance with the logical sequence. After the students answer the questions, teachers often ask students to give an explanation for the students' answers. Through these questions, the interaction created properly because students are encouraged to be active so it looks interactions between students. According Hasibuan above explanation can be interpreted that in asking questions of expert teachers make changes to the cognitive level questions, pay attention to the sequence of the question, has the skills to encourage student interaction in the classroom and often track the responses of the students [7]. According Hasibuan in asking a question, an expert teacher has good understanding of further questioning skills components [7].

      The novice teachers when asking questions often start with the question of understanding, but on other occasions teachers begin with the question of knowledge. Teachers will improve the cognitive level if the student is able to answer the questions previously posed questions teachers are in accordance with the logical sequence. After the students answer the questions, teachers do not always ask students to give an explanation for the students' answers. Through these questions, then the interaction of the atmosphere created in class pretty well. Hasibuan argued that the skills necessary to track owned by the teacher to know the extent to which the student's ability to answer put forward [7]. Thus, novice teachers understood well enough to ask further components but in terms of track is not always done by a novice teacher.

2. The Obstacle of Asking Question

   a. Expert Teacher

      In asking question the obstacle of expert teacher came from students' character. The character which is heterogeneous makes the teacher cannot maximize the opportunity to do a question and answer session. Lack of understanding ability, mental unfavorable and different backgrounds impede the teacher when asking questions.

b. Novice Teacher

In accordance with the results of an interview note that the obstacles by novice teacher in asking questions are the student's character and time allocation. The ability of the student who makes obstacle of novice teacher in asking questions. Levels of cognitive questions that achieved only at the level of understanding and not enthusiastic students when answering. In addition, the allocation of limited learning with students' lack of understanding capabilities and demands of finish material also inhibits teachers ask more questions.

4. CONCLUSIONS

Based on the research result, the conclusions are:

1. Teacher Asking Question Skill Components on Mathematics Learning Process

   Expert teachers understand the components of a basic questioning skills better than novice teachers. In presenting the question teachers ask questions in a clear and concise, providing suitable reference so that students answer correctly, focus the questions that asked by teachers, switch the student's turn to answer, pay attention to the direction and spread of the appointment of the question, give time to think fairly and provide guidance to students who have difficulty in answering. However, novice teachers do not always do the basic components which switch the student's turn to answer.

   Expert teacher understand the further skill components skills than the novice teacher. When asking questions teacher do the conversion level cognitive questions, pay attention to the sequence of the question, has the skills to encourage student interaction in the classroom. Often track the students 'answers. Meanwhile, novice teachers rarely seen track the student's answer.

2. The Obstacles in Asking Question

   The obstacles of expert teacher in asking questions came from the character of the students. The ability of understanding, mental and backgrounds influence the learning process, especially when the teacher asked a question. The obstacles of novice teacher came from the character of students and teaching time allocation. The ability of students who are less enthusiastic hinder the students in answer the teacher questions and the limited allocation of learning.

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Simulation of The Determination of The Professionalism Level of Teachers with Mamdani Fuzzy Inference Method Using Matlab Applications

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Abstract – A good quality education is effected by the quality of the teachers. Qualified teachers can be seen from the level of professionalism. Teachers ' professionalism is the condition, value, purpose, direction, and quality of an expertise and authority in the field of education and teaching. An assessment of professional level of teachers is important because teachers as change makers education system and preparing the nation for the better. To measure the level of professionalism of the teachers can be done by computerized systems using intelligent system with fuzzy logic inference Mamdani method. Determination of the variable input is a writing test results, practice test results, and the results of the workshops, each of which is determined on a low level, medium, and high. While the variable output is the level of professionalism that is composed of level is not professional, quite professional and professional.

1. INTRODUCTION

A qualified teacher will result in quality education. There are three general dimensions that become the competency of education personnel is as follows: 1) personal or private Competency a teacher must have a solid personality and exemplary; 2) professional competencies that a teacher should have a vast knowledge, depth of field of study is taught, is able to select and use a variety of teaching methods; and 3) social competence, teachers and the wider community[1].

Appropriate government regulation of Indonesia number 19 in 2005 about education standards on article 28 in paragraph 1 it is said that Educators must have an academic qualification and competence as an agent of learning, healthy physical and spiritual, as well as have the ability to realize the goal of national education. While the competence of educators who referred to in paragraph 1 is as mentioned in paragraph 3 of competence as an agent of learning including: competence of pedagogy; Competence of personality; Professional competence; and Social Competence. Thus then the teacher as a good educator must take on the role of environmental education in families, schools and communities. In the other research said that there are 5 knowledge that must be possessed by the teachers, namely personal knowledge, contextual knowledge, pedagogical knowledge, sociological knowledge, social knowledge[2]. Professional teachers are people who are well-educated and well-trained, and have a rich experience in the field[3]. So professional teachers are teachers who have the ability and expertise in the field of teacher training so as to be able to do the task and its function as a teacher with the ability to the maximum.

Given the importance of teachers as modifier education system and preparing the nation for the better development of the future then the need for an assessment to evaluate the professional level of teachers. To measure the level of professionalism of the teachers can be done by applying artificial intelligence using fuzzy logic. Fuzzy logic is used because the concept of fuzzy logic is easy to understand, very flexible, has a tolerance of inappropriate data, are able to model the non linear functions are very complex, can build up and apply the experience of the experts directly without having to go through the training process, it can, in cooperation with control techniques conventionally, and is based on a natural language[4]. In this paper will be more elaborated with fuzzy inference implementation used is mamdani and centroid defuzzification method to measure the professionalism of teachers.

Based on the background of the problems described above, the purpose of the writing of this paper is as follows: 1) How do the determination of categories of professionalism of teachers using Fuzzy Mamdani? ; 2) How the result of the calculation of the professionalism of teacher by application of fuzzy algorithm with mamdani method?; 3) How the level of accuracy is obtained using the method of fuzzy mamdani?

2. METHODS

A rule-based fuzzy system complete consists of three main components are: fuzzyfication, inference, and defuzzzication[5][6].

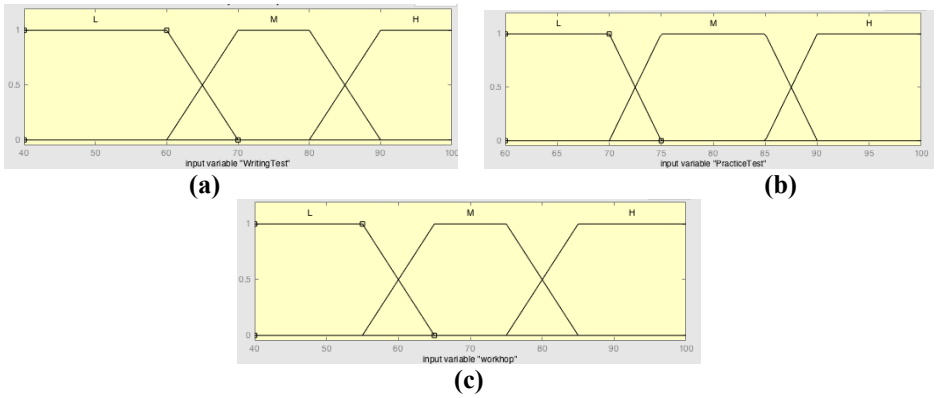
2.1 Fuzzyfication

Fuzzification changing inputs the truth values are definitely (crisp input) into the shape of the fuzzy input, in the form of linguistic semantic value is determined based on the spesific membership functions. Table 1 shows the crisps input.

Table 1 (The Set of Fuzzy)

Input	Writing Test	Low	<= 70
		Medium	60 - 75
		High	>=80
	Practice Test	Low	<=75
		Medium	70 – 90
		High	>= 85
	Workshop	Low	<=65
		Medium	55 – 85
		High	>=75
Output	Profesionalism Level	Not Profesional	<= 70
		Quite Profesional	65 – 85
		Professional	>=80

The determination of the membership of the crisp input on the set of fuzzy determined into low, medium, high by using a representation curve of the trapezium. In Figure 1 shows the picture graph membership function variable, (a) variable writing test, (b) variable practice test, and (c) variable workshop.



(a) (b) (c)

Figure 1 (Membership Function Variable)

2.2 Inference Engine

Inference do reasoning using fuzzy input and fuzzy rules have been determined , so as to generate fuzzy outputs. Of the three input in the determination of the professionalism of teacher have 27 rule is as follows at Table 2.

Table 2 (Rules of Fuzzy)

Rule	IF	Writing Test AND Practice Test AND Workshop	THEN	STATUS
1	IF	L and L and L	THEN	NP
2	IF	L and L and M	THEN	NP
3	IF	L and L and H	THEN	NP
		Until		
25	IF	H and H and L	THEN	QP
26	IF	H and H and M	THEN	P
27	IF	H and H and H	THEN	P

2.3 Defuzzyfication

The input of process defuzzification is a fuzzy set is obtained from compositionin of the fuzzy rules, while the generated output is some number in the fuzzy set of domains. From the process of defuzzyfication with fuzzy rules generate output in the form statement not professional, quite professional and professional.

3. RESULTS AND DISCUSSION

The calculation will be done manually and calculations using MATLAB application. Example the case of a teacher gets a score of writing test is 77, score practice test is 72, and score of workhop is 63.

3.1 Manual Calculations

Calculations membership function of writing test of 77 is located on the set of fuzzy medium with a value of 1. Calculation of membership function of practice test of 72 is located on the set of fuzzy low and medium.

$practicetest\ (L) = \frac{d\text{-}x}{d\text{-}c} = \frac{75\text{-}72}{5} = \frac{3}{5} = 0{,}6$ $\qquad$ $practicetest\ (M) = \frac{x\text{-}a}{b\text{-}a} = \frac{72\text{-}70}{5} = \frac{2}{5} = 0{,}4$

Calculations membership function of workhop of 63 is located on the set fuzzy low and medium.

$workshop\ (M) = \frac{d\text{-}x}{d\text{-}c} = \frac{65\text{-}63}{10} = \frac{2}{10} = 0{,}2$ $\qquad$ $workshop\ (H) = \frac{x\text{-}a}{b\text{-}a} = \frac{63\text{-}55}{10} = \frac{8}{10} = 0{,}8$

3.1.1 Inference Rule

The result obtained from the input is a writing test M(1), practice test L(0,6), practice test M (0,4), workhop L (0,2), workhop M (0,8). By the rules of selecting local minimum membership conjunction, the third input will be as follows.

[R10] IF writingtest is M (**1**) AND practicetest is L (**0,**6) AND workshop is L (**0,**2) THEN ProfesionalismLevel is NP
[R11] IF writingtest is M (**1**) AND practicetest is L (**0,**6) AND workshop is M (**0,**8) THEN ProfesionalismLevel is NP
[R13] IF writingtest is M (**1**) AND practicetest is M (**0,**4) AND workshop is L (**0,**2) THEN ProfesionalismLevel is NP
[R14] IF writingtest is M (**1**) AND practicetest is M (**0,**4) AND workshop is M (**0,**8) THEN ProfesionalismLevel is QP

Determine the value of $\propto predicate$ using the minimum value of μ (miu)

$\propto predicate10 = \mu writingtest\ \boldsymbol{M} \cap \mu practicetest\ \boldsymbol{L} \cap \mu workshop\boldsymbol{L}$
$= \min(1; 0{,}6; 0{,}2)$
$= 0{,}2$
$\propto predicate11 = \mu writingtest\ \boldsymbol{M} \cap \mu practicetest\ \boldsymbol{L} \cap \mu workshop\ \boldsymbol{M}$
$= \min(1; 0{,}6; 0{,}8)$
$= 0{,}6$
$\propto predicate13 = \mu writingtest\ \boldsymbol{M} \cap \mu practicetest\ \boldsymbol{M} \cap \mu workshop\boldsymbol{L}$
$= \min(1; 0{,}4; 0{,}2)$
$= 0{,}2$
$\propto predicate14 = \mu writingtest\ \boldsymbol{M} \cap \mu practicetest\ \boldsymbol{M} \cap \mu workshop\boldsymbol{M}$
$= \min(1; 0{,}4; 0{,}8)$
$= 0{,}4$

then using the value of disjunction by choose membership degree form maxsimum value of linguistik.

$\mu NP = \alpha predicate10 \cap \alpha predicate11 \cap \alpha predicate13$ $\qquad$ $\mu QP = \alpha predicate14$
$= \max(0{,}2; 0{,}6; 0{,}2)$ $\qquad$ $= \max(0{,}4)$
$= 0{,}6$ $\qquad$ $= 0{,}4$

3.1.2 Defuzzyfication

Defuzzyfication process using centroid method by determined the random point in the shaded area.

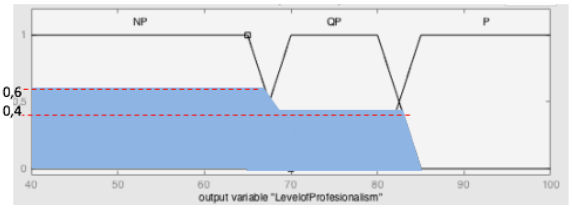


Figure 2 (Shaded Area from The Profesionalism Level)

$$y^* = \frac{\sum \mu(y)y}{\sum \mu(y)y}$$
$$= \frac{0{,}6(40 + 45 + 50 + 55 + 60 + 65 + 70) + 0{,}4(65 + 70 + 75 + 80 + 85)}{(0{,}6x7) + (0{,}4x5)}$$
$$= \frac{231 + 150}{4.2 + 2} = \frac{\mathbf{381}}{\mathbf{6.2}} = \mathbf{60,4}$$

3.2 Calculation Using MATLAB Application

The results of the simulation of the first case using matlab application can be seen in Figure 8. The case are 77 writing test, 72 practice test, and 63 workhop. And produce output results is 59,9.

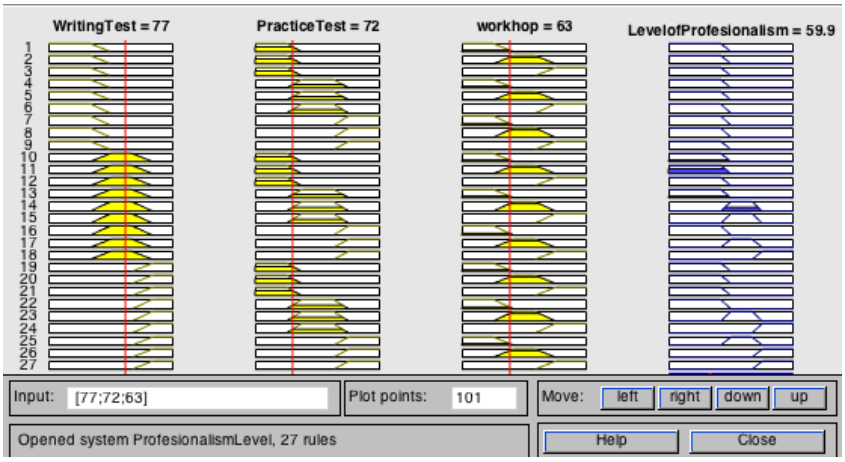


Figure 3 (The Result from Calculation using MATLAB)

3.3 Analysis

Comparison of the results defuzzy using manual calculations and calculation using matlab application can be seen in Table 3.

Table 3 (Comparison Of The Result Defuzzy)

Variabel		Perhitungan Defuzzy			Selisih
Uji tulis	Uji Kinerja	Workshop	Manual	MATLAB	
77	72	63	60,4	59,9	0,5
63	73	57	58	57,5	0,5
81	74	80	70	69,3	0,7
94	85	80	82,5	83,3	0,8
68	88	78	73,8	73,4	0,4
85	87	88	82,5	83,3	0,8
61	86	82	58,9	58,1	0,8
72	76	90	75	75	0
83	87	64	76,5	76,5	0
89	72	83	67	66,2	0,8

There are differences between manual calculations and calculation using matlab application. The most major difference is the manual calculation by calculation using matlab application is 0,8. So it's not have difference.

4. CONCLUSIONS

The use of fuzzy logic with mamdani method can count the determination of the level of teacher professionalism. There are rhree input variables and one variable output. On variable input is being used is a writing test results, practice test, and the result of workhops. Whereas, in the output variable is the result of the assessment. From the results of calculation that was performed on 10 experiment not too have significant differences. So the the concept offered can be used for decision support system of teacher professionalism

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Undergraduate theses: its research topics and statistics analyses methods

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Abstract – Education research is considered deserves very little attention among education manager in higher education institutions. Evaluation and assessment are important in providing information for developing a good management education system. The study was conducted by looking at topics research that is dominantly taken in undergraduates' thesis. The information will be benefited educational manager in related field of study. It could be one of an evaluation indicator of the appropriateness of the design of the curriculum in their institutions. The study will further focusing looking at the lecture of one of the important subject in scientifically atmosphere which is statistics. The result gained in the study will be used in capacity development of lecture in statistics.

A desk study was conducted at library of Fisheries and Marine Science Faculty of University of Brawijaya from August to September 2016. The thesis observed was from year 2008 to 2012 students of two study programs that are Fisheries Resource Utilization (FRU) and Marine Science (MS) of the Department of Fisheries Resource Utilization and Marine Science (FRUMS).

Statistical test for homogeneity of composition/proportion of undergraduate research topics was executed using the Chi-square test. The difference of the composition/proportion of research topics between each year group student were analyzed and tested statistically using Analysis of Variance (ANOVA) which will be followed by Tukey/Fisher test.

Statistical analysis Identification of the undergraduate thesis of FRU and MS were undertaken in order to describe the statistics analysis methods used and executed in their thesis. The appropriateness of the statistics analysis method then was observed and examined through some statistics principles that are: its research design, research questions, execution of statistics analysis used, research hypothesis, descriptive statistics, the statistics output, and conclusion or interpretation. Statistical test for homogeneity of composition/proportion of undergraduate thesis statistics principles was executed using the Chi-square test. The difference of the composition/proportion of statistics principles between each year group student were analyzed and tested statistically using Analysis of Variance (ANOVA) which will be followed by Tukey/Fisher test.

The dominant research topics of interest of FRU student are: social economics (25%), stock assessment (20%), fishing gear (17%), fishing port (12%), fish biology (8%), remote sensing (6%) and fishing boat-machine. While for MS students are: oceanography (27%), mangrove (19%), pollution/water quality (16%), corals (9%), remote sensing (8%), turtles (5%), and bio-active (5%). Students from both study programs prefer other programs for their thesis analysis rather than inferential statistics.

1. INTRODUCTION

Statistics have been accepted by scientist worldwide and become important part of the work of biologist as well as other related or applied science including fisheries. The important things are used in two senses. First is collecting and handling data. And second is organizing, summarizing or describing, analyzing, and inferencing or generalizing data. So that statistics is necessary when ones need to evaluate scientific works critically. Beside that statistical principle and methods is essential for ones doing scientific work for authorization (Fowler et al., 1998; Quinn and Keough, 2002).

As a Consequence, a higher level education student has to be able to design experiments and interpret and

evaluate the result of statistics literacy. They need to have skills through ability in design surveys and experiments; data collections; analysis and graphical presentations; descriptive statistics; hypothesis testing; ANOVA (Analysis of Variance) design; correlation and regression techniques; introduction to multivariate methods; analysis of frequency distributions; non-parametric equivalents and using of computer software for statistics analysis relevant for biologist(Underwood, 2001; Robson and Jhones, 2003). Therefore, biological research leading to experiments must start with validated observations, models of explanations or theories, hypothesis or predictions based on models, logical opposite to hypothesis, experiment or critical test of null hypothesis, and interpretations.

Statistics has limitations as it never proves anything rather the likelihood of the result of an investigation that comes from a chance. It can help to describe data design experiments, and test about relationships among things. It is a tool which helps to accept or reject the null hypothesis (Fowler et al., 1998). It might be one of the reasons why statistics has become an obligatory subject for higher degree (undergraduate and postgraduate) students (Nikiforidou et al., 2010).

Although lots of investment has been spent in higher education through the development fields of study but little have been spent to monitor what has been invested. It might lead to inadequate information and analysis of management of education system. It may because the education research has little priority compare to research in agriculture, science, health, economy and engineering (Puryear, 1995). Statistics as one of an obligatory subject for university students has been one of a good example of the absence of adequate evaluation and assessment. The study proposed will provide information on the learning process of statistics lecture and related subject (i.e. research design and analysis). The study is going to assess some complimentary criteria on statistical processes executed and undertaken by university students in their undergraduate thesis. Then, the information could be used to evaluate and improve the lecture on statistics and related field of study.

2. METHODS

2.1 Research topic

Research topics were grouped into study area of interests proposed in the thesis 'title. The research topics then were analyzed based on its dominance and its proportion. There were about 13 and 15 topics of fisheries resource utilization and marine science study program respectively have been identified. To determine whether research topics composition and proportion differ among the five groups of year of enrollment (i.e. 2008, 2009, 2010, 2011, and 2012), statistical test for homogeneity of composition/proportion was executed using the Chi-square test. Once the chi-square reject the $H_0$the analysis then followed by the test that try to address the difference between topics by each year. The difference of the composition/proportion of undergraduate research topics between each year group were analyzed and tested statistically using Analysis of Variance (ANOVA). The ANOVA test will be followed by Tukey/Fisher test to show which groups are differ between one and another.

2.2 Statistics analysis and procedure

Identification of the statistical analysis used in undergraduate thesis of fisheries resource utilization and marine science study program were identified in order to have general description of the statistics examination process or procedures should be undertaken. Then, the appropriateness of the statistics analysis methods used and executed in the undergraduate thesis was observed and examined through some statistics criteria such as: its research design, research questions, execution of statistics analysis used ,research hypothesis, descriptive statistics, the statistics output, and conclusion based on the statistics output.

3. RESULTS AND DISCUSSION

3.1 Research topic

The most dominant research topics of interest and its composition of FRU student are: social economics (25%), stock assessment (20%), fishing gear (17%), fishing port (12%), fish biology (8%), remote sensing (6%) and fishing boat-machine (5%) (**Error! Reference source not found.**). While for MS students are: oceanography (27%), mangrove (19%), pollution/water quality (16%), corals (9%), remote sensing (8%), turtles (5%), and bio-active (5%) (**Error! Reference source not found.**).

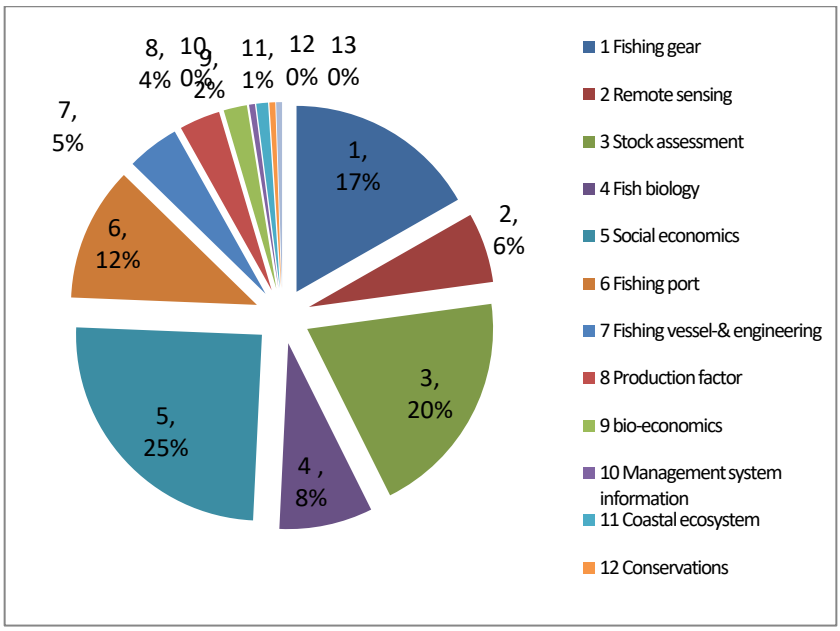

Figure 1 Composition/proportion of research topics of fisheries resource utilization students.

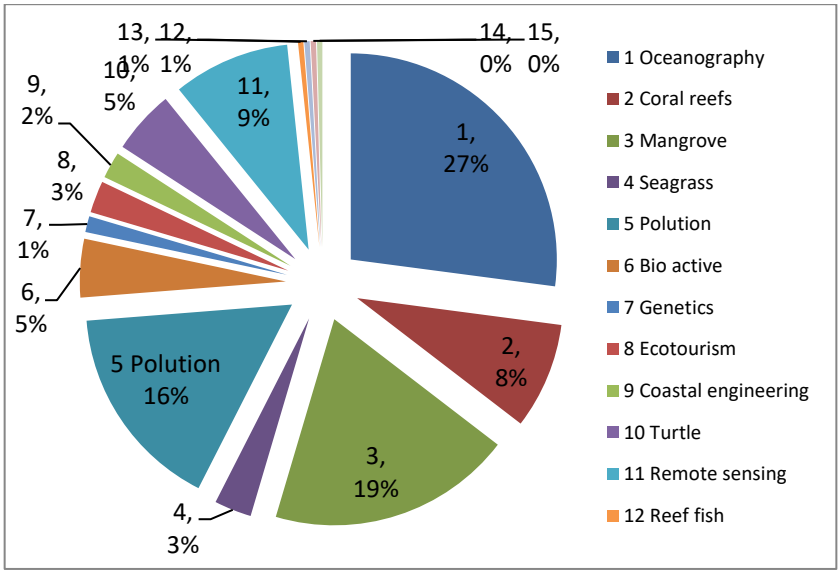

Figure 2 Composition/proportion of research topics of marine science students.

3.2 Statistics analysis

An MS student tends to explore the use of other programs such as: Arc-View, Arc-GIS, Reef-check, Shanon-Weaver, SWOT analysis, for their thesis to derive the conclusion and interpretation. other category of statistics analysis are PCA, correlations, regressions, ANOVA, t-test, and cluster analysis with frequency of 45%, 14%, 13%, 10%, 8%, 7%, and 3% respectively (See Figure 3).

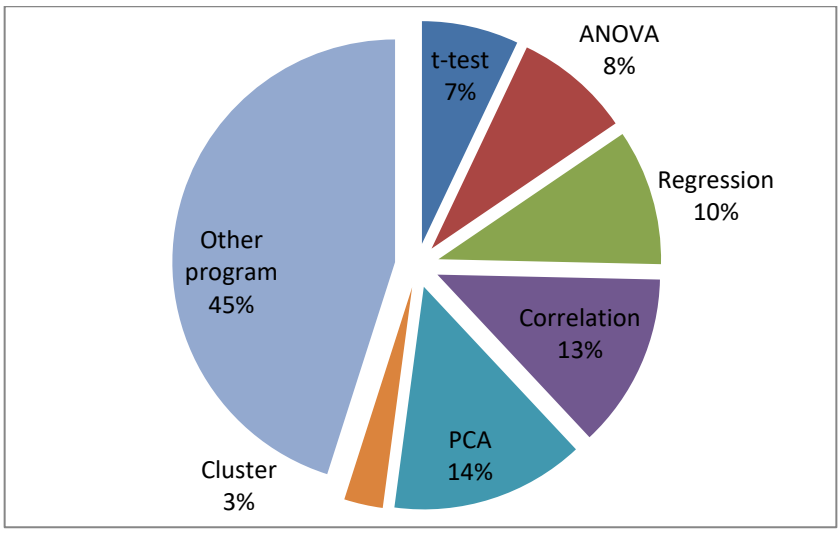

Figure 3 Composition/proportion of statistical analysis.

In general the compliance to the statistical principles is low (less than 40%). The compliance is higher/better in terms of designing research, stating the statistics analysis and explaining analysis methods (each term is more than 50%) and the rest principles are below 40% (Figure 4).

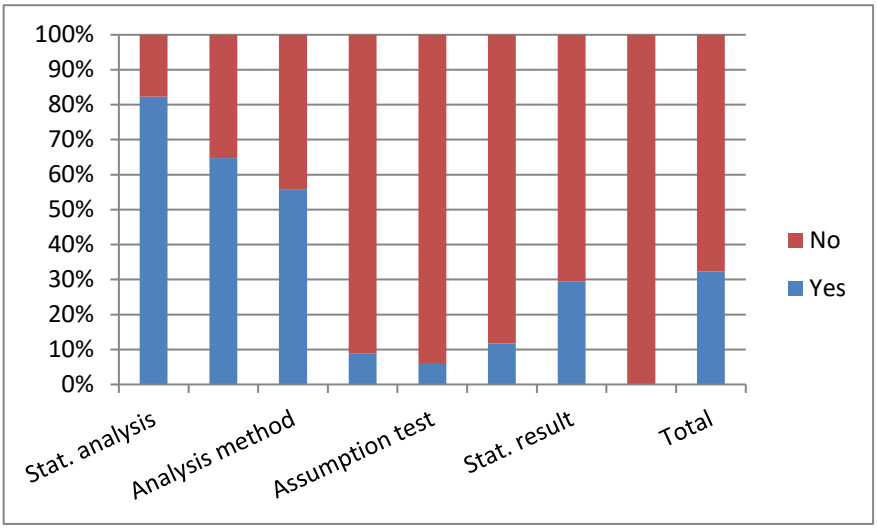

Figure 4 Compliance to statistical principles of MS students.

4. CONCLUSIONS

The most dominant research topics of interest and its composition of FRU student are: social economics (25%), stock assessment (20%), fishing gear (17%), fishing port (12%), fish biology (8%), remote sensing (6%) and fishing boat-machine (5%). While for MS students are: oceanography (27%), mangrove (19%), pollution/water quality (16%), corals (9%), remote sensing (8%), turtles (5%), and bio-active (5%). They prefer other analyses (45%) than statistics (Figure 2). However, compliance to the statistical analyses principles was low.

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Modified Adsorbents Based on Biomass for Ferrous Ion in Water

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Abstract – The huge amount of biomass waste could cause environmental problem due to the bad odor during degradation. Reuse the biomass as an adsorbent for metallic pollutant in drinking water sources has increased the economic and environmental values of the biomass. This article reviewed two adsorbents which were prepared from biomass, *i.e. Sansieviera trifasciata* and pineapple leaves. Modification was conducted by immobilizing tannin extracted from *S.trifasciata* in the active carbon and modified cellulose in activated pineapple leaves powder by introducing carbonyl groups from citric acid. From Infrared spectra it would be concluded that there were hydroxyl and carbonyl groups in the both adsorbents which were responsible for ferrous ion adsorption. Adsorption parameters including the initial ferrous ion concentration, pH of solution, adsorbent mass, and contact time should be studied to determine the adsorption of capacity for a new adsorbent.

1. INTRODUCTION

Iron is the second most abundance metal in the earth's crust but its concentration in water is small. In the groundwater, iron comes originally from the soil and during the rock formation [1]. The main anthropogenic sources of iron in the water system are various industrial sources, such as toothpowder manufacturing industries. The solubility and chemical behavior of iron in water depend on oxidation intensity in the system in which it is occurred [2], pH of water [3], and redox condition of groundwater. Iron is one of essential metals which is important for metabolism process in animals and plants. Iron has significant role as nutrient and for the formation of hemoglobin. High concentration of iron in the groundwater could cause red oxy-hydroxide precipitate that stain laundry, plumbing, dish wares, and glasses. At concentration more than 0.3 mg/l in groundwater, it can cause stringent odor to drinking water. In this case, removal of iron is due to aesthetic reason. Supply of domestic and industrial water is also affected by the presence of iron in the water due to fouling of well screens and pipping system [4].

In groundwater systems, iron occurs in one of two oxidation states: reduced soluble divalent ferrous iron (Fe2+) or oxidized insoluble trivalent ferric iron (Fe3+) with the concentration of ferrous ion is more abundant. Such methods have been adopted to remove iron for treatment of borehole water supplies including water softener using cation exchanger, complete aeration and filtration with pH adjustment [5], chlorination and aeration with pH adjustment/coagulation and filtration [6], ozonation and chelation, and adsorption. It depends on the form the iron is present in the water.

Such adsorbents have been produced and used to adsorb iron from the groundwater. Palm fruit bunch and maize cobs have been used to remove iron up to 79% [7]. Maize husk have been studied for removing iron solution at pH 4 with the capacity of 0.499 mg Fe/g adsorbent [8]. The iron removal efficiency was found to be more than 89% when using almond shell. Adsorbent doses, pH of iron solution, contact time, and initial concentration of iron solution were found to be the factors determined adsorption [9]. For ferrous ion itself, fly ash had been used as the adsorbent with 94% of removal efficiency at pH solution of 4.0 [10]. The use of biomass for adsorbent of ferrous ion can reduce the huge mass of biomass which may cause other environmental problems and improve the economic value of the biomass. However, the biomass adsorbent may be modified to enhance its adsorption capacity or the amount of ion adsorbed. This paper compared two modification ways of biomass from different sources and its removal efficiency for ferrous ion.

2. METHODS

2.1 Chemicals

Two biomass sources were modified as ferrous ion adsorbent. The *Sansiviera trifasciata sp.* and pineapples leaves were studied. Tannin of *s. trifasciata* were removed using distilled water. Lignin in pineapples leaves was removed using sodium hydroxide (Merck). Citric acid (Merck) was used to modify cellulose in pineapples leaves.

Buffer at pH 4.0 was prepared to adjust the iron solution pH. Ferrous ion solution was prepared from the solid salt of $FeSO_4$ (Merck).

2.2 Procedures

2.2.1 Adsorbent Preparation

The first adsorbent was prepared from *S trispasciata* leaf (Fig. 1a). It was prepared as described by Arif et al. 50 mL of crude extract of tannin was mixed with 20 gram of active carbon. The mixture was stirred for an hour followed by filtration. The solid phase of filtration was dried. [11].

The second adsorbent was from pineapple leaves (Fig. 1b). Lignin in pineapple leaf fiber was removed using the sodium hydroxide at 0.1 mol /L. The product was washed and filtrated until the pH of filtrate was neutral. The solid residue then was modified by mixing two grams activated pineapples leaves powder with 20 mL of citric acid 0.6 mol/L. The mixture was stirred for 200 minutes at 80^0C followed by filtration. The neutral solid residue then was oven dried. The functional groups of the adsorbents were characterized using IR spectrophotometer.

(a) (b)

Figure 1 The biomass sources for ferrous ion adsorbent: (a) *s.trifasciata*, and (b) pineapples leaves.

2.2.2 Adsorption Procedure

As described by Arif et al [11], the ferrous ion adsorption was carried out by mixing 25 mL of ferrous ion with 100 mg of adsorbent. It was shaken for an hour and followed by filtration. The concentration of iron in the solution was measured using atomic adsorption spectroscopy.

Meanwhile, 50 mL of ferrous ion 100 mg/L was mixed with 300 mg of modified pineapples leaves adsorbent. The mixture was shaken for an hour. After equilibrium was reached, the mixture was filtrated and the iron concentration in the filtrate was determined using atomic adsorption spectroscopy.

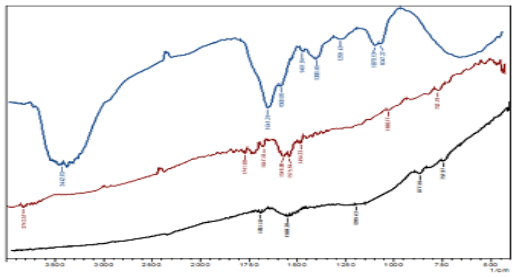
3. RESULTS AND DISCUSSION

Modification of adsorbent could be performed by introducing other with some functional groups available in the both adsorbents. It can be seen from Fig. 2 that the spectra of modified adsorbent from *s.trifasciata* (a, red) was similar to spectra of active carbon (a, black). It means that tannin extracted from *s.trafasciata* (a, blue) was physically adsorbed during immobilization in active carbon. Hydroxyl groups (3500 cm^{-1}) in tannin were adsorbed more than the carbonyl groups (1700 cm^{-1}). It can be seen from the decreasing of intensity and the shift of wavenumber for the both functional groups.

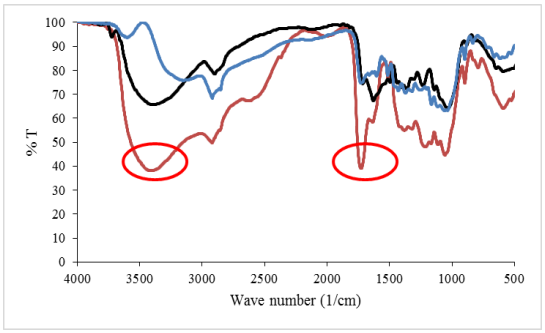
The modified adsorbent from pineapples leaves (Fig. 2, b). The dried powder of pineapples leaves showed hydroxyl groups of cellulose (b, blue). After rinsed with NaOH 0.1 mol/L, lignin has been removed so the intensity of hydroxyl groups was increased and shifted to the longer wavenumber (b, black). Modification by addition of citric acid has induced carbonyl groups to the adsorbent (b, red) which may have interaction with ferrous ion.

Interaction between ferrous ion with the adsorbents could be through physical interaction between hydroxil and carbonyl groups with ferrous ion. The isoterm adsorption should be investigated at equilibrium time to determine the actual interaction.

The adsorption profile of ferrous ion onto the modified adsorbents is shown in Table 1. For the second adsorbent, the adsorption parameter investigated was only the initial concentration of ferrous ion while other adsorption parameters had not been investigated yet. Hence, it is needed to investigate other adsorption parameters.



(a)



(b)

Figure 2 Infra red spectra of modified adsorbent prepared from biomass *s.trifasciata* (a) and pineapples leaves (b)

Table 1 Adsorption profile of ferrous ion onto the modified adsorbents

Adsorption parameters	*S.trifasciata* adsorbent	Pineapple leaves
Initial concentration of ferrous ion (mg/L)	75	80
pH	6	NI
Contact time (minutes)	60	NI
Adsorbent mass (mgram)	100	NI
Adsorption isotherm	Langmuir	NI

NI: not investigated

Based on the Table 1, the adsorbents adsorb ferrous ion at the similar initial concentration. pH of ferrous ion determines the species form of ferrous ion whether it is still in the ionic form or precipitate solid form. pH also affects the ionic charge of surface groups in adsorbents. Contact time determines the equilibrium. It should be determined firstly before determining the adsorption isotherm. It means, investigation of contact time will determine the adsorption capacity of developed adsorbents. Mass of adsorbent affects the adsorption capacity because it determines the amount of adsorbent functional groups which is responsible to interact with target analyte ion.

4. CONCLUSIONS

Many biomass sources could be used for adsorbent to adsorb ferrous ion originally. Modification of the adsorbent, either by immobilizing specific compound or addition of responsive functional groups, could increase the adsorption capacity.

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Application of Decision Support System for Selecting Candidates for Scholarship Using SAW in Uganda

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Abstract – Although Quality Education is an important strategy in improving people's standard of living for which higher education is an opportunity to obtaining a better life. Many students today especially in developing countries like Uganda experience economic constraints in pursuing higher education. Every year many undergraduate and postgraduate students in Uganda face severe financial constraints to continue enrolling for further studies like pursuing Bachelor's degree, Master's degree, Doctorate and PhD which has led to a serious decline in the quality of higher education in the country. This has necessitated the government, organizations and individuals urgently seek for new sources to curb the challenge. And the major solution has been seeking for international scholarships from national and international donors such as governments sectors, Non-government organizations, multinational corporations and individual donations and grants. Scholarship as a nonrefundable award of financial aid a student(s) to further their education especially university students who fulfill the specified criteria in accordance to the requirements. It is awarded basing on various criteria, which usually reflect the values and purposes of the donor or founder of the award.

Keywords*: DSS, Scholarship, SAW, MCDM, Uganda*

INTRODUCTION

Quality education is an important strategy in improving people's standard of living. Having a higher education is an opportunity to obtaining a better life. However, today many people especially in developing countries like Uganda experience economic constraints in pursuing higher education (can't afford access to high education especially low income earners at both under and postgraduate levels). To give hope to academically sound students is through in seeking for scholarships (nonrefundable money given to recipient) from national and international body donors such as governments, non-government organizations, multinational companies and individual donations and grants [1]. It is given basing on scholarship criteria such excellent performance, financial inability, Parent's number of dependents etc. However, the selection process of scholarship recipients in any given university is frequently carried out subjectively by scholarship council or committee. The selection process in the area of awarding scholarships often uses a manual framework to examine the candidate's qualifications according to that specified criteria by the donor. The task of short listing is conducted manually and consumes a lot of resources, time which results in unnecessary expenses to the organization and due to this manual selection process a certain degree of personal preference, perception and judgment is introduced into the process regardless of the criteria followed resulting into high degree of dissatisfaction from the general public towards the process. The focus of this paper is application of MCDM using SAW method in order to eliminate the core inefficiencies faced within this framework, thus improving the quality of the final decision results by facilitating the selection of the most deserving candidate and minimizing the degree of personal preference, perception and subjectivity or judgment [3]. The focus of this paper is to propose the application of SAW to shortlist the applications received via a web/online application which acts as an interface between the applicants and selection panel. Furthermore SAW was used to develop scores and their weights for each candidate basing on the selected criteria [2]. SAW method was selected because it is the simplest MCDM approaches to use [4]. Decision Making is a cognitive process for the selection of the best alternative among several potential alternatives or one action among a set of action possibilities. Decision Support Systems (DSS) is a computer-based system that is intended to facilitate the decision making process. It is adaptive, interactive, flexible, and specifically developed to support the solution of unstructured problem to improve the quality of decision making. Hence, DSS can be defined as an adaptive, flexible, interactive computer-based system which is useful to solve unstructured problems and thereby increasing the value of the decisions [5].

2. METHODS

Simple Additive Weighting (SAW) method is often also known as weighted summation method is a basic concept seeking to find weighted summation rating performance of each alternative on all attributes. SAW method requires a process of normalizing the decision matrix (x) to a scale which can be compared with all the rating alternatives that exist. SAW method has the following steps:

a) determining the criteria that will be used as a reference in the decision, namely Ci as given below:
C1 = Scores (CGPA) C2 = Number of Semesters
C3 = Number of Parent's Dependent C4 = Parent's Income

b) determining alternative weights on each criterion in order to obtain the matrix w.

c) making decisions based on criteria matrix (ci) that is create an alternative table that contains the value of each criteria of each individual student who asked to receive scholarships.

d) normalizing the matrix based on equations that are tailored to the type of attributes (attributes benefit or attribute costs) in order to obtain the normalized matrix r. the formula for determining the normalized matrix r are as follows:

$$r_{ij} = \begin{cases} \frac{Xij}{\text{Max } Xij} & \text{if j is benefit attribute} \\ \frac{Min_i\, x_{ij}}{Xij} & \text{if j is cost attribute} \end{cases}$$

Where, r_{ij} = Rating normalized performance, Max X_{ij} = maximum value of each row and column
Min X_{ij} = minimum value of row and column, X_{ij} = rows and columns of matrix,
If r*ij* is the normalized performance rating of alternatives on attributes ai cj; i = 1, 2, .., m and j = 1, 2, ……., n.

e). Determining final value
Calculate the value of the preference for each alternative (Vi) matrix by multiplying matrix *r* with matrix W set the recommended students to obtain scholarships. To get the final results, value that is obtained from the ranking process by summation of the normalized *r* matrix multiplied with the weighted vector in order to obtain the greatest value that can be selected as the best alternative (Ai) solution as given below: Preference value (Vi) = $\sum_{j=1}^{n} w_j r_{ij}$, Where, Vi = final value of the alternative, Wj = predetermined weight, rij = normalized matrix, and if the value of Vi is large shows that the alternative (Ai) is chosen. The design of a decision support system for determining a scholarship recipient using simple additive weighting (SAW) method follows those steps which has been described in the previous section.

3. RESULTS AND DISCUSSION

The input requirement analysis for data processing and selection of scholarship recipients conducted by the personnel of each academic year will depend on the following elements as given below:

1). Merit scholarship: a) CGPA ≥ 3.00, b) College Recommendation Letter of good Conduct. c) Two Minimal term.

2). Underprivileged scholarship: a) Two Minimal term, b) Total parents' income, c) Number of dependents. Based on the requirements above, the criteria specified by the user are:

(i) Photocopy of CGPA transcript, (ii) Photocopy of semester program study card, (iii) Photocopy of Parent's income certificate, (iv) Photocopy of parent's number of dependents based on the family card.

Through the application of SAW therefore, we can follow the above steps as follows:

a) Determine the reference criteria for decision making (Ci) based on the scholarship application requirements as seen below;
CGPA (Score) = C1 Semester = C2
Parent's no of dependents= C3 Parent's income = C4

b) Determine the suitability rating each alternative on each criteria, of these criteria made an interest rate criterion is based on the weights. Rating suitability of each alternative on each criteria is as follows:
Very Low (VL) = 0 (0; 25)
Low (L) = 25 (0; 25; 50)
Enough (E) = 50 (25; 50; 75)
Height (H) = 75 (50; 75; 100)
Very High = 100 (75; 100)

c) Make a decision matrix based on criteria, then normalizing matrix based on the equation adjusted for the type attribute to obtain the normalized matrix R. Criteria / Attributes are explained as follows:

1. Criteria Value GPA (C1), CGPA Criteria views student's scores obtained in GPA during the study period.

Table 1.Value Criteria CGPA

CPGA (C1)	Weight
2.75 - 3.00	0.25
3.00 - 3.25	0.50
3.25 - 3.50	0.75
3.50 >	1.00

2. Criteria Number of Semesters (C2). Criteria half of visits by the number of semesters that have been taken by students. Scholarships of Higher Education are usually held at the end of semester of every academic year.

Table 2. Semester Criteria

Semester (C2)	Weight
Semester 2	0.25
Semester 3	0.50
Semester 4	0.75
Semester 5	0.100
Semester 6	1.00

3. Number of Dependents of Parents criteria (C3). Criterion number of dependent parent visits based on the cost of living that still have to be borne by the parents of the overall number of children

Table 3. Parent's number of dependents Criteria

Number of Parent's dependents (CC3)	Weight
T = 1 Child	0.25
T = 2 Children	0.50
T = 3 Children	0.75
T = 4 Children	1.00

4. Parent's Income criteria (C4). Criteria on income of parents is seen by the amount of retained earnings in each month.

Table 4. Parent's Income criteria

Parent's Income (C4)	Weight
P 1.000.000	1.00
P 1.000.000 – 3.000.000	0.75
P 3.000.000 – 5.000.000	0.50
P 5.000.000 >	0.25

The final result can be obtained through the process of ranking the matrix multiplication and the sum of the normalized R with a weight vector in order to obtain the greatest value which can be selected as the best alternative as a solution. Weighting Decision is given by :

Table 5. Excellent Academic Achievement (EAA) Vector Weight

C1	C2	C3	C4
0.40	0.30	0.20	0.10

Table 6. Student's Learning Support (SLS) Vector Weight

C1	C2	C3	C4
0.10	0.20	0.30	0.40

Determining the value of a weight vector based on the level of importance of each criterion on a scholarship EAA and SLS. In Excellent Academic Achievement scholarship most preferred criteria is CGPA, then the value of the weight vector EAA (W1) is as follows: Wl = [0.40 0.30 0.20 0.10]

While in the Student Learning Support scholarship (SLS), criteria are most concerned about are the number of dependents of parents and salaries of the parents , then the value of the weight vector of Student's Learning Support (w2) is as follows: W2 = [0.10 0.20 0.30 0.40]

Simple Computation Analysis Example with SAW method

Table 7. Applicants' Data

Name of Applicant	CGPA	Semester	Parent's number of Dependents	Parent's Income (000)
Musazi	3.21	2	2	4.586
David	3.45	4	4	2.000
Karim	3.85	6	4	9.000

Suppose in Master's program for Vocational education in Informatics has a quota for each type of scholarship, and if there are three students applying for those scholarship slots, the student is assumed to follow a scholarship (EAA) and (SSL) with the following data as given in the table below:

Table 8. Clarification criteria of Applicants' Data

Alternative	Criteria			
	C2	C3	C4	C1
Musazi	3.21	2	2	4.586
David	3.45	4	4	2.000
Karim	3.85	6	4	9.000

Basing on the data in the table above, we can convert this given data into matrix format as below:

The matrix format of the above data is

$$X = \begin{bmatrix} 0{,}50 & 0{,}25 & 0{,}50 & 0{,}75 \\ 0{,}75 & 0{,}75 & 1.00 & 0{,}50 \\ 1{,}00 & 1{,}00 & 1{,}00 & 0{,}25 \end{bmatrix}.$$

Here is Screenshot of SAW criterion determination and matrix results on application

SIMPLE APPLICATION OF SAW METHOD

Name of Applicant	CGPA (C1)	Semester (C2)	Parent's number of Dependents (C3)	Parent's Income (C4)
Musa	3.21	2	2	4.586.600
David	3.45	4	4	2.000.000
Karim	3.85	6	4	9.000.000

Matrix X = $\begin{bmatrix} 0{,}50 & 0{,}25 & 0{,}50 & 0{,}75 \\ 0{,}75 & 0{,}75 & 1.00 & 0{,}50 \\ 1{,}00 & 1{,}00 & 1{,}00 & 0{,}25 \end{bmatrix}$

Then do the normalization matrix x to calculate the value of each criteria as an assumption of a benefit or cost criteria:

Musazi:

$$r1 = \frac{50}{Max\{50 \quad 75 \quad 100\}} = 0.5$$

$$r2 = \frac{25}{Max\{50 \quad 75 \quad 100\}} = 0.25$$

$$r3 = \frac{50}{Max\{50 \quad 100 \quad 100\}} = 0.5$$

$$r4 = \frac{Min\{75 \quad 50 \quad 25\}}{75} = 0.33$$

David

$$r1 = \frac{75}{Max\{75 \quad 100 \quad 50\}} = 0.75$$

$$r2 = \frac{75}{Max\{75 \quad 100 \quad 25\}} = 0.75$$

$$r3 = \frac{100}{Max\{100 \quad 100 \quad 50\}} = 1$$

$$r4 = \frac{Min\{50 \quad 25 \quad 75\}}{75} = 0.5$$

Karim

$$r1 = \frac{100}{Max\{100 \quad 50 \quad 75\}} = 1$$

$$r2 = \frac{100}{Max\{100 \quad 25 \quad 75\}} = 1$$

$$r3 = \frac{100}{Max\{100 \quad 50 \quad 100\}} = 1$$

$$r4 = \frac{Min\{25 \quad 75 \quad 50\}}{25} = 1$$

From this subsequently created matrix multiplication w * r, the sum of multiplication can be used to obtain the value of the best alternative by ranking of the largest value. If three students are tested based on the provision of scholarships weight vector PPA, the results matrix multiplication of W1 * r if computed manually is as follows:

V1 = (0.40*0.5) + (0.30*0.25) + (0.20*0.5) + (0.10*0.33) = 0.2 + 0.075 + 0.1 + 0.033 = 0.41. V2 = (0.40*0.75) + (0.30*0.75) + (0.20*1) + (0.10*0.5) = 0.3 + 0.225 + 0.2 + 0.05 = 0.78. V3 = (0.40*1) + (0.30*1) + (0.20*1) + (0.10*1) = 0.4 + 0.3 + 0.2 + 0.1 = 1

SAW RESULTS

ID No.	APPLICANT'S NAME	COMPUTATION OF RESULTS	TOTAL	STATUS
0001	Musa KM	(0.40*0.5) + (0.30*0.25) + (0.20*0.5) + (0.10*0.33)	0.41	Not selected
0002	David RB	(0.40*0.75) + (0.30*0.75) + (0.20*1) + (0.10*0.5)	0.78	Not selected
0003	Karim AM	(0.40*1) + (0.30*1) + (0.20*1) + (0.10*1)	1	Selected
----	----------	0	0	0

Here is Screenshot of SAW computation results by multiplying matrix R with matrix W and the final results on application

From the results obtained computation of V3 = 1 as the greatest value for the weight specified, thus the alternative 3 (Applicant 3) is an alternative that is chosen as the best alternative to get a scholarship award.

4. CONCLUSIONS

Decision Support System model with Simple Additive Weighting (SAW) method was evidenced to be useful and valid in the case of scholarship recipient selection process. In applying simple additive weighting (saw) method, the assessment criteria and their respective weighting should be determined in advance to obtain the final score of each candidates by is using matrix multiplication.

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Problem Based Learning Application in Geometry to Improve the Abstraction Skill of Student at Civil Engineering of The State Polytechnic Malang

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Abstract – Many factors cause the polytechnic student have math difficulties, among others basic math is low, there are too many topics, and less time allocation.. Therefore, efforts should be made to provide alternative styles of learning. One of the efforts to optimize the learning of mathematics is Problem Based Learning (PBL). PBL approach allows students to be more active learning in acquiring knowledge and developing thinking. In the implementation of PBL, the learning centers is on the student, the problem solving is done in groups and is presented in turns. The objective of this research is to describe Geometry topic with Problem Based Learning to improve student's abstraction. To ease the students to describe the shape of three-dimensional, teaching aids are used. The results obtained are: (1) The ability of the students is very heterogeneous, so the lecturer needs some strategy to help students who have low ability, (2) Students of SMK are more skill full in describing the three-dimensional compared to students of SMA, (3) The teaching aids of three dimensional is needed to solve problems of Geometry, and (4) Of the six groups, only one group is answering right about the volume of Concrete Construction.

Keywords: Strategy, Learning

1. INTRODUCTION

In polytechnic, mathematics is a general subject given at the beginning of the semester. The mathematics is also a subject to support the engineering materials. Many students have difficulties in mathematics. It can be seen from the result of the math scores, many students got C, even in the Department of Electrical Engineering more than 50 % of students got D, so the bad score could make the student to drop out. In the polytechnics there is no short semester, nor repetition. The lecturer needs to know the cause of the low ability in math and to find a solution, so as to reduce the number of drop outs. Widjajanti said (2010) that one of the factors that caused the difficulties was weak abstraction. The students have difficulties to construct mathematical problems into the mathematical model, such equations, formulation, charts, and pictures. Besides the difficulties in constructing the object, students also have difficulties in the formulation of the calculation. The students are not able to apply volume three dimensions formula. Therefore, there must be an effort to help students to solve the problems, especially formula Geometry Problems. According to Herman (2007), Problem Based Learning (PBL) is one of the learning approaches allowing students to learn more actively in acquiring knowledge and developing his mind by presentation with the relevant context. The objective of this research is to describe Geometry study with Problem Based Learning approach and to improve the abstraction ability. To facilitate the students to describe the shape of three dimensions, the learning of Geometry is assisted by teaching aid, group discussion, and presentation.

2. METHODS

2.1 Design of research

The research design used Classroom Action Research (PTK), because the problems appear in the researcher's class room. Using the design of PTK was based on the grounds that (1) This research was factual, the students have difficulties in mathematics and (2) the study was empiric, it meant that the implementation of the research was conducted in the real class room. This is consistent with the characteristics of Classroom Action Research, such as (1) It is conditional, it means that PTK is based on the results of diagnosis problem in a particular context, then try to find a solution, (2) PTK is a collaborative effort between researchers and relevant parties to solve the problem, (3) PTK is flexible, it means that it can be adapted to reality in class, it does not have to stick on the original plan.

2.2 Data

The collection of data obtained in this research were as follows: (1) The results of group discussion in a three-dimensional picture, (2) The observations were obtained directly during the learning of mathematics with a record sheet, (3) The questionnaire results were to know the response of the students to the research by PBL.

2.3 Data Analysis

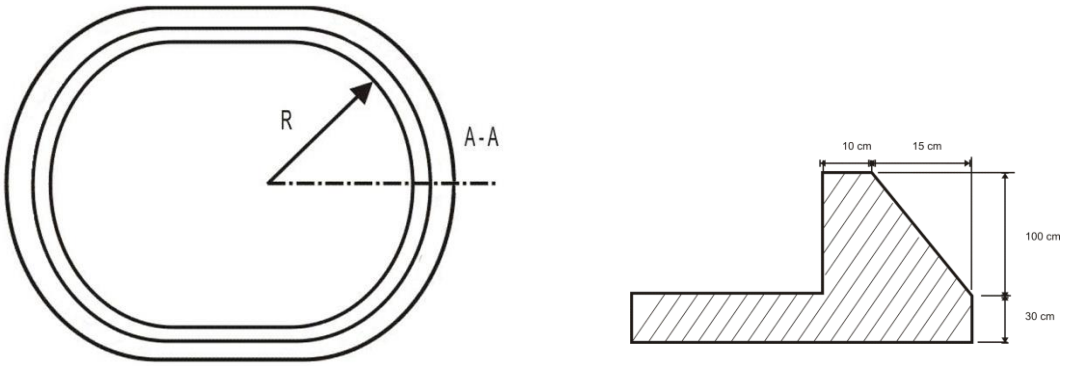
The application of PBL in mathematics learning used qualitative analysis developed by Miles and Huberman (Mujianto: 2007) which consists of three stages of the activities carried out in sequence, as follows:

1. Data Reduction
   Reducing the data is an activity to select and simplifies all data from the beginning.
2. Presentation of Data
   Presentation of data is done in narrative or verbal sentences.
3. Conclusions and Data Verification
   Conclusion is the activity to interpretation and evaluate. The data verification is an activity to test the validity of the data.

3. RESULTS AND DISCUSSION

In this research, the problem is how to improve the abstraction skill of students? One of the problems of Geometry is as follows:

Calculate the volume of the Concrete Construction of Picture 1, if R = 4 m.



Top View Cross Section A-A

Picture 1. The Concrete Construction look at Top View and Cross Section

This problem is solved in group discussion and presented alternately. To calculate the volume of concrete construction it is necessary to have three-dimensional picture. There were six groups in class IE (4 students / group) of the DIII program last year. The results were as follows: (1) all groups were unable to draw three-dimensional picture, (2) There were two groups that did computation correctly. To help students to solve the problem, the researcher gives teaching aid, as follows Picture 2.

Picture 2. The students to solve the problem with Teaching Aids

4. CONCLUSIONS

Based on the results of the research, the conclusion is as follows:

1) Having been given teaching aid, all groups are able to describe the dimensions correctly.
2) The results of the computation, there is only one group correctly.
3) The results of the questionnaire are as follows, lower score = 25, top score = 34, mean = 29. It means student have positive response.
4) The teaching aid can increase student abstraction. This is based on group discussion and observation.
5) The ability of students is very heterogeneous, so the lecturer needs a strategy to help students with low ability.
6) Student of SMK are more skillful in describing the three-dimensional than students of SMA.
7) The student needs teaching aid to solve math problems, especially Geometry.

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The Experimentation of *Learning Cycle 7E* (LC7E) Learning Model On The Linear Program Subject of Wonogiri Vocational School Students

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Abstract – The research aims to know the influence of LC7E learning model to the students achievement compared with direct learning method. The research is a quasi-experimental. The population of the research is vocational students grade XI in Wonogiri regency. Furthermore, the samples are chosen by stratified cluster random sampling. The data collection is conducting tests and documentation. Meanwhile, Data instrument is Mathematics achievement tests. Before used, the instrument are tested to get the valid data. Then, validator scoring system is used to get the validity of its content. The instrument reliability tests use the KR-20 formula. Test of distinguishing features use moment product correlation formula by Karl Pearson. The prerequisites test includes normality test with Lilliefors test method and homogeneity test which use Bartlett method. It can be concluded that the sample come from the homogeny and normal distributed population. Finally, by using LC7E learning model, it can be concluded that students achieve better result of studying Mathematics rather than by using direct learning method.

Keywords: Learning Model, LC7E, Students' Achievement

1. INTRODUCTION

Learning is a form of education in order to improve the quality of people through their experience in the environment. Slameto (2003:12) stated that learning is a process done by the people to change the whole attitudes as the result of their experience and interaction with environment. One of many purposes of learning is to gain and improve the students learning achievements. According to Syah (2003: 141). "Student learning achievement is the level of success in achieving the goals set in a program." So, achievement of each student must be different although the students are learning in the same class and also taught by the same teacher.

Learning model used is expected to be done interactively, inspirational, fun, challenging, motivating students to be participated actively, providing a sufficient space for innovation, creativity, independency based on the students' interest. One of learning model which applies constructivism is cyclical learning model. The cyclical learning is inquiry-based learning pattern, where the students are stimulated to be more active in conducting scientific inquiry through science skill to acquire knowledge and experience. The *LC7E* model is applied in the particular stages, 'engage stage' becomes 'elicit' and 'engage', meanwhile on 'elaborate stage' and 'evaluate' becomes three stages, which are 'elaborate', 'evaluate', and 'extend', so, stages on *LC7E* are 'elicit', 'engage', 'explore', 'explain', 'elaborate', 'extend' and 'evaluate'.

According to Kusumawati (Prihatiningrum, 2013: 6) her research in applying Problem Base Learning (PBL) learning model and Learning Cycle 7E (LC7E) to find out the effectiveness and integrity related to learning result which seen from the ability of self-regulation and students creativity on functional system material. The research stated that *LC7E* learning model can cover the lack of PBL learning model, so, it can improve the students learning achievement especially on functional system material. Another research related to application of *LC7E* learning model was conducted by Wahyu et al (2014). On his research, he want to know GI and NHT learning model on LC7E toward Mathematics achievement and achievement motivation in terms of adversity quotient. This research, NHT learning model in LC7E gains the improvement more than GI learning model on LC7E.

The process of applying *LC7E*, the teacher gives evaluation in a whole in the end of the lesson, so, the students who find difficulties can be known and immediately handled. In evaluation stage, the teachers make the conclusion of the entire process of giving learning material and continued by giving exercise without giving feedback on the students' works. The learning process has many stages and the students are expected to pass it well, it is very important for the teachers to know the level of students' understanding.

The purpose of the research is to know which gives the better Mathematics achievement between Learning Cycle 7E (LC7E) and direct learning.

2. METHODS

2.1 Chemicals

This research is a quasi-experimental. The population of the research is vocational students in Wonogiri regency, meanwhile the subject of the research is students grade XI in the first semester of year 2016/2017.

2.2 Procedures

The method used to collect the data covers documentation and test. Meanwhile, the instrument used Mathematics achievement test. The data analysis technique is variance analysis of the different cell. The prerequisite test which includes normality and homogeneity tests are needed to do before conducting the analysis. In this research, normality test used Lilliefors method, on the other hand, homogeneity test used Bartlett test.

3. RESULTS AND DISCUSSION

Hypothesis test used analysis of variance analysis of the different cell. Hypothesis test is done after the data is eligible to the normality and homogeneity test and reach significance level 5 %. The following is summary data normality test of Mathematics achievement which uses Lilliefors method.

Table 1. Normality test summary of achievement data

Class	L_{obs}	L_{α}	Test Decision	Conclusion
LC7E	0.0779	0.0886	H_0 approved	Normal population
Direct	0.0779	0.0865	H_0 approved	Normal population

According to data in the Table 1, it can be seen that all data come from normal distributed population. The following is the summary of data homogeneity test achievement using *Bartlett* test.

Table 2. The summary of Homogeneity test to achievement data

Homogeneity test	χ^2_{obs}	$\chi^2_{0.05;k-1}$	Decision	Conclusion
Learning Model	4.0694	5.991	H_0 approved	Homogene

According to data in Table 2, it is clearly seen that all data come from homogene variance population. The following is summary of hypothesis test using variance analysis of the different cell.

Table 3. Summary of Anava variance analysis of the different cell.

Source	JK	Dk	RK	F_{obs}	F_{α}	Decision
Model	10574.45	1	5287.22	16.8749	3.0000	Declined
Error	93995.90	191	313.32	-	-	
Total	**147555.62**	**192**	**-**	**-**	**-**	

According to Table 3, it can be seen that H_0 is declined, it means that there is difference of the student's achievement by applying LC7E and direct learning model.

Summary of average each learning model presented on Table 4.

Table 4. Average of Learning Model

Learning Model	Marginal Average
LC7E	71.7900
CONTROL	55.5905

According to marginal average of each learning model, it can be concluded that there is difference on student's achievement in studying Mathematics by using LC7E and direct learning method, by paying attention on its marginal average it is found that LC7E is better than direct learning method.

The result of research based on Hanuscin and Lee theory (2007: 1) stated that learning cycle is a student-centered learning model which addopted the constructivism principal. Learning cycle is a series of stages

activities which is organized in such way, so, the students can master the competence to be achieved in learning activity by doing active role.

The result is in line with Polyiem, et.al (2011) which concluded that learning with Learning Cycle 7E approach improve .01 level significant toward exam score, scientific thinking skills, and moral compared with direct learning model. The result is in line to Tuna and Kacar (2013) which conclude that students achievement which is applied Learning Cycle 7E is better than the students achievement applied direct model on trigonometry material.

4. CONCLUSION

According to the finding and discussion of this research, it comes to the conclusion that student's achievements on Mathematics by applying LC7E is better compared to the direct model. The suggestions from this research are (a) The applying of LC7E learning model should be adapted to the material taught (b) The research is expected to be continued in the broader scope. In the research, applying of LC7E learning model only focusing on linear program material, so, it is expected to the future research to apply in the different material.

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Portraying Pedagogical Content Knowledge (PCK) of Science Teacher Using CoRe

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Abstract – The purpose of this research is to portray Pedagogical Content Knowledge (PCK) of science teacher in environmental pollution topic. The research method in this research is descriptive method. Subjects were 6 science teacher of junior high school in Padang Pariaman District of West Sumatra. Instrument to capture the PCK of those six teachers is Content Representation (CoRe) format presented by Loughran *et al.* (2004). The findings showed that the mean percentage of CoRes' aspect achievement is 57%. There are four aspects of CoRe that can describe teachers' PCK well. They are the explanation about concepts to be taught to students, related concept that teacher don't intend students to know yet, other factors that influences teaching this concept, and the explanation about teaching procedures. The CoRe is a window into capturing and portraying science teachers' PCK. The conclusion is five teachers are categorized as *growing PCK* and one teacher is categorized as *pre PCK.*

1. INTRODUCTION

Teacher is a determining factor for successful learning process. Improving the quality of teachers have a positive impact on educational quality. Kind (2009) notes The best teachers are those who have specialist subject knowledge and a real passion and enthusiasm for the subject they teach. A recent report from England's Office for Standards in Education (OFSTED) points to the fact that successful teaching, as measured by the numbers of 5 – 16 year olds achieving specific levels on the science National Curriculum, barely changed between 2005 and 2007. The report observes that given the extensive subject knowledge of most secondary science teachers much teaching paid scant regard to what and how pupils were learning. In many lessons, teachers simply passed on information without any expectation of pupils 'direct engagement in the process. The objective appeared to be to get notes into books, then leave the learning to the pupils. Bucat (2004) said that There is a vast difference between knowing about a topic, and knowing about the particular teaching and learning demands of that particular topic.

Shulman (1986) introduced a distinctive form of teacher's professional knowledge, Pedagogical Content Knowledge (PCK). This knowledge is different from the subject matter knowledge or the knowledge of the general principles of pedagogy. Shulman (1986) defined PCK as special amalgam of content and pedagogy that is uniquely the province of teacher, their own special form of professional understanding. Loughran, *et al.* (2012) states that PCK is an idea that is rooted in the belief that learning requires more than just provide the content knowledge to students, and students learn more than just absorbing information. Therefore, teachers should have a good ability of PCK. PCK will greatly assist in the development of more professional competence of teachers. Loughran, *et al.* (2012) said that PCK is the knowledge that teachers develop over time, and through experience about how to teach particular content in particular ways in order to lead to enhanced student understanding.

Loughran and colleagues developed a framework which they contended could capture and portray teachers' PCK. A resource folio consisted of a Content Representation (CoRe) and any number of Pedagogical and Professional-experience Repertoires (PaP-eRs). A CoRe is a table which sets out to represent science teachers' understanding of the content for a particular topic. The CoRe enables a solid base around which an overview of teachers' PCK for a topic can be articulated, and provides insights into decisions that teachers' make when teaching a particular topics including the linkages between the content, students, and teachers' practice. PCK that teachers had will categorized into a certain level. This categorization aims to determine the development of teachers PCK. Anwar (2014) created a category PCK based on the four components in the learning activities. The components include learning objectives, the selection of important concepts, pedagogy and evaluation. Anwar (2014) classifies the development of PCK teachers into three categories, they are *pre PCK, growing PCK, and maturing PCK.*

2. METHODS

The research method in this research is descriptive method. Subjects were 6 science teacher in Padang Pariaman District of West Sumatra. Instrument to capture the PCK of those six teachers is Content Representation (CoRe) format presented by Loughran et al. (2004). CoRe developed by asked teachers to determined the "big idea" of the environment pollution topic. These "Big Ideas" form the column headings. The rows consist of eight prompts which aims to reveal the teachers' reasoning behind pedagogical choices/activities, knowledge of their students (such as alternative conceptions, difficulties, and points of confusion) and ways of assessing student understanding that will shown in Table 1. Teacher answers were analyzed to see how teachers' perspective on environmental pollution topic and find teachers' PCK category. This data analyzed using CoRe rubrics developed by Anwar (2014).

Table 1 CoRe Format

Aspects	Big Idea 1	Big Idea 2	Big Idea 3	Big Idea …….
1. What do you intend the students to learn about this idea?				
2. Why is it important for students to know this				
3. What else do you know about this idea (that you do not intend students to know yet)?				
4. What are the difficulties/ limitations connected with teaching this idea?				
5. What is your knowledge about students' thinking that influences your teaching of these ideas?				
6. Other factors that influences your teaching of this idea				
7. What are your teaching procedures (and particular reasons for using these to engage with this idea)?				
8. Specific ways of ascertaining students' unders-tanding or confusion around this idea				

3. RESULTS AND DISCUSSION

The results showed that the mean percentage of CoRes' aspect achievement is 57%. There are four aspects of CoRe that can describe teachers' PCK well. They are the explanation about concepts to be taught to students, related concept that teacher don't intend students to know yet, other factors that influences teaching this concept, and the explanation about teaching procedures. The results also showed that five teachers are categorized as *growing PCK* and one teacher is categorized as *pre PCK.*

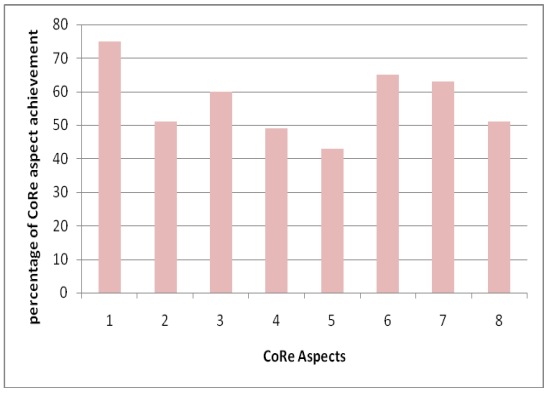

Figure.1.Teachers achievement in developing CoRe

The first thing teachers' do in developing CoRe is to determine "Big Idea" of environmental pollution topic. The big idea is an idea or concept that teacher thing it's important for students to build students' understanding the topics. The findings showed that the number of big ideas set by the teacher as much as 3-5 big idea. According Louhgran, *et al.* (2012) the number big ideas do not establish with certainty, but teachers are generally set 5-8 big idea. Too little big idea show that too many concept that include in a single big idea, while if too many big ideas led to the topic broken down into chunks of information that are not related.

In this study, teachers completed their own individual CoRe that focused on the topic of environmental pollution. The interpretation of teacher's responses at each of CoRe's prompts offer a way into a science teachers' thinking

about their professional practice and portray their PCK's development. This is how we can describe teacher's pedagogical content knowledge according to CoRe aspects.

What do you intend the students to learn about this idea?

This prompt is very helpful to unpacking science teachers' understanding of what matters in environmental topic and why. Being specific about what a particular group of students should be able to learn is an important aspect of well developed PCK. Most of teachers' response at this prompt were provide specific detail. It shows that teacher have a good understanding about the topic.

Why is it important for students to know this?

Almost all teachers can't reveal the importance of studying the big idea. The reasons behind why they thought it limited to the general concept. Only one teacher that give the reason relevant to students' everyday lives. Bringing the concept into students daily lives will make the concept easy to comprehend by student.

What else do you know about this idea (that you do not intend students to know yet)?

In this prompt, most teachers know the concept that too complex for students to grasp. Teachers' response were related to the prompt. Teachers have ability choose and prioritize the portions of material that are too broad or deep, so it is not urgent to be known by the students.

What are the difficulties/limitations connected with teaching this idea?

Teachers listed some difficulties but they didn't give the solution to that problems. Expert science teachers use this knowledge and information to shape the manner in which they teach particular concept and topics. Without this feature of PCK it could well be argued that teaching is not genuinely responsive to constructivist views of learning and is therefore not concerned with students processing, structuring, synthesizing and reconstructing their knowledge (Loughran *et. al,* 2012).

What is your knowledge about students' thinking that influences your teaching of these ideas?

These prompt help to illustrate the teacher's knowledge based on teaching experience in a subject and how this knowledge influence their thinking in teaching. Based on the analysis, six teachers describe misconception is already associated with the concepts being taught. But, teachers have not been trying to anticipate or plan activities to improve it. Loughran (2003) states that a good science teacher will plan learning according to their understanding about student's knowledge of the topic.

Are there any other factors that influence your teaching of these ideas?

Most of teachers have a good response in describing several factors like factor related the environmental pollution concept, time allocation, facilities like instructional media and student ability. This prompt will unpacking teachers' contextual knowledge about students to explore their approach in planning their teaching.

What are your teaching procedures (and particular reasons for using these to engage with this idea)?

Teachers describe variety of teaching procedures with description of what was involved. They explain the particular reasons for using the approach described. Most of the teachers use scientific approach in their teaching.

Specific ways of ascertaining students' understanding or confusion around this idea

Most of teachers listed written test as a way of ascertaining students' understanding. Teacher describe that written test is the best way to gather information about students' understanding.

4. CONCLUSIONS

There are four aspects of CoRe that can describe teachers' PCK well. They are the explanation about concepts to be taught to students, related concept that teacher don't intend students to know yet, other factors that influences teaching this concept, and the explanation about teaching procedures. The CoRe is a window into capturing and portraying science teachers' PCK. Five teachers are categorized as *growing PCK* and one teacher is categorized as *pre PCK.*

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Bolemia (Bolpoin Elektrokimia): An Alternative Learning Media of Electrochemistry That Interesting and Meaningful For Senior High School Students

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Abstract – Electrochemistry is one of materials that is abstract and has a high difficulty level to be understood by students. This characteristics requires facilities that can support the learning process suited with the curriculum in 2013, which is student center. But in the reality, learning process involving the interaction of students, can not be applied maximally. Because students only receive the knowledge as information from the teachers and they are unaccustomed to engage actively with what is learned. Therefore, we need a solution that can increase the involvement and the activity of student in learning process. One method that can be used in this learning process, which considered quite difficult material for student, is experimental method. By using the experimental method, students not only get an active role in the learning process but also they can enhance their creativity. The purpose of this paper is to provide an alternative solution that is Bolemia (Electrochemistry Pen), an electrochemical learning media, to increase student's activity during the learning process. The method used in this paper is a qualitative descriptive approach based on the study of literature. It is expected the using of the learning media can make students become more active during the learning process and ultimately improve student understanding.

Keywords: Bolemia (Electrochemistry Pen), Students Activity and Creativity, Electrochemistry Materials.

1. INTRODUCTION

Electrochemistry is one of materials that is abstract and has a high difficulty level to be understood by students. Electrochemistry is also considered as one of materials that is difficult to be studied both by students and teachers [3]. This characteristics requires facilities that can support the learning process suited with the curriculum in 2013, which is student center.

Curriculum 2013 is a curriculum that requires students to actively participate in the learning process, because in this curriculum focuses on the student (student centered). Teachers act as facilitators or mediators as well as planner of learning to enable students actively seek new knowledge [6]. But in the reality, learning process involving the interaction of students, can't be applied maximally. Because there are many students who are passive during the learning takes place and are likely waiting for material from the teacher. They are less active in preparing the material and find the knowledge and skills independently. Because students only receive the knowledge as information from the teachers and they are unaccustomed to engage actively with what is learned. So, the knowledge will be easily forgotten and less meaningful in their everyday life.

This is contrary to the spirit of curriculum development stipulated in Government Regulation No. 19 in 2005 which states that: Standard learning process at unit level of education was held interactively, fun, challenging, and motivating the students to actively participate and provide space for innovation, creativity, and independence according to the talents, interests, physical and psychological development of learners.

To strive the standards of learning process quality, we need an alternative that can be used as a learning medium that attract students to be actively involved in the learning process, so that the knowledge and experience obtained during the learning process can be meaningful to them. Meaningful learning is expected to present a concrete experience in understanding chemical concepts which according to some students is an abstract concept and difficult to apply in the environment. One method that can be used to make an effective learning process of the material that is considered quite difficult for students is experimental method. Using the experimental method, students not only get an active role in the learning process but also they can enhance their creativity.

In this modern world, students are accustomed to use digital devices such as smartphones, tablets, and other advanced equipment in their learning process every day. So, expected with using Bolemia (model touch pen or

stylus that anti mainstream and have unique characteristics) may increase students interests to learn about electrochemistry by the experimental method. Making Bolemia as an experimental tool only requires materials and chemical equipment are cheap and easily obtained. In addition, experiments using Bolemia can also be done with a few variations of the experiment so it will give different results that can be easily observed by eye directly [4].

The purpose of this paper is to provide an alternative learning method that can actively engage students in the learning process and provides the opportunity for students to develop their creativity and ability to apply the knowledge they've gained. So expected the implementation of this learning method can improve students understanding of electrochemistry materials.

2. METHODS

The method used in this paper is a qualitative descriptive study based literature. Selection of this approach is expected to describe accurately the state or certain symptoms of the object of study. In this case I tried to create an overview of the concept of Bolemia (Electrochemistry Pen) as a learning media for senior high school students to learn about electrochemistry subject.

2.1 Materials and Equipment

(1) Batteries (1.5 volt) as a source of direct current in the electrochemistry process, (2) the empty whiteboard markers as the place for the electrochemistry process, (3) a copper wire as an anode that is connected to the positive pole of the battery, (4) electrolyte solution as an intermediary for this process, (5) foam or cotton to endure electrolyte solution that directly fall down or leak, (6) markers tip as a contact between the electrolyte solution with a metal plate, (7) isolation, (8) crocodile clips as a connector between the source of direct current with a metal plate, (9) metal plate as the cathode (metal coated).

2.2 Procedures

The first step is to prepare all the tools and materials needed. Then the second step is assembling the inside of Bolemia. That include batteries, copper wire, the empty whiteboard markers, content marker, electrolyte, foam, a place for electrolyte solution, marker tip, isolation, and others. The first copper wire is wrapped to the marker content, and then put it into place of electrolyte, the next is electrolyte solution is poured in this place, then it is closed with a cover that has been given a small hole for the release of copper from the electrolyte. Furthermore, the copper wire is connected to the positive pole of the battery (current source) as the anode and the negative pole of the battery is connected to the other copper wire that will be connected with the other metal plate as cathode. Then insert the finished sequence into a large markers and covered with a lid that has been given a small hole as a way out of copper wire. Then, copper wire is connected with the metal plate using crocodile clip. This metal plate as a cathode. And the last step is evaluation and modification. The evaluation process was conducted to evaluate the working system of the tool. So, after a prototype is complete, and there is a mismatch of the function of the tool, so these tool must be corrected until all the parts can do their function as good as possible. While modifications stage was made to obtain the experimental results that are different, so the students can make a conclusion from these learning process about electrochemistry. This modification process may include a potential difference of the battery used and the type of metal plate used.

3. RESULTS AND DISCUSSION

Electrochemistry is the study of energy changes, especially the changes of chemical energy into electrical energy or changes of electrical energy into chemical energy [10]. Electrochemistry is part of science that studies about the relationship between the chemical reactions with an electric current. Electrochemistry can be applied in a variety of human needs, such as the manufacture of materials both organic and inorganic chemicals, pharmaceuticals, polymers, automotive, jewelry, mining, waste management, and field analysis. According to [8] an electrochemical cell divided into two kinds, namely voltaic cell or galvanic cells and electrolysis cells. Voltaic cell is a cell that is based on a chemical reaction to produce an electric current. For example the battery and fuel cells. While electrolysis cells are cells based on chemical reactions that require electric current. The reaction in the electrolysis cell is not a spontaneous reaction because only proceed if there is a current source. The examples of the using electrolysis cell are:

Electrodeposition is metal deposition on the surface of the electrode. This technique is used for the manufacture of nanotechnology materials, electroplating, corrosion prevention, jewelry, and car accessories.

- Electroanalysis is the application of electrolysis for analysis, such as polarography, voltammetry, potentiometric, Normal Pulse voltammetry (NPV), Differential Normal Pulse voltammetry (DNPV), Square Wave voltammetry (SWV), anodic stripping voltammetry (ASV), Cathodic stripping voltammetry (CSV) and adsorptive stripping voltammetry (AdSV).

- Electrosynthesis is the synthesis of organic and inorganic compounds by electrolysis. This technique can overcome some of the weaknesses of synthesis in the usual manner. Some organic compounds can be synthesized in a way electrosynthesis include acetic acid, adiponitrile, alkyl tetra plumbum and tetrafluoro-p-xylene, while the synthesis of inorganic compounds include Ti, Al, Na, MnO_2 and Cl_2.
- Electro degradation is the decomposition of organic and inorganic waste. Decomposition of waste with this technique is more efficient and energy saving. The final result of the decomposition of organic waste is water and CO_2, while the inorganic waste such as metals to be deposited at the cathode. Metal that has been deposited at the cathode, the metal can be separated by dissolving them in strong acids, then separated into pure metal by precipitation.

The word 'Media' comes from the Latin language, medius, which literally means "middle", "intermediate", or "introduction". Technology and Communication Association (AECT) restrict media as forms and channels used to convey a message or information. Media is all forms of intermediaries used by the disseminator of ideas so the idea arrive to the receiver, while learning media is software or hardware that has a function as a learning tool [2]. While the definition of learning media is a communication tool that can be used to convey information from the source or teacher to the students who aim to stimulate them to pursue actively in the learning process.

Learning media can improve students learning process so it is expected to enhance the learning outcomes achieved. Learning media include physically tool that is used to convey the contents of the subject matter, such as books, tape recorders, cassettes, video cameras, video recorders, films, slides, photographs, images, graphics, and others. Learning media that is relevant will make the learning process to be effective and efficient.

Implementation of Bolemia in learning process. In the implementation of learning process using Bolemia, the author uses cooperative learning methods aided demonstration. One type of cooperative learning is cooperative problem solving (CPS). CPS is a problem-solving learning combined with cooperative learning. In addition, this method can be interpreted as a teaching method that causes a lot of learning activities. Because students are faced with the problem, then they have to formulate and test the truth of the hypothesis until they can get the conclusion as an answer from that problem. One of the methods that can be used is demonstration [1]. Demonstration is a teaching method where the teacher or instructor shows a process. Teachers can create a problem that will be solved by the students. In addition, demonstrations method will make students be easier to examine emerging issues [5]. The methods that have been aided by demonstration is chosen because demonstration methods used to help students understanding about the materials and to train their creativity thinking. This method is appropriate to be used if the facilities of laboratory is minimum or the limited time for doing experiments in laboratory [9].

The implementation begin with giving a demonstration using Bolemia by the teacher in front of the class. After the demonstration carried out, students were divided into some groups. Then, students are welcome to do an experiment using Bolemia with metal constituent and the different potential used. Then they observed the results. After they get the result, they can share and discuss with the other group. Because each group is provided Bolemia with different kinds of metal and the potential difference, the observations of one group and other groups will be different. This result will lead every member in a group to be active in the discussion to find out what is the factors that make the differences in these observation. With the positive interaction between students, so students are able to interact with other students in group discussions that will make students more active during these discussions (affective aspect) [7]. After the time trial and discussions are finished, then doing the evaluation process. This evaluation process was conducted to determine how students understanding of the electrochemistry material after implementation of learning method using Bolemia (Electrochemistry Pen) as a medium of learning. This evaluation process is done by giving a test, could be in the form of a written test or the other (Fig 1).

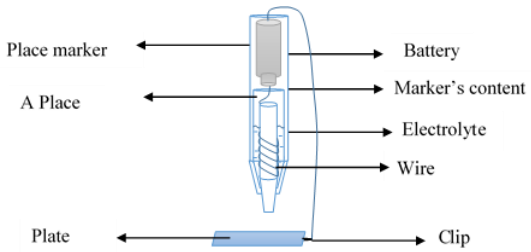

Figure 1. The Explanation and Design of Bolemia

4. CONCLUSIONS

It is expected that the implementation of learning process by using Bolemia (Electrochemistry Pen) as a learning medium can help students to understand about electrochemistry material and enhance their activity and creativity thinking. With these learning process is also expected to make learning process more meaningful for students.

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Experimentation of Teaching Math Model Think Talk Write (TTW) with Numbered Head Together (NHT) on The Probability Based on Emotional Intelligence of 11th Grade Students Senior High School in Sukoharjo

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Abstract – The research was a quasi-experimental research with a 2 × 3 factorial design that aims to determine the comparation of the Think Talk Write with Numbered Head Together (TTW-NHT) and the direct learning in terms of emotional intelligence for students' learning mathematics achievement. The population of this research was all of Senior High School Students grade 11th in Sukoharjo Regency schools in the academic year 2016/2017. The research sample was obtained by stratified cluster random sampling. The collection of data was methods of documentation, emotional intelligence questionnaire, and mathematics achievement tests. The analysis data technique used was two-way analysis of variance with unbalanced cells. Based on the research results, the conclusion is mathematics achievement of students who were subject to TTW-NHT learning model was better than the students who were subject to direct learning model.

Keywords: emotional intelligence, NHT, the mathematics learning achievement, TTW

1. INTRODUCTION

Education is a very important component in the formation and development of human resources in dealing with the progress. In this globalization era, all attempted field was to experience changes for the better, not only in the education in general, but also in the education of mathematics. Mathematics is one of the compulsory subjects that students need to learn from formal education. Mathematics also has a special place in our daily lives because math has a close connection with our lives. However, what is happening now is not what expected; mathematics is still a subject that is considered difficult by most students. A teacher of mathematics in particular should do efforts to make students happy with the subject. Students can receive, retain and further develop the teaching materials, the way of teaching and learning should be appropriate, efficient and effective in order to make a significant effect on increasing learning achievement.

Based on the observation of some of state senior high schools in Sukoharjo, there are many teachers who still apply direct instructional model. A teacher is still dominating in the learning activities, so students are passive and less enthusiastic in learning. Furthermore, based on interviews with some math teachers in state senior high schools of Sukoharjo regency, there are many students who have difficulties in learning opportunities. The difficulties that experienced by the students existed because of the lack of students' understanding of the material. The students are reluctant to ask of the material that has not been understood by them, and only a few teachers give students the chance to communicate their ideas. Students' difficulties in understanding the material affected on the chances of students' achievement on national exam results. The absorptive capacity of students in the material of opportunities at the national level was 45,46%, at the provincial level was 45,32% and at the level of regency, Sukoharjo in this case, was 45,05%. Therefore, some efforts are needed to improve the quality of learning in the material of opportunities.

One attempt to improve the mathematics learning achievement is the implementation of innovative learning model. The innovative learning model that can be implemented in the classroom is Think Talk Write (TTW) with Numbered Head Together (NHT) learning model. TTW learning model is basically built through think, talk and write. Huinker and Laughlin (1996: 81) argue, thinking and talking are important steps in the process of bringing meaning into student's writing". The learning model Numbered Head Together (NHT) could encourage students to work together and provide the opportunity to provide ideas. Suprijono, A (2013: 92) argues, "there are four stages of implementing NHT technique. They are numbering, asking questions, thinking together, and answering". The learning model has been exercised students' ability to think and speak.

The other important thing to improve the mathematics learning achievement is the support of emotional intelligences or Emotional Quotients (EQ). Martin (Ibrahim, 2011), revealed that most of the low achievement of

students in Indonesia in mathematics at school was not caused to the intelligence of the intellectual or their IQ but on emotional control. Winata (2014: 6) quotes the Shaw's opinion, "more emotionally supportive classrooms are associated with better academic achievement and in classrooms with lower levels of emotional support; children with poor attention tend to have lower achievement". Kalhotra (2012) argues, "those children who have high emotional intelligence will also be high academic achievers". Nasir (2012) said that, "emotional intelligence has a mediating effect on the relationship of cultural adjustment and academic achievement of international students. Emotional abilities help international students deal with difficulties in the process of adjustment to a new environment and make adjustment process easier, thereby, improve their academic performance". This indicates that emotional intelligence can help accelerate the students in the process of adjustment to the new environment, helping to improve their academic work. Ibrahim (2011: 64-65) argues, "An effort to improve emotional intelligence and cognitive skills can be performed simultaneously, even both support each other". Based on these images, it can be observed that the ties and cooperation between the emotional and mind would lead to their complementary between the two. The results of this study can be used as a reference for mathematics teachers of that mathematics learning should be managed so that is able to realize the intellectual and emotional balance.

The aims of the study is to figure out: (1) which learning model that gives better mathematics learning achievement, between the TTW-NHT learning model and the direct learning model on the probability, (2) which students that have better mathematics learning achievement between those who have high, medium, or low emotional intelligence on the probability, (3) which students that have better mathematics learning achievement between those who have high, medium, or low emotional intelligence who were subject to the TTW-learning model NHT and the direct learning model on the probability, (4) which learning model that gives the better mathematics learning achievement, between the TTW-NHT learning model and the direct learning model to the students who have high, medium, and low emotional intelligence on the probability.

2. METHODS

This research was conducted in some state high schools in Sukoharjo in the first semester of the school year 2016/2017. This type of research is a quasi-experimental research. . The populations of this research were all of Senior High School Students grade 11th in Sukoharjo Regency schools in the academic year 2016/2017. The sampling in the research was done by stratified cluster random sampling. After sampling was obtained, it was found that SMAN 3 Sukoharjo represents a school with a high group, SMAN 1 Polokarto represents a school with a medium group, and SMAN 1 Mojolaban represents a school with a low group.

The data collection technique that was used is documentation, test and questionnaire methods. Before performing the experiment, the normality, homogeneity, and balance test were done to gain the prior knowledge data of mathematics using ANOVA one way with unbalanced cells. While data on the mathematics learning achievement were analyzed using t-test after normality test and homogeneity test had been done. The normality test on the prior knowledge data and learning achievement data is done using Lilliefors test and the homogeneity of the population variance using Bartlett test. Research hypothesis testing used was two-way analysis of variance technique with unbalanced cells. If the result of the analysis of variance showed that the zero hypotheses was rejected, the researcher would use a further test of post-ANOVA; namely Scheffe test.

3. RESULTS AND DISCUSSION

The prerequisite test results concluded that all the samples were taken from a normal distribution population and the population had the same variance. Furthermore, the balance test found that the samples of the experimental group and the control group were in a state of balance. Then, the hypotheses test was done. The average between cells and average marginal is shown on Table 1.

Table 1: The average between cells and average marginal

Learning Model (A)	Emotional intelligence (B)			Average Marginal
	High (b_1)	Medium (b_2)	Low (b_3)	
TTW-NHT (a_1)	66,9565	64,9143	65,8889	65,7872
Direct (a_3)	63,0000	54,6667	56,8889	58,0952
Average Marginal	64,5424	59,3247	62,0317	

The resume of two-way analysis of variance with different cell is shown in Table 2.

Table 2: The resume of two way analysis of variance with different cell

Source	SS	df	MS	F_{obs}	F_a	Determination
A	2857,1345	1	2857,1345	17,2984	3,8901	H_{0A} is rejected
B	898,380	2	449,4690	2,7213	3,0427	H_{0B} is accepted
A*B	353,2528	2	176,6264	1,0694	3,0427	H_{0AB} is accepted
Error	31877,2549	193	165,1671			
Total	35986,5803	198				

Based on the Table 2, it can be concluded that (a) there are differences in the mathematics learning achievement between the students who were subject to the TTW- NHT learning model and the direct learning model, (b) there is no difference in the mathematics learning achievement among students with high, medium, and low emotional intelligence, (c) there is no interaction between the learning model and the emotional intelligence of the students' the mathematics learning achievement.

Based on calculations was obtained H_{0A} is rejected. It means that there are differences in the mathematics learning achievement between the two models of learning. Then, based on the average marginal in Table 1 it is concluded that TTW-NHT learning model is better than the direct learning model in the mathematics learning achievement of students. These results are supported by Kosko and Wilkins (2010: 1) argues, "Both writing and discussion are seen as a way for individuals to reflection or explain in detail certain mathematical ideas". Idris (2009: 42) argues, "Expository writing is an effective and practical tool for teaching math problem solving.

Based on calculation, it shows that H_{0B} is accepted. It means that there is no difference in learning mathematics achievement between the students who have high, medium, and low emotional intelligences. Therefore, the further test of Anava between columns is not necessary to be done. The conclusion is students with high, medium, and low intelligences have similar learning mathematics achievement in terms of competencies.

Based on test result, it shows that H_{0AB} is accepted. Therefore, there is no interactions between the learning models and the students' emotional intelligences, so that the further Anava test between cells is not necessary to be done. In each level of emotional intelligences, the TTW-NHT learning model has better learning mathematics achievement than the direct learning model has. In addition, in each teaching models, the students with high, medium and low intelligences have similar learning mathematics achievement in terms of competencies.

4. CONCLUSIONS

Based on the result, it can be concluded that: (1) the students who were subject to the TTW-NHT learning model have better learning mathematics achievements than those who were subject to the direct learning model. (2) the students with high, medium, and low intelligences have similar learning mathematics achievements in terms of competencies, (3) in each emotional intelligences, the TTW-NHT has better learning mathematics achievement than the direct teaching model has, (4) in each learning models, the students with high, medium and low emotional intelligences have similar learning mathematics achievements in terms of competencies

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The Effect of Project Based Learning Toward Student's Self Efficacy at The Material of pH Range Natural Indicator

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Abstract – This study aim analyze the effect of project-based learning (PjBL) model toward student's self efficacy at the material of pH range natural indicator. The method used is quantitative method with pretest posttest non equivalent control group design. Subject of the study consisted of 37 students who received the experimental group learning PjBL model, and 36 students as a control group who used learning of convensional . Instrument is used such as observation and self-efficacy questionnaire. The enchancement of learning outcome was measured based on differences of score pretest and posttest using the formula of N-gain. The different between two means were analyzed by using t-test in significant degree of 0,05. The results showed that there is effect of model ,PjBL toward student's self efficacy at the material of pH range natural indicator in which the average N-gain group of experiments was higher than the control group in the medium category.Teachers are expected to be able to implementing the model of PjBL specially at the material of pH range natural indicator which is students-centered in improvement self-efficacy.

Keyword : PjBL, self efficacy, natural indicator.

1. INTRODUCTION

Education is not only a process of "transfer of knowledge" but it is also "transfer of values". This is under Act number 20, 2003 on National Education System Article 3, which explains that national education serves to develop the ability and build character to educate life of the nation (Kementrian Pendidikan dan Kebudayaan, 2013). Building character is creating a character such as self-efficacy.

Active and productive learning will bring some activities. These activities can be influenced by internal factors. Internal factors consist of physiological and psychological aspects. Self-efficacy is one of physiological aspects. The learning process is influenced by self-efficacy (Purwanto, 2004). The self-efficacy is used by students to organize their own learning and to master academic activities, so that would affect to the achievement and motivation (Bandura, 1993). Students who have a high efficacy tend to be active and productive since students who have low self-efficacy tend to be passive and not productive. Self-efficacy plays a cognitive mechanism that allows a person to control themselves against pressure or a difficult condition. The stronger the individual's beliefs towards memories capacity, the more powerful assembled efforts for processing and increasing the memory capacity of the individual (Bandura, 1997). Self-efficacy is indispensable in the learning process.

Good learning is learning that can involve the students through the activities of learning, so that students become more active and productive. The learning process is carried out in schools still tend to be centered on the teacher (Ngalimun, 2015), the learning is still going in one way. Teachers more dominate school activities, so students become passive and inoperative. Students only receive information from teachers. So, necessary to look for alternative models of learning process that can increase the student's spirit to learn. One of learning process model that can involve students in the learning process is a model of Project-based Learning (PjBL).

Studies of the effects of PjBL model toward student's self-efficacy had been ever done. The research subjects were divided into two groups: the experimental group was and control groups. The results of the research show that there are effects of PjBL model on self-efficacy which self-efficacy of experimental group using the PjBL model is higher than control groups (Bas, 2011; Chen & Chan, 2011; Ilter, 2014; Bilgin, et al., 2015; Chen, et al., 2015; Shain, 2015; Gacanja, et al., 2016; Goldstein, 2016).

Based on the problems mentioned above, necessary to study on how the PjBL model effects toward student's efficacy. The research was conducted on the material of PH range natural indicator. Through the material of PH range natural indicator with learning of PjBL model which comprises the steps in determining the

fundamental questions, designing the project planning, scheduling project design, monitoring students in planning the project, examining results and presentations, and testing experiences, which ultimately produces results, expected that student's self-efficacy can be increased. This study analyzes the effects of PjBL model towards self-efficacy of high school students at the material pH range natural indicator.

2. METHODS

The research is conducted using quantitative approach. The method used is quasi-experiment, with pretest-post-test design, non-equivalent control group (Wierma&Jurs, 2009). The technique used is purpose sampling. This study is conducted in one of the National High School in Bandung. The sample comprises two groups, experimental group and control groups. The experimental group is Class XI of MIA I which comprises 37 people, and the control group is class XI of MIA II which comprises 36 people. The instruments used in this research include observation sheets and questionnaires of the student's self-efficacy adapted from efficacy questionnaire developed by Uzuntiyarki and Aydin (2009). Aspects of self-efficacy measured in this research include aspects of cognitive abilities, psychomotor abilities, and applications in daily life.

Technique of collecting data is conducted in two ways: fieldwork and literature research. Technique of collecting data to decide the effects of the PjBL model toward student's self-efficacy at the material of pH range natural indicator uses self-efficacy questionnaire. Analyzing data in this research is processed using software support of MS Excel 2013 and Predictive Analytics (PASW Statistics 18). The data of test results of mastering concepts and student's self-efficacy are analyzed by using statistical tests. The processed data in this research is N-gain data. Having got N-gain data is then performed statistical tests to find differences of increasing student's self-efficacy between the experimental group and the control group.

3. RESULTS AND DISCUSSION

The effects of PjBL model towards students' self-efficacy at the material of pH range natural indicator is measured using self-efficacy questionnaire. To know how the effect of student's self-efficacy, so self-efficacy questionnaire is distributed to students before and after the learning with PjBL model.

The results of data analysis of overall student's self-efficacy can be seen in Table 1. Based on Table 1, got the results are as follows; in the experimental group, the average pretest score is 90.03 and increase up to 134.95 after using PjBL learning. The score of N-gain is 0.45. Based on the criteria of Hake (1999), that increase is in middle category. In the control group, the average pretest score is 95.86 and increase up to 123.50 after using conventional learning. The score of N-gain is 0.30, that increase is in the low category.

Table 1 (Student's Self-Efficacy Overview)

Group	Statistic	Student's Self-Efficacy		
		pretest	Post-test	N-gain
Experiment	$\bar{x}$	90.03	134.95	0.45
	st dev	17.16	11.54	0.07
Control	$\bar{x}$	95.86	123.50	0.30
	st dev	7.78	13.74	0.13

Ideal Maximum Score = 189

The results of data analysis show that the average score pretest experimental group and control group is relatively the same while it will be a diversity in the average posttest score both on experiment group and on control group. Average score of posttest efficacy in experimental group is bigger than the control group. The. Based on standard deviation score of N-gain, student's self-efficacy scores in experimental group are better than control group, but this increase is not significance.

If the overview of the student's self-efficacy is described by its aspects, so it will be presented in Table 2 as follow.

Table 2 (Overview of Student's Self-Efficacy Aspects)

Group	Statistic	Aspect 1			Aspect 2			Aspect 3		
		pretest	posttest	N-gain	pretest	posttest	N-gain	pretest	posttest	N-gain
Experimental	$\bar{x}$	28.59	44.84	0.47	30.95	45.73	0.46	30.49	44.38	0.42
	st dev	5.40	3.71	0.09	7.03	4.30	0.12	7.21	5.44	0.13
Control	$\bar{x}$	29.36	41.75	0.37	33.81	43.50	0.32	32.69	38.25	0.18
	st dev	4.22	4.94	0.13	4.33	5.44	0.20	5.41	6.11	0.18

Ideal Maximum Score = 63

Based on Table 2, the results show that the experimental group both in self-efficacy condition by cognitive abilities (feature 1), in self-efficacy condition by psychomotor abilities (feature 2), and in self-efficacy condition by applications in daily life (feature 3), it will be an increase of student's self-efficacy before and after implementation of learning with PjBL model. N-gain score of each feature is in middle category. In control group both in self-efficacy condition by cognitive abilities, in self-efficacy condition by psychomotor capabilities, and in self-efficacy condition by applications in daily life, it will be an increase of students in self-efficacy before and after implementation of conventional learning. N-gain score of each feature is in middle category, except in feature 3 in low category. The increase in experimental group was greater than the increase in control group.

The results of the data analysis of pretest average score based self-efficacy condition by cognitive abilities in experimental group and control group is relatively the same. Based on the data of the pretest and posttest average score on this feature indicates that it will be an increase in self-efficacy both in experimental group and control group. N-gain score of feature 1 in experimental group is 0.47, while in control group is 0.37. The increase in experimental group is greater than in control group but the increase in student's self-efficacy is not statistically significant.

The next feature is self-efficacy condition by psychomotor abilities. Based on the data of the pretest and posttest average score on this feature, it shows that it will increase self-efficacy both in experimental group and control group. The increase in experimental class is greater than in control group but the increase in student's self-efficacy on this feature is not significant.

The feature 3 is the self-efficacy condition by ability of applications in daily life. Based on the data of pretest and posttest average score in this feature, it indicates that it will be increasing self-efficacy both in experimental group and control group. N-gain score in the ability of applications in daily life in experimental group is 0.42, in control group is 0.18. The increasing in experimental group is greater than in control group but the increasing in student's self-efficacy in this feature is not significant. Based on the analysis of self-efficacy above, both its overall and by its aspects, the PjBLmodel gives effect towardsstudent's self-efficacy at material of pH range natural indicator. Testing the effects ofPjBL model towards student's self-efficacy is conducted by statistical tests.

Statistical test of the effects PjBL model towards student's self-efficacy at the material of pH range natural indicator is conducted using independent t test. This section will test the variation of self-efficacy N-gain based on learning approach. Before the test, firstly conducted the prerequisites test, namely normality and homogeneity of variance test of the two groups of data samples N-gain. The test results are presented in Table 3 and Table 4. Table 3 shows that both groups have a sig. (2-tailed) value that is greater than 0.05, so H_o is accepted. Thus, according to the N-gain data of student's self-efficacy of both groups of learning, population is normally distributed. Having in mind that the data is normally distributed, conducted the homogeneity test of N-gain data of students' self-efficacy of both groups of learning with the following hypotheses.

H_0: There is not difference variance score of N-gain self-efficacy in terms of group learning.

H_1: There is difference variance score of N-gain self-efficacy in terms of group learning.

Table 3 (Normality Test of N-gain Self-Efficacy Data of Both groups Learning)

Data	Learning	n	Average	Sig. (2-tailed)	H_o
Overall	PjBL	37	0.454	0.846	Accepted
	Conventional	36	0.299	0.407	Accepted
Aspect 1	PjBL	37	0.469	0.863	Accepted
	Conventional	36	0.365	0.998	Accepted
Aspect 2	PjBL	37	0.462	0.402	Accepted
	Conventional	36	0.321	0.927	Accepted
Aspect 3	PjBL	37	0.424	0.561	Accepted
	Conventional	36	0.178	0.865	Accepted

Testing criteria is if a probability value (sig.) is greater than $\alpha = 0.05$, then H_0 is accepted, and H_0 is rejected. To test this hypothesis, Levene test is used (Levene's Test for equality of Variances). Results of homogeneity test calculations are presented in Table 4.

Table 4 shows that the sig. (2-tailed) N-gain data is less than 0.05, so H_0 is rejected. Thus, the two sets of data variance N-gain of students' self-efficacy in both groups learning is not homogeneous. So, to test the data N-gain of students' self-efficacy in both groups learning, t-independent statistic of equal variance not assumed is used.

Table 4 (Variance Homogeneity Test of N-gain Self-Efficacy Data in Both groups Learning)

Data	Levene Statistic	Sig.
Overall	10.452	0.002
Aspect 1	6.522	0.013
Aspect 2	10.708	0.002
Aspect 3	4.549	0.036

To test the hypothesis, then the hypothesis is proposed to examine differences in students' self-efficacy N-gain in both groups learning. The testing criteria is if the probability value (sig.) is greater than $\alpha = 0.05$, then H0 is accepted, in other cases H1 is rejected. The test results of N-gain significance of differences in students' self-efficacy by using the t-independent test of equal variance not assumed are presented in Table 5.

Table 5 shows that the value of the probability or sig. (2-tailed) is smaller than $\alpha = 0.05$, so H0 is rejected. Thus there are differences in self-efficacy increase both overall and in every feature of the students who receive PjBL learning with conventional learning. If seen from the average increase, it was found that the average increase in PjBL learning models is higher than conventional learning. It means that there is PjBL effects towards students' self-efficacy of pH range natural indicator both overall and in each feature.

Table 5 (Difference N-gain Test of Students' Self-Efficacy in Both groups Learning)

Data	Learning	N	Equal Variance Not Assumed					H_o
			Average	St Dev	Average Differences	t	Sig. (2-tailed)	
Overall	PjBL	37	0.454	0.075	0.155	6.324	0.000	Rejected
	Conventional	36	0.299	0.127				
Aspect 1	PjBL	37	0.469	0.091	0.105	3.881	0.000	Rejected
	Conventional	36	0.365	0.135				
Aspect 2	PjBL	37	0.462	0.118	0.141	3.706	0.000	Rejected
	Conventional	36	0.321	0.196				
Aspect 3	PjBL	37	0.424	0.127	0.246	6.806	0.000	Rejected
	Conventional	36	0.178	0.176				

Self-efficacy is the confidence and assessment of one's ability or ability in the face of an obstacle to achieve a goal. Confidence in this case is the conviction in assessing the ability of students to do a task that is confidence in the ability of different cognitive, psychomotor, and the ability of applications in daily life.

According to Bandura (1997), that self-efficacy is the confidence in one's abilities to drive motivation, cognitive resources and a set of actions required to meet the demands of the condition at hand and motivation, cognitive resources, and a set of actions that can affect learning. According to Bandura (1997) through the motivational process, individuals who have high self-efficacy will intensify its efforts to overcome the challenges showing positive efforts and existence. Further, through cognitive sources, that the self-efficacy of individual will affect the mindset that is helping or hindering. While a set of actions result from affective processes, in which self-efficacy influences on how much pressure is experienced by individuals in a threatening condition. Individual who believe he can solve conditions that threaten and he perceives, will not accept anxious and disturbed by the threat, so he will do activities.

The activities in the learning process conducted in this research include activities in the stages of PjBL model. The stages include determining the fundamental questions, designing the project planning, designing schedule project planning, testing results (experimental) and presentation, and testing the experience.

The stages in the PjBL model will improve self-efficacy both self-efficacy of good cognitive abilities, self-efficacy of psychomotor abilities, and self-efficacy of application in daily life.Student's self-efficacy in assessing the cognitive abilities, self-efficacy in assessing psychomotor abilities, and self-efficacy in real life is not always the same, it depends on the level of complexity and the breadth of the material (Bandura, 1997). Student who has high self-efficacy will act rapidly and focus, especially if the goal is a clear goal. The high self-efficacy is perceived will motivate individuals cognitively (Bandura 1997). Instead of student who has a low efficacy cannot act rapidly and focus.

Student who has high self-efficacy himself grow confidence that, "I know, I am able to do this", so that student who has high self-efficacy will show active belief patterns, able to manage the condition and face the obstacles, set goals, plan, prepare and apply, strive and diligent, productive and able to solve problems, learn from failures and show success. Instead of student who has low confidence beliefe patterns cause him to be passive, shy away from the difficult task, not able to develop aspirations and low commitment, he focuses on his shortcomings, does not want to try and does not want to struggle, is discouraged by the failure, blames the failure on inability, is anxious, is stressed and depressed, so the result is a failure (Kreitner and Kinicki, 1989).

Results of research on show an increase in students' self-efficacy though it as not significant, can be caused by various factors, may come from internal and external factors. According to Purwanto (2004), internal factors comprise physiological and psychological aspects. Physiological aspects are the physical condition of the student upon receipt of learning such as health condition and so on. External factors that affect students' activities are condition of family, educators and teaching in this case, educator is and a researcher, subject matter, social environment and school environment.

4. CONCLUSIONS

Based on the results of research and discussion, it can be concluded that the effects towards students' self-efficacy at material of PjBL model in pH range natural indicator in the experimental group show that N-gain score is better than the control group but not significant.

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Development Intertextual Learning Strategy with Guided Inquiry on Hydrolysis of Salt to Enhance the Concept Mastery of Students

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Abstract – This study aims to develop intertextual learning strategy with guided inquiry on hydrolysis of salt to Enhance the concept mastery of students. The method used is the Research and Development (R & D) with a modified models of the 4-D (four D models). This study was designed with 4 stages on 4-D models items, namely (1) define, (2) design, (3) develop and (4) disseminate. Application of 4-D in this study just until the preliminary field testing that is part of the development stage. The object of this study is the intertextual learning strategy with guided inquiry on hydrolysis of salt to enhance the concept mastery of students. The instruments used were validation sheets to know the congruency between learning activities with guided inquiry learning steps and also between activities with concept mastery aspects, concept mastery test, and observation sheet. The result of intertextual learning strategy development with guided inquiry on hydrolysis of salt was claimed as valid by 4 validator with some correction based on the validators' suggestion. The test is done to the students of class XII SMA (Senior High School) in Bekasi. In this study used pre-experimental method to one group pretest-posttest design. The trial results showed that the intertertextual learning strategy with guided inquiry on hydrolysis of salt can enhance concept mastery of students with N-gain Obtained at 0.38 which include medium criteria

Keyword: intertextual strategy, guided inquiry, concept mastery, hydrolysis of salt.

1. INTRODUCTION

Chemistry is one of the branches of Natural Science that studies the composition, properties, and the transformation of materials and how the composition of a material affects its properties [4]. The chemistry is a science that is quite difficult to learn, it is because the concepts in chemistry are abstract [13]. One of the reasons for student difficulties in understanding the chemistry that is associated with the use of the level of representation used to explain the chemical phenomena [5]. Difficulties experienced by students in chemistry can cause errors in understanding a concept that is often called misconceptions [1]. Based on previous research, misconceptions experienced by students occur on some chemical concepts to the material of which the hydrolysis of salt. One of the causes of misconceptions students in understanding chemical concepts is the inability of students in linking the three levels of representation used to explain the phenomena in chemistry [5]. Misconception reflect mastery of concepts is lacking in the concept. Whereas one of the essential character of chemistry include understanding the representation of students at three levels, namely the macroscopic, submicroscopic and symbolic as well as the relationship between the three levels should be explicitly taught [8]. Level macroscopic representation of a chemical that can be obtained from direct observations *(visible)* to a phenomenon that occurs in everyday experience, the level of submicroscopic namely the representation of chemistry that explain the observation of phenomena to the sense on the level of particles (atoms, molecules, or ions), and symbolic level represents a quantitative and qualitative chemical, such as chemical symbols, chemical formulas, images, and equation [12]. Submicroscopic representation is a key factor in the ability of multiple representation, inability to represent submicroscopic can hamper the ability to solve problems related to the phenomenon of macroscopic and symbolic representations [6].

Hydrolysis of salt is one of the chemical materials that require third-level representation (representation macroscopic, submicroscopic and symbolic) to be able to understand this material well. This material is the application of the concept of chemical equilibrium that occurs in solution with water solvent. Contextually, the concepts in this material plays an important role in biological processes and the environment, one example is controlling the pH of the water should remain 5.5 in order to plant and aquatic life is going well [11]. The phenomenon can be more easily understood, involving the three levels of representation.

In the process of learning chemistry, students should be guided to connect between the three levels of representation both visually and verbally, so that students can understand the chemistry involving a conceptual relationship between the representation of the macroscopic, submicroscopic and symbolic. Teachers can use learning strategies intertextual in helping students understand chemistry concepts by linking the chemical representation, everyday experience, and the events experienced by students in the class, so as to provide an understanding of chemical concepts intact and correctly [16]. Intertextual learning strategies can be applied with inquiry learning. Learning by inquiry can connect science fact with science concepts [3]. So with the inquiry learning students can connect real phenomena found in the students' everyday life and science concepts using macroscopic level, submicroscopic and symbolic. The relationship between the three levels of the chemical representation need to be taught explicitly in the context of the inquiry learning process [16]. Inquiry learning encourages students to test his hypothesis with data obtained from direct observations such as the experimental data. Students will use the symbols and find their own answers to the questions, so that the two levels of representation are directly applied in inquiry learning. Learning with the inquiry can involve students actively in the investigation in the form of practical activities that can support to enhance students' skills in linking the three levels of representation from observed macroscopically to build symbolic representations [2]. In this study of inquiry used is *guided inquiry* (guided inquiry), based on considerations which students study place have yet to get a learning experience with guided inquiry learning, so they needed the help of teachers as facilitators to guide students in conducting investigations. Guided inquiry can be given to students who do not have experience in conducting such an inquiry [3]

Based on these problems, it is necessary learning strategies that can associate the three levels of the chemical representation of macroscopic level, submicroscopic and symbolic, so as to improve the mastery of concepts. One strategy is the right strategy intertextual learning with guided inquiry. Based on the identification of the problem, it can be formulated the main problem is "How the development of learning strategies intertextual with guided inquiry on material salt hydrolysis to improve students' mastery of concepts".

2. METHODS

This study uses the approach of *Research and Development* (R & D) with a modified model of the 4-D *(four D models)* [15]. Model 4-D consists of four main stages, namely: (1) *define,* (2) *design,* (3) *develop* and (4) *disseminate*. Application of 4-D in this study was not conducted in full, but just until preliminary field testing that is part of the development stage due to limited study time. Based on the above research and development, this research is to develop learning strategies intertextual with guided inquiry on hydrolysis of salt concept to increase mastery concepts of students are tested to the students of class XII science in one high school in Bekasi.

The steps of research and development as follows.

1. *Define*

At this stage, the study of literature and field study. Studies conducted literature is (i) an analysis of the content standards based curriculum 2013 on hydrolysis of salt concept; (ii) a literature review guided inquiry learning model; (iii) review of the literature intertextual learning strategy; (iv) review of the literature aspect mastery of concepts;

2. *Design*

The design phase of intertextual learning strategy with guided inquiry is based on the results of preliminary studies. At the design stage have made the initial draft strategy intertextual with guided inquiry learning on hydrolysis of salt concept. Before designing learning strategies intertextual with guided inquiry, first performed an analysis of the matters which acts as a support in designing learning strategies intertextual with guided inquiry that is the translation of multiple representations of the material salt hydrolysis, analysis misconceptions salt hydrolysis of various journals / articles, analysis concept description from various books *of general chemistry,* analytical indicators of mastery of concepts conformity with the description of the concept. Intertextual learning strategies developed by guided inquiry is designed to improve the mastery of the concept of students.

3. *Develop*

The purpose of this phase is to improve the design obtained from the previous stage. Results from the design stage *(design)* is seen as an early version that must be refined before it becomes a final version as appropriate. At this stage there are two things to do that validation and test strategies intertextual learning with guided inquiry on material salt hydrolysis.

a. Validation was conducted to determine the validity of the strategy developed. A total of four experts (validator) with expertise in the field of chemical education required to validate the suitability of the learning activities with guided inquiry syntax and conformance aspects of concept mastery in

learning activities. At the suggestion of improvement of validator, intertextual learning strategy with guided inquiry developed to be more precise, effective, and high quality and can be used in learning.

b. Testing strategies intertextual learning with guided inquiry in the form of a trial is limited to 35 students at one high school in Bekasi. The study design used at that stage is *pre experiment. Pre experiment* design used for this study is a pilot study that in this study there was no control group for comparison. *Pre experimental* design used was *one group pretest-Posttest.* Before learning takes students must first conduct *a pretest* and *posttest* after the study is *completed.* Instruments used in the trial that is about mastering the concept, questionnaire responses of students and teachers questionnaire responses.

At the time of the study, the instrument is a major tool in data collection. The instrument used in this study is the validation sheet, test mastery of concepts.

2.1 Data analysis technique

The data obtained in this study is the result of the validation of learning strategy intertextual with guided inquiry on hydrolysis of salt concept, the results of tests mastery of concepts, the results of the questionnaire responses of students and teacher responses to the trials of learning strategies intertextual with guided inquiry on the hydrolysis of salt. The data was analyzed in detail as follows.

1. The results of the validation strategy

Data obtained in the form of words and symbols. The symbol is shown with a check mark in the column validation while the words shown on the comments and suggestions provided by the validator. The data obtained are described and taken into consideration for fixing the validated content.

2. Results of tests mastery of the concept

Results of tests mastery of the concept is obtained before the study *(pretest)* and after learning *(posttest).* Calculating an increase in test mastery of the concept is calculated by *N-gain* (Normality Gain). Increasing mastery of the concept of students according to criteria (Hake, 1999).

3. The results of the observation sheet and videotape

The results of observation sheets and video recordings acquired during the learning described into data trial learning strategies intertextual with guided inquiry on hydrolysis of salt, in addition to video recording is also used sound recordings as supporting data to describe the activity of each student in the group during the learning takes place

3. RESULTS AND DISCUSSION

Results of research and discussion on learning strategies intertextual with guided inquiry on hydrolysis of salt to increase mastery of the concept of students. Results and discussion includes several stages.

Step Development Intertextual Learning Strategy with Guided Inquiry on Hydrolysis of Salt Concept.

1. **Analysis of Curriculum Content Standards**

The development of learning strategy begins with an analysis of Content Standards (SI) to the curriculum in 2013 related to the concept to be taken, namely the concept hydrolysis of salt. Analysis of content standards in this study refers to the content of the curriculum standards in 2013 which includes the analysis of core competencies (KI) and the Basic Competency (KD) chemical subjects.

2. **Determination Mastery Indicators Concepts Concepts and Descriptions Salt Hydrolysis**

The structure of the curriculum includes core competencies (KI) and basic competence (KD), core competencies include the four dimensions of one of them is the number three core competencies are knowledge dimension. Indicators mastery of concepts derived from the Basic Competence 3.11 (Analyzing the balance of ions in the salt solution and calculate pH). Indicators mastery of concepts formulated by KD 3.11 consists of the following three indicators as below: explaining the concept of salt hydrolysis, analyze the nature of the acid, alkaline or neutral of a saline solution based on equilibrium reactions of ions in the salt solution, and calculating the pH of salt solution undergo hydrolysis. Once formulated indicators of mastery of concepts is carried elaboration on the concept description salt hydrolysis matter by referring the literature that describe a precise concept that is of textbooks *General chemistry*

3. **Draft Activity Learning Strategies intertextual with Guided Inquiry on Salt Hydrolysis Material to Improve Concept Mastery of Students .**

The design of learning activities intertextual use of guided inquiry on material salt hydrolysis to improve the control concept was developed based on indicators of mastery of concepts, descriptions of concepts and

representations that have been chemically developed earlier. The stages in the learning activities using the following syntax guided inquiry. Syntax guided inquiry to use based on the results of the study of literature. As previously described, the syntax of inquiry used consisted of six stages that lead to problems, formulate hypotheses, design experiments, conduct experiments, analyze data, and draw conclusions.

Results Validation draft Learning Strategy on material intertextual Hydrolysis Salt to Improve Student Mastery Concept.

The design of intertextual learning activities with guided inquiry on material salt hydrolysis to increase mastery of the concept are arranged in tabular form validation conformity with the syntax of the learning activities of guided inquiry and compliance aspects of the mastery of the concept in learning activities. The table validated by 4 validator.

Description Trial Learning Strategies intertextual with Guided Inquiry on Salt Hydrolysis Material to Improve Concept Mastery.

An overview of the learning process of learning strategies intertextual with guided inquiry on material salt hydrolysis can be described from the activities of teachers and students during the learning activities with learning strategies intertextual with guided inquiry underway. To describe the activities of teachers and students clearly it is supported by video recordings and sound recordings of each group.

Mastery of Concepts in Materials salt hydrolysis after Trial Students Learning Strategy intertextual with Guided Inquiry on Salt Hydrolysis material.

Learning material salt hydrolysis in this study has three indicators mastery of concepts, which explains the concept hydrolysis of salt, analyze the nature of the acid, alkaline or neutral of a saline solution based on equilibrium reactions of ions in the salt solution, and calculate the pH of salt solution undergo hydrolysis. Each indicator is analyzed ketercapaiannya based on % *N-gain* that has been calculated from the score of *pretest* and *posttest scores* and % *N-gain* for each indicator mastery of concepts shown in the following table.

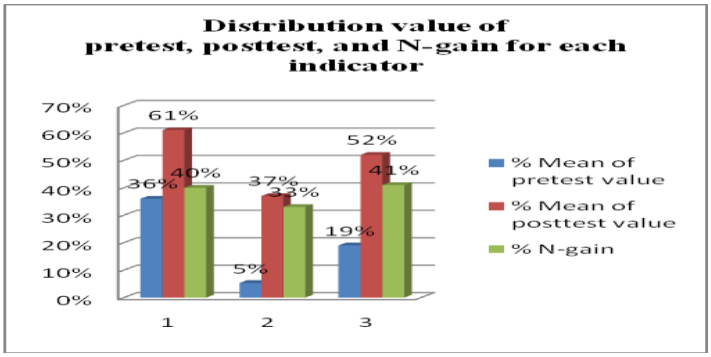

Figure 4.1. The percentage of the value *pretest, posttest* and N- *gain* for each indicator

3.1 DISCUSSION

The draft strategy intertextual learning with guided inquiry on material salt hydrolysis to increase mastery of concepts validated by the validator 4 people skilled in the field of chemical education. The results of the validation of conformity with the syntax of learning activities guided inquiry and compliance aspects of the concept of mastery learning activities declared invalid by all validators with some suggestions and improvements. suggestions and improvements of each validator in the form improve the content in the form of correspondence between the syntax learning activities with guided inquiry and compliance aspects of the concept of mastery learning activities.

The draft revised based on suggestions and feedback from the validator, and then conducted trials intertextual learning strategy with guided inquiry on material salt hydrolysis, a test of mastery of concepts students through learning strategies intertextual with guided inquiry on material salt hydrolysis. Based on trial results learning strategies intertextual with guided inquiry on material salt hydrolysis visible from the student activity very enthusiastic and active in learning, as in step experiment to develop skills at the level of macroscopic, and analyze data that can develop your skills at the level of submicroscopic and symbolic form of the questions in analyzing the data, so as to make students actively discussing to find their own answers in developing the concept. In trials to measure students 'mastery of concepts in order to obtain information on students' mastery of concepts consisting of about the description. This matter represents three indicators of learning that explains the concept of salt hydrolysis, analyze the nature of the acid, alkaline or neutral of a saline solution based on equilibrium reactions of ions in the salt solution, and calculate the pH of salt solution undergo hydrolysis. Each indicator is analyzed reached based on % N-gain that has been calculated from the score of pretest and posttest scores. Increased mastery of concepts for indicators 1 comprising from third question (question no 1.2. And 3)

obtained N-gain of 40%, which included low criteria according to Hake [7], increasing mastery of the concept of the indicator 2 is comprised of 3 questions (question no 4,5, and 6) acquired% N-gain of 33% were classified as moderate category. increasing mastery of the concept of the indicator 3 consists of 3 questions (question no 7,8, and 9) acquired% N-gain of 41% were classified as moderate category. Overall improvement of students' mastery of the material concept hydrolysis of salt obtained by calculating the value of N-gain of 0.38, which means the trial intertextual learning strategy with guided inquiry on hydrolysis of salt can improve students' mastery of concepts on the medium criteria.

4. CONCLUSIONS

Based on the research that has been done, it can be concluded that it has generated textual learning strategy with guided inquiry on material salt hydrolysis to increase mastery of concepts that are appropriate, effective, and of high quality. Try out intertextual learning strategy with guided inquiry on material salt hydrolysis include learning process with textual learning strategy with steps on guided inquiry. During the learning process, students follow the learning process by step systematic and structured learning. In general, almost all students are active, interested and enthusiastic about learning with learning strategies intertextual with guided inquiry on material salt hydrolysis. Trial intertextual learning strategy with guided inquiry on material salt hydrolysis can improve students' mastery of concepts in these materials. Increasing students 'mastery of concepts to the material obtained by salt hydrolysis *of N-gain* value calculation, the overall values obtained *N-gain* of 0.38, which means the trial intertextual learning strategy with guided inquiry on material salt hydrolysis can improve students' mastery of concepts on the criteria being.

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Developing Worksheet and Problem sheet Based on Creative Thinking Skills With Multimodal Representation for Physics Learning in High School on The Topic Static Fluid.

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Abstract – This research aims to develop creative thinking skills oriented worksheet and problem sheet using multimodal representation on the topic static fluid for physics learning in high school. Through the research and development (R&D) design, worksheet and problem sheet have developed using the Representational Approach Learning to Write development method. Instrument used in this study consist of comprehensibility test, quality questionnaire, and creative thinking skills test. Data of comprehension and quality test collected were analyzed by calculating the score percentage, meanwhile data of the creative thinking skills test were analyzed using n-gain, t'-test statistic, and Cohen's effect size. The results showed that comprehensibility and quality of worksheet and problem sheet were categorized as feasible. In addition, based on the result of main field testing stage, worksheet and problem sheet that developed can improve students' creative thinking skills with n-gain value is 0.34 and categorized as moderate. The null hypothesis tested at 0,05 level of significance indicated that there is significant difference between creative thinking skills of those who were taught with creative thinking skills oriented worksheet and problem sheet using multimodal representation and those who were taught with worksheet and problem sheet that usually used in that school. Cohen's effect size value is 1,59 and categorized as big. Based on the research results, it was recommended that worksheet and problem sheet are effective in improving secondary high school students' creative thinking skills.

Keywords: creative thinking skills, multimodal representation, problem sheet, static fluid, worksheet

1. INTRODUCTION

Demand of globalization periods in the 21st Century, people meet the many challenges because of a rapidly changing world. In order to deal with the new situation arising in their life, the government should put great emphasis on education. Through education, people should be taught in the proper way to improve some skills for facing 21st Century. The important skills for facing the 21st century is called 21st century skills. As one of 21st century skills, creative thinking skills is the important skills that should be taught in the classroom [1]. Creative thinking defined as a process of becoming sensitive to a problem, deficiencies, gap in knowledge, missing elements, disharmonies, and so on; identifying the difficulty; searching for solutions, making guesses, or formulating hypothesis about the deficiencies; testing and retesting these hypotheses and possibly modifying and retesting them; and finally communicating the results [2]. Speaking of creative thinking skills, new requirements for working and living in the 21st century is the ability of students to become independent learners, so that they can work in groups, to work as good communicator, willing to take risks, committed to lifelong learning and is also capable of critical thinking and creative [3]. In addition, creative thinking skills are essential to success in learning and success in life [4]. In Indonesia, regarding the theories of the importance of creative thinking skills, Indonesian Ministry of Education and Culture has established a regulation No.54 year 2013 about graduate the competency standard for primary and secondary students, which said that the qualification of school graduate includes the skills dimension which consists of the ability to think and act effectively and creatively [5].

Based on the result of field study in a number of schools in Kabupaten Ciamis, revealed that creative thinking skills have not been taught yet in physics learning. Teachers used transmission model as the dominant model in physics learning. Through the transmission model, students do not have much practice applying the knowledge to new context, using it to solve problems, or using it as a platform to develop creativity. In addition, the teacher has not used instructional material included worksheet and problem sheet that facilitate creative thinking skills as well. Whereas, worksheet and problem sheet that arranged effectively would play an important role on student achievements and student attitude toward learning as the result of some research done by Ayvaci and Yildiz [6]

Some research about fostering creative thinking skills have done by Mokaram, et al., who designed computer application named electronic slide [7]; Neira and Soto, who used V diagram and concept map in learning [8]; and Sulaiman implemented problem based learning [9].

Among the efforts about fostering creative thinking skills, either learning model or instructional material, should fit with what within the curriculum. As noted in Ministry of Education and Culture (59) year 2013, learning activity should concern on student centered learning [10]. Through student centered learning, the teacher gave students opportunity to develop their skills [11]. In student centered learning, the teacher should manage a whole classroom activity effectively and give student opportunity to construct their own knowledge by collecting and applying in new situations. Furthermore, instructional material has strong influence on student learning directly [12]. Students interact with learning material more often, not only in the classroom, but also out of classroom. One of instructional material that help student learn actively is a worksheet [13]. Worksheet defined as printed materials that can be used to support learning in an informal learning environment [14]. Worksheet also defined as printed materials used for guiding students in a laboratory activity [15]. Problem sheet also make student more active in learning as well [16].

Teacher should make worksheet and problem sheet based on characteristic of physics subject. Physics is one of subject that involved phenomena and relation between its variables. Multimodal representation play a role to make student more understand about phenomena provided in worksheet and problem sheet. Multimodal representation of knowledge is not only grow up student motivation, but also deeper understanding about learned material [17].

Based on the facts that worksheet and problem sheet was not designed yet based on creative thinking skills as Ministry of Education and Culture noted and there were not research yet about developing creative thinking skills oriented worksheet and problem sheets. In short, three research purposes of this paper are to examine:

What is the quality and comprehensibility of creative thinking skills oriented worksheet and problem sheets using multimodal representation?

What is the improvement in creative thinking skills of students who use creative thinking skills oriented worksheet and problem sheets using multimodal representation vs. worksheet and problem sheets that usually used by the teacher?

What is the effectiveness of creative thinking skills oriented worksheet and problem sheets using multimodal representation in improving students' creative thinking skills?

2. METHOD

Method of this study followed Borg and Gall Research and Development [18], there were: 1) Research and information collecting, which consists of doing literature study about curriculum and analyzing teacher's worksheet and problem sheet; 2) Planning, which consists of determining core competency and base competency of curriculum 2013, determining type of worksheet and problem sheet, and determining whose creative thinking skills framework was used; 3) Develop preliminary form of product, which consists of developing product using Representational Approach Learning to Write method [19], doing quality test of worksheet and problem sheet involved 11 physics teacher in Kabupaten Ciamis and 3 physics lecturers and doing trial test of creative thinking skills involved 15 students; 4) Preliminary field testing, which consists of doing comprehensibility test of worksheet and problem sheet involved 17 students of secondary high school; 5) Main product revision; and 6) Main field testing which involved high schools students in Kabupaten Ciamis. Researcher chose sample using random sampling technique and then 54 samples were grouped as 28 students of experiment class who were taught using creative thinking skills oriented worksheet and problem sheet using multimodal representation and 26 students of control class who were taught using worksheet and problem sheet usually used in that school. Research designed used was *pretest posttest control group design* [20].

Instrument used in this study consists of rating scale questionare for measuring quality of worksheet and problemsheet, comprehensibility test, and creative thinking skills test. The questionare were used consists of 20 descriptors which were categorized into three component, there were: 1) suitability of base competency and indicator (K1), 2) suitability of worksheet and problemsheet writing style (K2), and 3) suitability of learning activities within worksheet and problemsheet (K3). Questioner also was completed with the space of giving suggestion related to worksheet and problemsheet. Comprehensibility test consists of four questions, there was about main idea, additional explanation to the main ideas, unfamiliar words, and less understandable sentences. Meanwhile, creative thinking skills test consists of five essay question that followed Torrance framework. In this study, creative thinking skills criterion includes fluency, flexibility, elaboration, and originality.

Data of comprehensibility and quality test collected were analyzed using descriptive method by calculating score percentage, meanwhile data of creative thinking skills test were analyzed using n-gain, t'-test statistic, and Cohen's D effect size.

3. RESULT AND DISCUSSION

Based on quality test of worksheet and problem sheet performed by experts, revealed percentage of worksheet and problem sheet quality as showed in Figure 1. There were percentage of each component of quality test. Each component has high percentage with average percentage is 87%, categorized as suitable. But there were some part should be revised following the experts' suggestion, include less understandable sentences, less understandable image selection, additional information of picture, less suitability of some activities with indicator.

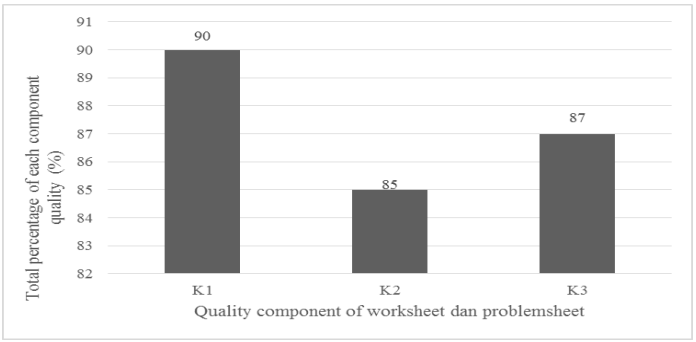

Figure 1. Result of quality test of *worksheet* dan *problemsheet*

Based on comprehensibility test revealed in Table 1, level of comprehensibility worksheet and problem sheet was 56%, categorized as most part of it were comprehensible. Some parts should be revised following the result of comprehensibility test, include less understandable sentences, less understandable image selection, and additional information of the picture.

Table 1 (Result of comprehensibility test of worksheet and problem sheet)

Worksheet	Topic	Percentage of comprehensibility	
		Main Idea	Additional Explanation to the main idea
1	Hydrostatic pressure	53	81
2	Atmosfer pressure	63	63
3	Pascal Laws	70	84
4	Archimedes Laws	41	34
5	Surface tension	45	33
6	Viscocity	70	40
	Average	57	56

Then, the revised worksheet and problem sheets could be used in main field testing stage involved 28 students of experiment class and 26 students of the control class. In this main field testing, both classes tested with the creative thinking skills test as a pretest, then experiment class were taught using creative thinking skills oriented worksheet and problem sheets, meanwhile control class were taught using worksheet and problem sheets usually used by the teacher. In the last part of this stage, students given posttest. Data of pretest and posttest were analyzed using n-gain as revealed in Table 2. Control class got n-gain value 0,10 that categorized as low, meanwhile experiment class got n-gain value 0,35 that categorized as moderate. These results indicated that experiment class performed better than control class. Creative thinking skills improvement of experiment class was higher than control class.

Based on the identification of students' posttest answers, control class can not generate an original idea of giving solution related to the problems. Students also cannot generate more than one idea for each test item. Class control can not explain more detail the idea so the explanation of the idea less understandable. Meanwhile, experiment class can generate more various original idea more. Furthermore, students of experiment class can explain more detail the ideas using their own picture.

Table 2 (N-gain of experiment and control class creative thinking skills)

Class	Mean of pre-test score	Mean of post-test score	*N*-gain	Category
Experiment	7,64	25,71	0,35	Moderate
Control	6,54	12,08	0,10	Low

In this study, the null hypothesis stated that there is no significant difference between creative thinking skills of students who were taught using creative thinking skill oriented worksheet and problem sheets using multimodal representation and students who were taught using worksheet and problem sheet that usually used by the teacher.

Tabel 3 (t'test of experiment class and control class n-gain)

Data Source		Normality Test		Homogenity Test		t'-test	
		Sig.	Ket.	Sig.	Ket	Sig.	Ket
N-Gain	Eksperiment class	0,20	Normal	0,002	not homogen	0,000	Ho was rejected
	Control clas	0,20	Normal				

The null hypothesis tested using t'-test at 0,05 level of significance revealed that null hypothesis was rejected. This is indicated that there is significant difference between creative thinking skills of those who were taught with creative thinking skills oriented worksheet and problem sheet using multimodal representation and those who were taught with worksheet and problem sheet that usually used in that school. Beside statistic test, the researcher also calculate effect size as showed in Table 5. The result showed effect size score was 1,59, categorized as big.

Table 4 (Effect size of n gain experiment class)

M_{Ek}	$M_{Kontrol}$	S_{pooled}	D
0,3436	0,1035	0,1503	1,59

The improvement of students' creative thinking skills influenced by the arrangement of worksheet and problem sheet itself. In the beginning part of worksheet and problem sheet, there is a space of asking question. Students should make many questions related to the given picture. Asking questions activity is one activity that will encourage students' creative thinking skills [24]. Another part of worksheet and problemsheet is a problem solving activity. Providing a problem is a way to stimulate creative thinking skills. This way in line with the definition of creative thinking skills which said that creative thinking skills is a process of becoming sensitive to the problem. Then, students should generate different solutions following the given questions. The questions are arranged well looking at four criteria of creative thinking skills. This question will guide students to think divergent and generate many different responses to a given situation. Making student to think divergent is one of strategy to enhance creative thinking skills [25].

4. CONCLUSION

Based on comprehensibility test and quality test in preliminary field testing stage, developed worksheet and problem sheet categorized as feasible. Furthermore, based on the result of the main field testing stage, developed worksheet and problem sheet can improve students' creative thinking skills with N-gain value is 0.34 and categorized as moderate. The null hypothesis tested at 0,05 level of significance indicated that there is significant difference between creative thinking skills of those who were taught using creative thinking skills oriented worksheet and problem sheet using multimodal representation and those who were taught using worksheet and problem sheet that usually used in that school. Cohen's effect size value is 1,59 and categorized as big. Based on the research results, it was recommended that worksheet and problem sheet are effective in improving secondary high school students' creative thinking skills.

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Application of Combination of NPK Fertilizer *Nano Chisil* and on the Growth of Maize (Zea mays L var. Pioneer 27).

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Abstract – Maize (*Zea mays* L) is one of the food commodities in Indonesia. Corn hybrid varieties P-27 is a development of hybrid corn seed P-21, which has a high economic value. Maize plant growth can be improved by the addition of combination of Nano chisil and NPK fertilizer *Nano Chisil* is a fertilizer that contains micro-nutrients Si, while NPK fertilizer contains macronutrient such as N, P, and K. This study was conducted to determine the effect of optimal combination of Chisil Nano fertilizer and NPK fertilizers to improve maize plant growth (varieties P-27). There are 5 treatments in this study: P0 (without any fertilizer); P1 (25% *Nano Chisil* + 75% NPK); P2 (50% *Nano Chisil* + 50% NPK); P3 (75% *Nano Chisil* + 25% NPK); P4 (100% *Nano Chisil* + 0% NPK); P5 (0% *Nano Chisil* + 100% NPK). Each treatment is repeated 3 times. The parameters of this study consisted of growth; plant height, number of leaves, leaf length, leaf color, fresh weight and dry weight. The data were analyzed using Analysis of Variance (ANOVA) if there is a significant difference result, the result can be followed by Duncan Multiple Range Test (DMRT) at the 95% significance level. The results showed that the combination of *Nano Chisil* fertilizer and NPK fertilizer had effects on plant fresh weight. The most optimal combination of fertilization was 25% *Nano Chisil* fertilizer and 75% NPK fertilizer which most effectively increase the weight of wet corn crop varieties P-27, but it had no effect to it had no effect to plant height, leaf number and dry weight of the corn crop varieties P-27.

Keywords: Maize (Zea mays L var P-27), Nano Chisil, NPK fertilizer, growth.

1. INTRODUCTION

Maize is one of the important commodities in Indonesia. This plant is one source of carbohydrates after rice and wheat. Maize has a high economic value in the industry, particularly as raw materials in the manufacture of animal feed. Due to the developments in the industrial sector, the market demand for maize will increase as well (Rashid et al, 2010). Corn productivity in 2011 in Indonesia reached 3,864,692 tons per year, the year 2012 is as much as 3,957,595, while in 2013 the production decreased up to 35 tons 3,857,359 tons per year. The demand for maize as one of the industry from year to year has increased, but the level of productivity corn from year to year fluctuations increased and decreased to 3% per year (Central Bureau of Statistics, 2014). One of the varieties of maize plant that has potential is p-27. P-27 is a development of a new hybrid P-21 with some advantages, including better maize crop. Maize plant stems and fruit of this variety of large and more robust roots that can still produce well under extreme weather conditions and less fertile land. P-27 pioneer superior species has large corn cob, such resistant to extreme weather, resistant to pests and fungus Caucasians. Yields of maize crop could reach 8-10 tons per hectare and cropping period is shorter, such as 95-100 days (Anonym, 2012).

Nano Chisil fertilizer is a mixture of chitosan and silica nanoparticles which made in nanoparticles size with nanotechnology by CV Dipon Nanotech (Nanosil 99) (Anonym, 2006). Chitosan is a substance made from the shells of shrimp, crab and lobster which is a byproduct of the fishing industry, so it is a very abundant availability of raw materials (Bitteli et al. 2000). Nanotechnology can break chitosan and silica into smaller particles. The particle size is reduced raw material to make fertilizer can be absorbed easily by the cell membrane leaf cells so as to improve the quality of maize productivity. One product of fertilizer applied nanotechnology and solving chitosan and silica is *Nano Chisil.* Silica is a component of micro-elements in the soil that are required in small amounts by plants, silica is known as the beneficial element that protects plants from drought and pathogens without damaging the land, especially for plants that accumulate Si such as Gramineae. The addition of Si is sufficient to reduce the tendency Cereal crops to wither in drought conditions

may be due to a decrease in water vapor permeability top of leaf epidermal cell walls (Yukamgo and Yuwono, 2007). Some of the benefits contained in chitosan, among others slow down the release rate of fertilizer nutrients with covering most of the pores so that the water can still enter to dissolve through the pores that are not closed. Therefore been chitosan as a coating for chitosan water insoluble and able to coat the fertilizers also have properties biodegradable, biocompatible, non-toxic and environmentally friendly (Mingzu, 2007).

The mechanism of absorption by the plant through the leaves in the form of acid monociliate. After entering through the stomata monociliate acid polymerizes into a silica gel and accumulate in the epidermis. Silica gel in the leaf epidermis cell wall associated with cellulose to form a double layer that serves to limit excessive water loss can cause dryness. Drought water on the leaves causes a decrease in the content of photosynthetic pigment chlorophyll and conductance stomata.

Chlorophyll synthesis limited to the lack of water and induce the production of peroxidase enzymes associated with degradation of chlorophyll in a thylakoid membrane. Lack of water in plants can lead to decreased absorption of nitrogen which is an important element in the synthesis of chlorophyll. Silica gel in the epidermal layer of leaves associated with cellulose so thick a layer of silica gel which helps hold or slow the loss of water by evaporation.In addition, Si also strengthens the walls of epidermal cells that can suppress the activities of transpiration and water stress can be reduced while the Si accumulation in stem tissue causes increased cell wall thickness thereby increasing the strength of the rod. Agricultural land in Central Java generally lacks the element silica, making it necessary additions from the outside to support the growth and production of corn plants.

Nano Chisil fertilizer can be applied to crops of rice, sugarcane, and corn. Silica is one element of the necessary plant nutrients and one of the greatest material content needed by plants. Silica benefits, among others, can stimulate photosynthesis, soil fertility and translocation of CO2 (Suwardi, 2007).

Excess consumption of NPK fertilizer that is with one application of fertilizer may include multiple elements, resulting in the more efficient use of fertilizer when compared to single. The use of NPK fertilizer can be alternative solutions and in improving the growth of plants, but it is expected to provide ease of application in the field and can be used directly by plants (Anonym, 2011). Nutrients N, P, and K are generally returned to the soil through fertilization, but the elements silica (Si) and microelements are not returned to the soil. While the provision of NPK alone is not sufficient nutrients such as silica that are beneficial to the growth of corn plants. Therefore, an intensive land still requires additional nutrient fertilization silica as a combination of the corn crop in small amounts. Based on the description of the background, this study aims to determine the effect of the combination of Nano Chisil fertilizers and NPK fertilizer on plant growth of maize varieties P-27 and knowing the combination and NPK fertilizer Nano Chisil most optimal in increasing the growth of maize plants varieties of P-27.

2. EXPERIMENTAL DETAILS

2.1 Time and Place

This research was held at PT. Tossa Shakti, Jalan Raya Semarang-Kendal Km 19, Nolokerto, Kendal. This research began in November 2015 to January 2016. As for the analysis of plant growth is done in Laboratory of Biological Structure and Function of Plants, Department of Biology, Faculty of Science and Mathematics, University of Diponegoro, Semarang.

2.2 Equipment and Materials

The equipment used in the research are tractor, camera, pH meter, thermo-hygrometer, plastic bags, labels, meter, tank sprayers, digital scales, hoes, buckets, measuring cups, stationery, and oven. The materials used for this study is corn seed varieties P-27, which is manufactured by PT DuPont Indonesia, Nano Chisil fertilizer made from chitosan and crystalline silica produced by CV Dipon, organic fertilizer (cow manure), fungicides and insecticides.

2.3 Methodology

Soil preparation

Soil is prepared beforehand by in a tractor. Soil that has been tractor then an initial fertilization using NPK fertilizer is given with a ratio (2: 3: 2) prior to planting. pH of the soil in the area is checked by using pH meter. Soil flattening performed using tractor Kulti. Land that has been ready then conducted by making current flow will start planting. Preparation Seed. Maize seed varieties used are P-27, manufactured by PT DuPont Indonesia that has been soaked in a solution of fungicide and insecticide. Seeds soaked in a solution of fungicide and insecticide for 24 hours. Cultivation P-27 maize varieties planted one item in the planting hole with a spacing of 20 cm was measured by using a ruler, then the closure of the land with a thickness of approximately 5 cm. The

distance between beds is 75 cm. While planting is done by making a hole in the ground, the holes filled with the seed, and then covered again with soil and watered to initiate seed to be germinated.

Fertilization

Fertilization takes place on day 10 and day 25. Spraying fertilizer done in the morning. P-27 maize varieties given fertilization treatment with a variety of fertilization as follows: P0: Control (Without *Nano Chisil* and NPK fertilizers); P1: 25 ml of *Nano Chisil* fertilizer in 1.5 L of water to 3.93 g NPK; P2: 2,5 mL of *Nano Chisil* fertilizer in 1.5 L of water to 2.62 g NPK; P3: *Nano Chisil* fertilizer 3.75 mL in 1.5 L of water to 1.31 g NPK; P4: 5 ml *Nano Chisil* fertilizer in 1.5 L of water; P5: 5.25 g of NPK fertilizer. Fertilization of *Nano chisil* is done by spraying the leaves and stalks of corn plants. NPK fertilizer is given on the edge of the corn crop at a distance of 5 cm from maize, while the dose is given by calculating fertilizer requirement per plant by a dose of fertilizer per plant. Treatment plant P-27 maize varieties performed by removing the weeds. Watering is not performed if the soil is sufficiently moist by rain.

3. RESULTS AND DISCUSSION

Results of ANOVA (*Analysis of Variance*) ON 95% significance level (Table 3.1) shows the addition of Nano Chisil combination and NPK fertilizer influential Against Real Wet weight Maize P-27 ($p < 0.05$), whereas the High Against Plant, Period leaves, leaf color and plant dry weight did not provide any real effect, but tends to increase in each of the observed variables

Table 3.1.The average of rest weight number of leaves, plant height, leaf dry weight and color P-27 maize plants after 47 days of planting.

Treatment	Plant height (cm)	Number of leaves (leaf)	Wet weight (Kg)	Dry Weight (kg)
P0 (without the addition of NPK and *Nano Chisil*)	185	12,67	0,6^{c}	0,12
P1 (25% *Nano Chisil* + 75% NPK)	198,33	15	1,74^{a}	0,15
P2 (50% *Nano Chisil* + 50% NPK)	206,67	14,33	1,39^{ab}	0,13
P3 (75% *Nano Chisil* + 25% NPK)	206,67	13,67	1,06^{bc}	0,13
P4 (100% *Nano Chisil* + 0% NPK)	217,67	15,33	0,73^{c}	0,14
P5 (0% *Nano Chisil* + 100% NPK)	194	15,33	0,76^{c}	0,13

Description: The numbers followed by different letters in the same column indicate significant differences by Duncan test at 95% confidence level.

3.1 Plant height

The results showed higher average data of treatment plant at 100% *Nano Chisil*, tend to have a higher yield compared to control or without fertilizer, 75% and 25% *Nano Chisil* can be shown in (Table 3.2).

Table 3.2. The average of plant height P-27 after the addition of *Nano Chisil*fertilizer and NPK

Combination treatment	Plant height (cm)
P0 (without the addition of NPK and *Nano Chisil*)	185
P1 (25% *Nano Chisil* + 75% NPK)	198,33
P2 (50% *Nano Chisil* + 50% NPK)	206,67
P3 (75% *Nano Chisil* + 25% NPK)	206,67
P4 (100% *Nano Chisil* + 0% NPK)	217,67
P5 (0% *Nano Chisil* + 100% NPK)	194

Description: The numbers followed by different letters show significant differences by Duncan test at 95% confidence level

The results showed the highest rates in the P4 plant (100% + 0% *Nano Chisil* NPK). This reveal that the treatment of liquid manure *Nano Chisil* with 100% concentration on maize P-27 adds a high average better than the other treatments. Elements of silica can improve the plant growth and crop production to help the absorption of light more effectively through a more upright leaves and sturdiness. Higher plants will increase as a result of mechanical reinforcement so that the network more robust and erect stems. Higher plants have an influence on increasing the number of leaves for increases stem segments. Anticipated increase in runway length segments by division rate intercalary meristem cells affected by turgor pressure in the vacuole. In the opinion of Savant et al, (1999) which states that the Si was allegedly involved in the elongation or cell division. The result of the high average corn crop P-27 can be seen in Figure 4.1

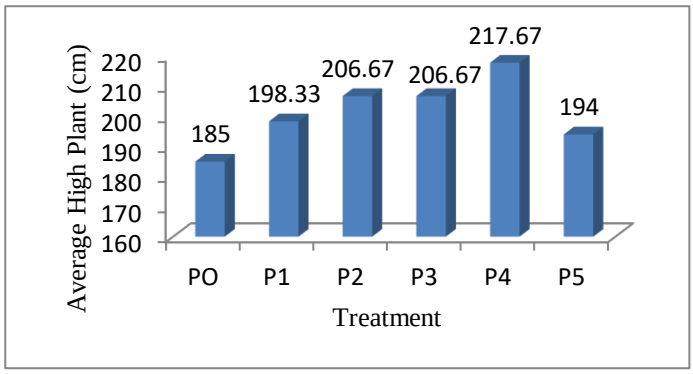

Figure 3.1 Histogram high average corn crop P-27 after being given fertilization treatment *Nano Chisil* and NPK different P0 = Control (without *Nano Chisil* and NPK), P1 = 25% *Nano Chisil* + 75% NPK, P2 = 50% *Nano Chisil* + 50% of NPK fertilizer, P3 = 75% *Nano Chisil* + 25% NPK, P4 = 100% + 0% *Nano Chisil* NPK fertilizer, P5 = 0% *Nano Chisil* + 100% NPK

The role of silica nutrient for plants that can stimulate photosynthesis and translocation of carbon dioxide (CO2). Silica which is accumulated in the leaves catching the sunlight can help in the process of photosynthesis and translocation of CO2. The higher the rate of photosynthesis of plants, the higher the rate of plant growth. This is consistent with the Sarief (1989) statement that the application of fertilizers in the form of nano silica or commonly can increase the height of maize plants. Control treatment shows lower plant height than other treatment. There is a tendency that the plant is so high plant N deficiency is not optimal. This is consistent with the reference of Campbell et al (2008) stated that the plant will be short or stunted if grown in nitrogen-poor soils.

3.2 Number of Leaves

The amount Of leaves On treatment P4 (100% NanoChisil + 0% NPK) and P5 (0% NanoChisil + 100% NPK) have Better result in average Better result in average compared with Control treatment P0 (without NanoChisil and NPK) has a number of leaves at least.The results showed that the number of leaves was not significantly different at each treatment, both in the fertilizer or not in fertilizers tend to increase the amount of leaves. According Iriany and Takdir (2006) leaves of maize plants, would be formed if the needs of the content of macro- and microelements met. P0 treatment shows deficiency of macro elements and microelements in the soil, so that the leaf formation is inhibited.

Average of number of leaves P-27 maize plants can be seen in Table 3.3

Combination treatment	Number of leaves (leaf)
P0 (without the addition of NPK and *Nano Chisil*)	12,67
P1 (25% *Nano Chisil* + 75% NPK)	15
P2 (50% *Nano Chisil* + 50% NPK)	14,33
P3 (75% *Nano Chisil* + 25% NPK)	13,67
P4 (100% *Nano Chisil* + 0% NPK)	15,33
P5 (0% *Nano Chisil* + 100% NPK)	15,33

Description: The numbers followed by different letters show significant differences by Duncan test at 95% confidence level

Control treatment (without NanoChisil and NPK) shows that the number of leaves of the plant are lower than other treatment. This is due to crops suffer from lack of nutrients N. According to Prasetyo (2009), the leaf is an organ that originate from cells that have cleavage meristematic, thus forming a leaf buds are developing and shaping the end result leaves a certain amount to a plant. The average yield of maize crop leaf number P-27, which can be seen in Figure 3.2

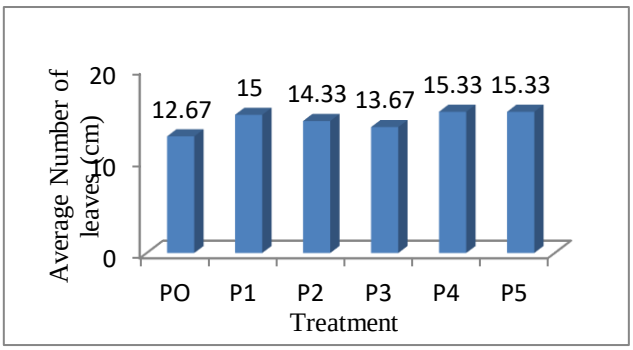

Figure 3.2 Histogram average number of leaves corn crop P-27 after being given fertilization treatment *Nano Chisil* and NPK different P0 = Control (without *Nano Chisil* and NPK), P1 = 25% *Nano Chisil* + 75% NPK, P2 = 50% *Nano Chisil* + 50% of NPK fertilizer, P3 = 75% *Nano Chisil* + 25% NPK, P4 = 100% + 0% *Nano Chisil* NPK fertilizer, P5 = 0% *Nano Chisil* + 100% NPK

The amount of the leaf on treatment P4 (100% *Nano Chisil* + 0% NPK) and P5 (0% *Nano Chisil* + 100% NPK) shows better result in number of leaves than other treatment, so it can be used as an alternative in combination fertilization because the amount needed fewer and more efficient in fertilization. The more the number of leaves on the plant, the more leverage in the process of photosynthesis (Iriany and Takdir, 2006).

3.3 Wet Weight

Results of ANOVA (Analysis of Variance) shows the addition of a combination of Nano Chisil and NPK had significant effect on the wet weight of the corn crop P-27 ($p < 0.05$) (Appendix 1). Duncan advanced test combination treatment *Nano Chisil* fertilizers and NPK fertilizers can be seen in (Table 3.1).

In general, the addition of *NanoChisil* fertilizers and NPK fertilizer on maize P-27 had better result in average than the control treatment P2 treatment (25% and 75% *Nano Chisil* NPK) Shows the highest result in wet weight compared to other treatments with An average of 1.74 kg. While the corn crop P0 control (without fertilizer *Nano Chisil* and NPK fertilizer) is a plant of the most low-weight wet with average of 0.6 kg. According Sudjana and Sudjadi (1991), fertilizers Nanosilika have a silica content that can reduce transpiration in plants, so that the water content of a plant can be saved, and the resulting differences in fresh weight in maize P-27.

Average of wet weight maize P-27 can be seen in Table 3.4

Combination treatment	Wet weight (Kg)
P0 (without the addition of NPK and *Nano Chisil*)	0,6^{c}
P1 (25% *Nano Chisil* + 75% NPK)	1,74^{a}
P2 (50% *Nano Chisil* + 50% NPK)	1,39^{ab}
P3 (75% *Nano Chisil* + 25% NPK)	1,06^{bc}
P4 (100% *Nano Chisil* + 0% NPK)	0,73^{c}
P5 (0% *Nano Chisil* + 100% NPK)	0,76^{c}

Description: The numbers followed by different letters show significant differences by Duncan test at 95% confidence level

The result of the combination of fertilizer and NPK fertilizer NanoChisil can be used as an alternative. According Maruapay (2002), an increase in wet weight of both weight without husk and cob weight salable allegedly closely linked to the amount of fotosintat are translocated to the cob. The greater the cob fotosintat are translocated to the increased weight also fresh cobs. The results mean wet weight in maize P-27 can be seen in Figure 4.3

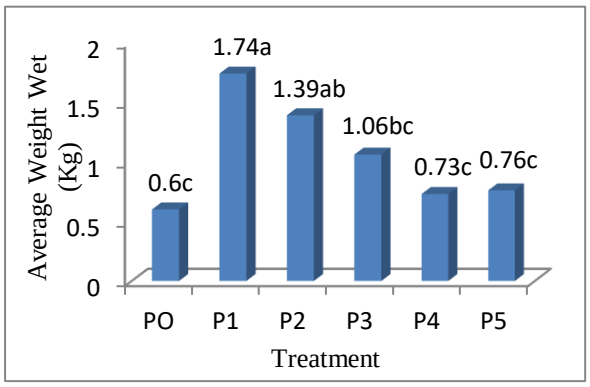

Figure 3.3 Histogram average wet weight of the corn crop P-27 after being given fertilization treatment *Nano Chisil* and NPK different P0 = Control (without *Nano Chisil* and NPK), P1 = 25% *Nano Chisil* + 75% NPK, P2 = 50% *Nano Chisil* + 50% of NPK fertilizer, P3 = 75% *Nano Chisil* + 25% NPK, P4 = 100% + 0% *Nano Chisil* NPK fertilizer, P5 = 0% *Nano Chisil* + 100% NPK

According to Lakitan (2005), silica and water absorbed by the roots and transported through the xylem to the top of the plant, especially the leaves. A similar opinion was delivered by Yukamgo and Yuwono (2007) that some nutrients and water entering the plant parts used for photosynthesis while the other (water and mineral salts) released through the process of transpiration. Silica is involved with water will stay on and integrate with the cells of the epidermis. Silica associated with cellulose accumulate in cells of the epidermis. Accumulation in the cell will provide power stems and leaves on corn plants. Control treatment (without fertilizer *Nano Chisil* and fertilizer NPK) lower wet weight compared to the plant with treatment (Table 4.1). Plants that are not given fertilizer treatment had lower wet weight compared to those given fertilization (Sudjana and Sudjadi, 1991).

3.4 Dry Weight

Results of ANOVA (*Analysis of Variance*) shows the addition of a combination of *Nano Chisil* and NPK has no real effect on the dry weight of the corn crop P-27 ($p < 0.05$).

Average of dry weight of the corn crop P-27 can be seen in (Table 3.5).

Combination treatment	Dry Weight (kg)
P0 (without the addition of NPK and *Nano Chisil*)	0,12
P1 (25% *Nano Chisil* + 75% NPK)	0,15
P2 (50% *Nano Chisil* + 50% NPK)	0,13
P3 (75% *Nano Chisil* + 25% NPK)	0,13
P4 (100% *Nano Chisil* + 0% NPK)	0,14
P5 (0% *Nano Chisil* + 100% NPK)	0,13

Description: The numbers followed by different letters show significant differences by Duncan test at 95% confidence level

The highest result in dry weight are showed in P1 (25% *Nano Chisil* + 75% NPK) with an average of 0.15 kg dry weight. While the P-27 corn plants dry in treatment P0 (0% *Nano Chisil* + 0% NPK) is a plant with the lowest dry weight of 0.12 kg. Production plants are usually more accurately stated the size of the dry weight than the weight of the wet, because the wet weight is influenced by conditions of humidity (Sitompul and Guritno, 1995). A comparison of the dry weight of the fertilizer *Nano Chisil* compared with organic fertilizer as a whole has the result of fertilizer *Nano Chisil* more has a dry weight compared with the use of NPK fertilizer. Fertilizer *Nano Chisil* have a component of silica as a coating plants so that when transpiration is no evaporation of excess. The results of photosynthesis in corn plants will be optimized and the results can be saved. Reserves of food can be further optimized stored in the form of carbohydrates and the formation of body cells of plants.

According Iriany (2006) increased fotosintat formed growing too dry weight of plants for 90% of dry matter of plants derived from photosynthesis. The use of fertilizers *Nano Chisil* can be used as an alternative for fertilizing corn crop P-27. Being able to increase the dry weight of plants, other than that use relatively less can be used as a substitute for the use of NPK fertilizer. Data mean dry weight of the corn crop P-27 can be seen in Figure 3.4

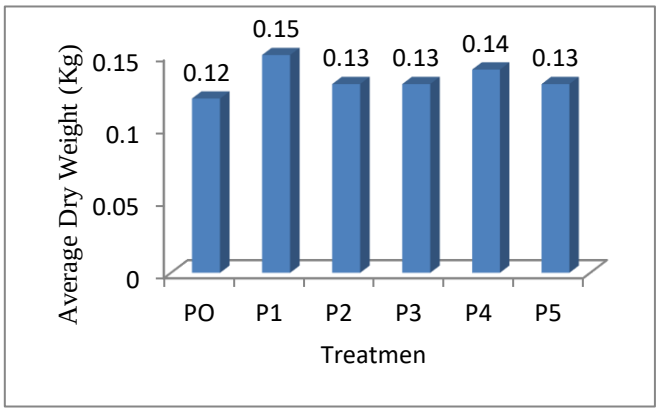

Figure 3.4 Histogram average dry weight of plants corn P-27 after being given fertilization treatment *Nano Chisil* and NPK different P0 = Control (without *Nano Chisil* and NPK), P1 = 25% *Nano Chisil* + 75% NPK, P2 = 50% *Nano Chisil* + 50% of NPK fertilizer, P3 = 75% *Nano Chisil* + 25% NPK, P4 = 100% + 0% *Nano Chisil* NPK fertilizer, P5 = 0% *Nano Chisil* + 100% NPK

The results show that the average dry weight of P1 (25% *Nano Chisil* + 75% NPK), P2 (50% *Nano Chisil* + 50% NPK), P3 (75% *Nano Chisil* + 25% NPK), P4 (100% *Nano Chisil*+ 0 % NPK) and P5 (0% *Nano Chisil* + 100% NPK) tend to have better average result than the P0 control treatment (without *Nano Chisil* and NPK). This shows that the treatment with the addition of combination of *Nano Chisil* and npk towards the control treatment had a significant result.

3.5 Leaves Color

Leaves color of P1 (25% *Nano Chisil* + 75% NPK), P3 (75% *Nano Chisil* + 25% NPK) and P4 (100% *Nano Chisil* + 0% NPK), has the same general color which is dark green leaves compared with P0 (without *Nano Chisil* and NPK), P2 (50% *Nano Chisil* + 50% NPK) and P5 (0% *Nano Chisil* NPK + 100%), which indicates less than optimal result which has color light green leaves. *Nano Chisil* liquid fertilizer that has an element of silica will play a role in the process of photosynthesis thus spurring the growth of leaves. Elements of silica in nano size is more easily absorbed by the roots through the process of absorption of nutrients and into the xylem and transported to the leaves. Elements of silica in leaf organs will bind water molecules and forms a protective layer so that the water molecules are not easily lost through transpiration process. In plants obtained assortment pigment that absorbs light energy role. Photosynthetic pigment found in chloroplasts consisting of chlorophyll a, b, santofil, carotenoids, bacteriochlorophyll in bacteria. Pigments absorb the color or wavelength of light is different. Chlorophyll is the green pigment found in chloroplasts. This pigment is useful for photosynthesis in plants. Various shapes and sizes of chloroplasts found in various plants (Salisbury and Ross, 1995). Color dark green leaves containing chlorophyll a (P1, P3 and P4), while the color of light green leaves containing chlorophyll b (P2 and P5).

Table 4.6. Leaf color P-27 corn plants 47 days after planting

Treatment	Color Leaves
P0 (without the addition of NPK and *Nano Chisil*)	Yellowish green
P1 (25% *Nano Chisil* + 75% NPK)	Dark green
P2 (50% *Nano Chisil* + 50% NPK)	Light green
P3 (75% *Nano Chisil* + 25% NPK)	Dark green
P4 (100% *Nano Chisil* + 0% NPK)	Dark green
P5 (0% *Nano Chisil* + 100% NPK)	Light green

Nutrients silica in nano size able to fit into plant cells and affects the metabolic activity of cells such as increasing protein synthesis and the formation of chlorophyll causing leaf color becomes greener. Formation of chlorophyll that encourage increased rate of photosynthesis, resulting in a greener leaf color. Elements silica get into the cell to bind with water molecules and improve the content of the element N. Leaf color is good for the growth of the corn crop is green leaves. Based on the color of the leaves in Figure 4.5 indicates that the P0 plant leaves appear yellowish green compared to other plants. Leaves N-deficient plants are characterized by a yellowish color due to the presence of symptoms of deficiency. According to (Grace, 1997) deficiency symptoms in plants with yellow leaves and looks a bit burned a shortage of nitrogen, in addition to the shortage of the element nitrogen is also shown by the plants look stunted.

While the leaves of plants that do not require additional N more dark greens, such as the treatment P1, P3 and P4. While the leaves of corn plants that light green color indicates a good crop growing conditions such as the treatment of P2 and P5. The green color of the leaves can be affected by NPK fertilizer, because the content of

the fertilizer N in both able to increase the green color of leaves (Anonym, 2011). This is in accordance opinions Engelstad (1997) states that the provision of optimal nitrogen can increase plant growth, increase protein synthesis, chlorophyll formation which causes the color of the leaves become greener and improve the ratio canopy roots. Therefore the optimal nitrogen applications can increase the rate of plant growth. Color dark green leaves contain chlorophyll a, while the color of light green leaves contain chlorophyll b.

4. CONCLUTION

The combined application of fertilizer *Nano Chisil* with NPK fertilizer had an effect Against Real Wet weight of Maize var. P-27, but did not significantly affect Plant height, Term leaves and dry weight. Combination treatment *Nano Chisil* 25% And 75% NPK (P1), raise the wet weight ON growing maize var. P-27

5. ACKNOWLEDGMENTS

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Is Heating in Estuary Waters Potential to Influence Glass Eel Migration?

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Abstract – Completing their life cycle, freshwater eels of the genus *Anguilla* glass eel enter estuaries. Some power plants are in the estuaries area which glass eels usually enter. While increasing water temperature was found in the outlet of the discharged waste. However it is well understood that environmental processes in estuaries modulating glass eel migration patterns. Therefore behavioral response to higher water temperature (34^0C) than the natural one (28^0C) was investigated on glass eel of *Anguilla sp* in order to determine the water temperature preference. Accumulation of the time fish spent in each test area was recorded. Wilcoxon test was performed to the data of the spending time to distinguish the preference. The result showed a quite significant different (P<0,05) in the preference of the glass eel to the waters. The glass eel was tend to avoid the water with 34^0C in temperature and spent much of its activity moving along in the water with 28^0C in temperature. Compared to few fish fish spent more in water of 34^0C. A number of 15 fish spent more in water of 28^0C (about 75% of used samples). This finding showed that glass eel could sense and then perceive the environmental information resulting in its particular response. Different of 5 -7 degree in water temperature could directly affect to the glass eel migration.

1. INTRODUCTION

The freshwater eels of the genus *Anguilla* (family Anguillidae) are considered to be of the greatest commercial importance for food [1]. Eels are widely distributed throughout the world except for the eastern Pacific and south Atlantic [2]. Using Mitochondrial DNA analysis Aoyama and Tsukamoto (1997) stated that freshwater eels originated in the present-day Indonesian region during the Cretaceous. Furthermore Auyama and Tsukamoto suggested that a group derived from this ancestor dispersed westward, probably by larval ransport in the global circum-equatorial current through the northern edge of the Tethys Sea. This group split into the ancestor of the European and American eels, which entered into the Atlantic Ocean, and a second group, which dispersed southward and split into the east African species and Australian species [3].

Recruitment of both the three northern hemisphere eel species (European eel Anguilla anguilla, American eel Anguilla rostrata and Japanese eel Anguilla japonica) and the southern hemisphere eel species have reduced significantly since the 1970s and 1980s [4]. Decline of the stoks include glass ell, yellow eel as well as silver eel. European glass eel recruitment (measured Europe-wide) has declined to<5 % of the average level from 1969 to 1980 [4]. The causes of the decline in recruitment of the anguillid species are not well understood, and have been attributed to different factors affecting recruitment, growth and/or the eel's reproductive stages [5]. Eventhough The major causes for the decline are now thought to be habitat destruction and obstruction of migration routes by dams and other chemical or physical obstacles [6].

This species is catadromous, migrate between freshwater growth habitats and offshore spawning areas [7]. The spawning areas of the genus *Anguilla* are all located in tropical regions [3]. Temperate anguillids have well-defined spawning seasons and that they make long migrations out into the open ocean to spawn [8]. The long migrations to these spawning areas have fascinated scientists because each eel must migrate thousands of kilometers back to the same area for spawning [9]. However until recently, remarkably little was known about the spawning areas of tropical eels. In contrast to the long migrations made by temperate eels, tropical eels make much shorter migrations to spawn in areas near to their freshwater habitats [10]. The leptocephalus stage of temperate eels is of long duration, and appears to be highly adapted to a marine planktonic life [11]. The larvae use stream to assist their journey across ocean, and the glass eel approach the coast and enter estuaries [12]. In contranst to Glass eel of temperate anguillid eel species that recruit to inshore and freshwater areas seasonally, usually being from early winter to late spring, tropical glass eels were reported almost throughout the seasons in fluctuation abundances [13] Changes in glass eel density were reported to be partially driven by local environmental variables, such as turbidity, rainfall and temperature [4].

Some power plants are built in the estuaries area which glass eels usually enter. While increasing water temperature was found in the outlet of the discharged waste [14]. It is well understood that environmental processes in estuaries modulating glass eel migration patterns [7]. Therefore in this study temperature preference was observed in glass eels of Anguillid species in order to discover potential effect from heating of estuary waters by powerplant waste to migration of the glass eels.

2. METHODS

2.1 Samples

Glass eels were collected from estuary of Serayu river in Cilacap, Southern of Central Java, Indonesia, using triangular shaped Net. Then fish were put into a plastic bag with ambient oxigene and transfered to the experimental place. Fish were reared and addapted to the laboratory condition for preparation of the experiment. Forthy fish of 4.1 to 5.5 mm length were used for the experiment. Laboratorial observation was conducted in December 2015 to January 2016 at Marine Science Laboratory, Marine Science Department, Faculty of Fisheries and Marine Science, Diponegoro University.

2.2 Experimental Setup

The independent variable was water temperature. There were two level i.e. the natural habitat temperature (28^0C) and the not natural habitat temperature (34^0C). The natural habitat temperature was determined based on the observation of the estuary water in Cilacap not adjacent to human activity. While the not natural habitat temperature was determined based on literature review of study of water temperature near the waste discharge at Paiton Power Plant in Sitobondo, East Java [14]. Water Ttemperatures of the estuary water with radius ± 125 from the waste discharge pit was 34.10^0C. The distance was chosen because it's the radius for glass eel to migrate from salt water to fresh water and their activity is very dynamic in the radius mentioned above before glass eel enters the estuary [15]).

Those temperatures were then set into two water chamber, chamber A for temperature of 28^0C and chamber B for temperature of 34^0C. Experimental aquaria was a modification from Atema et al (2002) (Fig. 1) [16]. The aquaria was made from glass material. It was divided into two sections with another glass of 5 mm in thickness and 10 cm long. The glass was placed in the middle of water tank so the two water columns of different temperature would not be mixed one another in the main compartment. The angle of the tilted glass was 15^0 and approximately 42 cm long. The tilted glass was perceived as a manipulation component of the water tank to create water movement when the water was channeled into the water tank. The water movement was functioned as factor which was capable of triggering glass eel natural behavior to move up against water current [15].

Previously, colour and temperature tests were undertaken. Colour test was performed using two different dyed current water flow trough each channel in the same time [16]. As the water flew into the main container, undissolved artificial coloring were mixed with the water flow. This step was aimed to see the pattern of water current in the water tank to estimate how a different temperature would be mixed one another in the main compartment (Fig. 1).

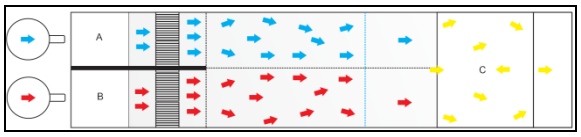

Fig. 1. Water flow scheme in the water tank interpreted using undissolved dye in the colour test

Temperature test aims to ensure feasibility of the experimental setup (Fig. 2). Test was done in a number of controlled points in the water tank. Experimental setup became the main focus of the test to control and stabilize water temperature in the water tank. Temperature controlling was placed in several points, such as chamber A (A1, A2, and A3), chamber B (B1, B2, and B3), and chamber C (C1, C2, and C3). Temperature controlling was conducted along with the experiment three times in each point mentioned above. Temperature recording was done in three main points that became the crucial parts in the experimental setting proper test. These points were A3, B3, and C3. These points were chosen because of their importance in determining glass eel preference during the experiment process.

Fig. 2. Temperature control points observed in the water tank

Subsequently, experiment was perform using individually forthy sample glass eels at the night time and at the day time. I is well known that eels are noctural, they are more active at the night time rather than at the day time. Acclimatization in C3 was lasted for three minutes without water flow [17]. During the process, glass eel was put into the water tank (C3) and isolated using a transparent plastic cup. After three minutes the cup was lifted up and then water flowed. Water current flew into the main compartment along with its different temperature. Fish could freely choose both different temperature areas (A2 or B2). Accumulated times the fish spent in each area (A2 or A3) was recorded. Each experiment was conducted for three minutes [17] using single fish. Twenty fish were tested individually.

2.3 Data Analysis

The result of this study was interpreted in graphics. Data was statistically analysed using Wilcoxon test for two identical measurement subjects on a particular treatment.

3. RESULTS AND DISCUSSION

3.1 Feasibility of the experiment setup

Result of the preliminary study for temperatur test was presented in tabel 1 and Fig. 3. Temperature recording was done in three main points that became the crucial parts in these test. These points were A3, B3, and C3. These points were chosen because of their importance in determining glass eel preference during the experiment process. This method was chosen because the test calculates a quantitative analysis, so the result could be more powerful, objective, and apart from user subjectivity (Field, 2005). The result of the test at the night has shown that: 1.) water temperature in A3 and B3 was significantly different ($P < 0.05$); 2.) Water temperature in A3 and C3 was not significantly different ($P > 0.05$); and 3.) Water temperature in B3 and C3 was significantly different ($P > 0.05$). Therefore, the experimental setting was determined feasible to be used in the experiment.

Table 1. Temperature of compartment A3, B3, and C3 during the temperature test for 60 minutes in order to test feasibility of the experimental setup. Tests were conducted at the night time and the day time.

MINUTES	NIGHT			DAY		
	A3	B3	C3	A3	B3	C3
10	28,83	34,17	29,00	29,17	34,17	29,00
20	28,17	34,00	30,00	29,50	34,00	30,00
30	27,17	34,50	30,83	28,50	34,50	30,83
40	27,50	33,17	28,83	29,00	33,17	28,83
50	28,17	34,00	29,33	28,33	34,00	29,33
60	27,33	34,50	28,00	27,67	34,50	28,00
AVERAGE	27,86	34,06	29,33	28,69	34,06	29,33

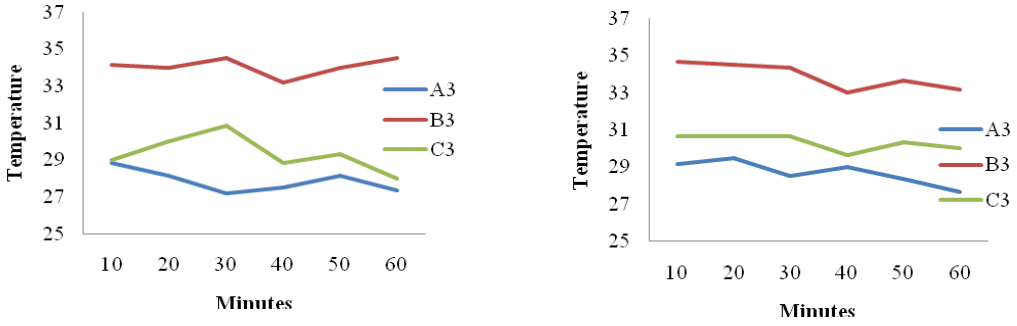

Fig. 3. Temperature of compartment A3, B3, and C3 during the temperature test for 60 minutes in order to test feasibility of the experimental setup. A. The night Time; B. The day time.

Stability desirable temperature during a trial was also depends on the surrounding condition. Minimun temperature of the experiment setup were 27,56– 27,89 ^{0}C during the night time test and 27,44– 28,22^{0}C during the day time test. From the preliminary study, the natural habitat temperature was recorded as 27 – 33.5^{0}C at the night time and 30– 32,8^{0}C at the day time as well.

3.2 Temperature preference of Anguilla sp glass eels

Result of the data statistic nalyses using Non-parametric test statistics showed there was a significant different in the temperature preference of the glass eels not only at at the night time ($P = 0.023$; $P < 0.05$) but also at the day time ($P = 0{,}029$; $P < 0.05$). Accumulation of spending time was calculated from 20 times experiment using individually fish each experiment (Fig. 4). The glass eel spent time for 4.91 seconds and 3.95 seconds in the naturaltemperature habitat of 28^0C and in the not natural temperature habitat of 34^0C respectively during the nitght time experiment. While during the day time experiment glass eel spent time for 5,27.91 seconds and 3,53.95 seconds at 28^0C waters and at 34^0C water respectively.

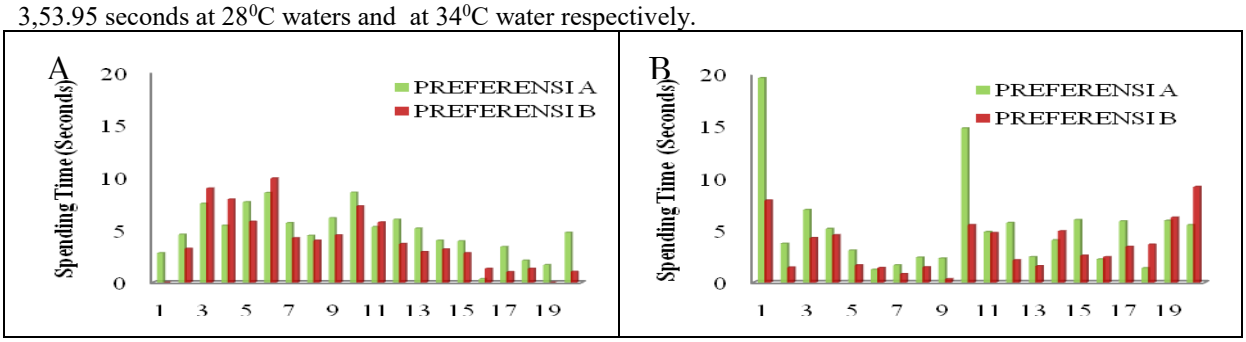

Fig. 4. Spending time of glass eel in the natural habitat with temperatur of 28^0C waters (preferensi A) and in the not natural habitat with temperature of 34^0C (preferensi B). A. The night time; B. The day time.

Moreover, glass eel was tend to avoid the water with 34^0C in temperature and spent much of its activity moving along in the water with 28^0C in temperature. In the night experiment, compared to few glass eel spent more in water of 34^0C, a number of 15 fish spent more in water of 28^0C (about 75% of used samples), while in the day experiment, 70% of glass eel spent more in water of 28^0C (Fig. 5).

Analysis of non-parametric statistics was continued to diagram analysis of glass eel preference on each water temperature. This analysis was calculated by combining and comparing temperature control records during the experiment with the accumulated time of glass eel preference in respond with the system. Ffluctuation of water temperature up to 2^0C at 28^0C and 1.4^0C at 34 during the experiment were not significantly affect glass eel preference to choose water column in each water temperature and condition. Experiment on the preference of glass eel to different habitat temperature has shown that there was a significant role of water temperature to affect glass eel habitat preference between water temperatures at 28^0C and 34^0C. At least there are four particular reasons underlying this behavior. Firstly, physiological adaptation of glass eel (*Anguilla* sp.). This physiological adaptation is closely related to glass eel temperature adaptation and tolerance responding to the disturbance of their natural habitat. Even though glass eel have good adaptability to water temperature change, glass eel need time when they were faced with extreme environmental condition (water temperature at 34^0C). In relatively short interval, as well as the experiment, glass eel would consistently choose water column with temperature at 28^0C. Physiological adaptability in responding water temperature would be increased along with the development of glass eel's body [18]. However, glass eel stage is very vulnerable and sensitive to extreme water temperature changes. This happened because some of the organ in glass eel's body has not been developed enough and their adaptability is not as good as the adults.

Secondly, glass eel (*Anguilla* sp.) ecological adaptation. Glass eel is very sensitive to the changes of water physical characteristics. The estuary as the natural habitat of glass eel, in which also many factors would affect it, give a significant impact on the behavior of glass eel. Glass eel will rest during the day with water temperature at 28 – 30^0C [18]. Glass eel will adapt to their environment and move actively at night in the water column that has an identical water characteristic, in this case water temperature, where they had rest on the day. Thus, glass eel will tend to avoid water column with high temperature, in this case water temperature at 34^0C in the experimental setting, and find an appropriate water temperature while they enter the main flow of the river. Destructed physiological characteristics of the water due to human activity distinguished by the higher water temperature, if that happened in a wide coverage area and in a long time, would definitely inhibit and reduce the movement of glass eel. Glass eel would be trapped in a less favorable environment and die due to predators and inability to adapt. Thus, extreme water temperature changes that occurred in the natural habitat would disconnect glass eel migratory chain, decrease their abundance, and cause the death of glass eel before being able to grow onto the next phase.

Thirdly, enzymatic activity and mechanism in glass eel's body. Enzymatic activity would optimally processed with temperature ranged from 30 – $40\ ^0$C and would eventually stopped when the temperature of the system reached beyond its optimal range [19]. When glass eel were dealing with water temperature at 34^0C, their body reacts so quickly and immediately refrain from it. Glass eel body size and their simple organ decreased their adaptability to respond a spontaneous water temperature changes. Water temperature at 34^0C is an optimum temperature for their body to process any enzymatic activity in their body. When they were trapped in such condition, their physiological and enzymatic activity would be decreased continually and even would be stopped.

This condition had been a major cause for glass eel to avoid water temperature at 34^0C as could be seen during the experiment. Observation on glass eel preference in the experiment has shown that glass eel would eventually choose water temperature at 28^0C in the very first time as many as they would choose water temperature at 34^0C with the same intensity. However, glass eel still had a tendency of preference to water temperature at 28^0C after several experiments with different sample.

Behavioural and environmental processes could modulate glass eel migration patterns [7]. Concerning to the world-wide decline of the anguillid eels, fishery managements have been endeavour by European Commission trough some members of the International Council for the Exploration of the Sea/European Inland Fisheries Advisory Commission (ICES/EIFAC) Working Group on Eels (WGEEL), as well as Japanese government [1]. The catch of glass eel has been more or less in decline since 1994 [2]. These declines have been linked to overfishing, the fragmentation or limitation of habitat caused by dams, and pollution as a result of urbanization [20]. Physical pollution on environment such as unsuitable temperature suspected to influence glass eel recruitment in estuary. This study proposed that Different of 5 -7 degree in water temperature could directly affect water temperature preference and it may be potential affect their migration trought a complex mechanism. Glass eels could sense and then perceive the environmental information resulting in its particular response. Regarding construction of some powerplants close to estuary, place of recruitment of the glass eel, environmental condition should be suitable for glass eel. It is strongly recommended that waste from power plant should be threated before it is released to open sea.

4. CONCLUSIONS

There was a quite significant different (P<0,05) in the water temperature preference of Anguilla sp glass eels. The glass eels tend to avoid the water of 34^0C and in contract spent much in the water of 28^0C. Moreover, 75% and 70% of used samples fish spent more in water of 28^0C at night time and day time experiment respectively.

Different of 5 -7 degree in water temperature could affect to the water temperature preferences of Anguilla sp glass eels. Glass eels could sense and then perceive the environmental information resulting in its particular response.

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Development of Assignment Worksheet of Natural Science Elementary School Based on Android Multimedia Through *Augmented Reality*

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Abstract – Worksheet is one of learning tools which supporting in learning objectives achievement optimally. Data field in Elementary School showed that worksheet which is given to students is less optimal in supporting learning objectives achievement, especially in natural science subject. Worksheet that presented was not capable to give significant influence in maximizing the learning process. This study aims to develop worksheet using assignment method through *Augmented Reality (AR)* that can be accessed via Android. This research is development research that consists of needs analysis and first research, planning, development of first form product, revision and first field experiment, revision and main field experiment, and revision and realization field experiment. Subject of this research is students of Surakarta Elementary School in academic year of 2016/2017. The research data obtained through interview, test, and questionnaire based on the result of the research that was conducted before. The conclusion shows that worksheet developed is stated eligible and able to give significant influence towards the improvement of students' achievement.

Keywords: *Assignment Worksheet, Augmented Reality (AR), Android*

1. INTRODUCTION

Based on the result of PISA (2009), Indonesia is on the second lowest rank from the 65 countries. There are three aspects that were researched by PISA, they are: the ability of reading, mathematics, and science. The result showed that science is on the lowest rank. This is apprehensive that aspect which is measured by PISA is including the special skill in science field that is very needed in facing the global era challenge. In this case, science learning should be revised to be better. From those reasons, it becomes challenge for sciences teachers to teach science maximally to the students. One of the efforts is revising the learning system.

In education field, science learning is called as natural sciences learning. Natural science (IPA) is collection of knowledge that is arranged systematically about natural indication. Natural science development is not only about fact, but also scientific method and scientific attitude. It is hoped that natural science education can be tool for students to learn surrounding nature. Also, the continually prospect development can be implemented in daily life. (Depdiknas, 2008).

Based on the result of survey in Surakarta, it is showed that the score of natural science is lower than the others subjects. In conducting examination, the score of natural science is in the lowest rank compared with other subjects. On the national examination in academic year of 2013/104, the average of Indonesian Language is 8,26 , mathematics 7,38, while natural science is 7,29. Then, on the national examination in academic year of 2014/2015, the score of natural science has significant decreased with average 62,43.

Based on the result of interview to the elementary school teachers in Surakarta, the data showed that natural science subject belongs to the difficult subject. There are some topics that difficult to understand by the students. Then, the teachers have limited time to explain the subject clearly. Besides, the learning system is still use teacher centered. Based on preliminary survey to students, it is known that topic that difficult to understand is topic that needs analysis process towards picture and cycle, one of those is digestion system topic.

One of efforts that can be conducted to maximize natural science learning in elementary school is by developing learning material in form of worksheet. According to Andriani that cited from Belawati (2003) stated that there are four functions of worksheet, they are: (1) as learning material that can minimize the role of teacher, but more activate the students; (2) make the students easy to understand the topic given (3) as learning material that brief and rich of assignment to exercise. (4) make the learning process more effective. According to Isnaningsih and Bimo's research in implementing worksheet based science process skill showed that it can increase the students' learning achievement. Furthermore, Nurina (2015) showed that worksheet based assignment can increase the

students' learning motivation. To solve the limited time of teacher in explaining the topic, it can be developed an assignment worksheet. It is worksheet that used assignment approach. Syaiful Sagala (2009:201) explained that assignment approach is way to present the learning material where the teacher gives certain assignment to make students do learning activities and should responsible about it.

In this modern era, the advance of technology information gives a big influence in various fields, including in education field. In education world, the advance of technology information has a positive impact. We can see that because of the development of technology information, the education world shows significant change. There are so many changes if it compared before. Now, distance and time are not the mean problem in getting knowledge. There are many applications to facilitate this. Therefore, the advance of technology information can be maximized in supporting learning process.

Nowadays, one of the trending technologies is *Augmented Reality*. Suryawinata (2010) stated that *Augmented Reality* is combination between virtual and reality world made by computer. The virtual object can be text, animation, 3D model or video that gathered with the real environment, so that the users can feel the virtual object is in their environment. *Augmented Reality* belongs to new technology branch. However, the development is fast. So far, this technology is used in various fields, especially in military and advertisement. And now, it started applying in education field. Elango (2015) in his research showed positive result in implementing *Augmented Reality* on mathematics learning. Then, Chiang, Yang & Hwang (2015) stated that there is increasing of students' understanding in learning science through media based *Augmented Reality.* Also, research by Kucuk, Zilmas, and Goltas (2015) showed positive result in utilization of *Augmented Reality* in learning language.

In short, object can be seen more real through *Augmented Reality.* Therefore, it is very interesting if it is applied in learning process, especially in natural science, for it has many topics that can be learned through picture or visual. Besides, *Augmented Reality* can be accessed via OS Android in phone facility.

Therefore, in this research, the researcher developed new innovation in natural science learning in elementary school. It is hoped that the problems above can be solved. The researcher developed assignment worksheet that utilize technology by using *Augmented Reality* system and can be accessed via *android.*

2. METHODS

Research that was conducted is Research and Development (RD) that developing assignment worksheet of Natural science in elementary school based multimedia android using *Augmented Reality.* The development that conducted is using procedural model that adapting Borg and Gall model development.

The development according to Borg and Gall (1983) consists of ten steps: (1) research and information collecting (2) Planning (3) Develop preliminary form of product (4) Preliminary field testing (5) main product revision (6) main field testing to validate development product in large scale and compared with control product (7) operational product revision (8) operational field testing. It is validation test towards operational product that produced (9) Final product revision (10) dissemination and implementation product.

Respondent in this research is class 5 elementary school students in Surakarta. In the trial of small scale, the product is tested to 10 students and 1 teacher. In the trial of middle scale, the product is tested to 25 students and 2 teachers. Then, in the trial of large scale, it is tested to six classes (control class and experiment class). Instruments that used in this development research are questionnaire, questions, validation sheet, assessment sheet between students and observation sheet. Data processing in this research is conducted using descriptive analysis, including: expediency analysis and analysis of learning test result data. The method of collecting data in this research is questionnaire technique to know the expediency product (assignment worksheet based android multimedia) from topic experts, language experts, learning experts, media experts, and also the teacher and students' respond, learning result assessment, psychomotor, and behavior. Test technique to assess cognitive learning result, and assessment technique inter students to psychomotor and behavior. In the step draft development I, product is revised based on suggestion/input from experts. Before tested, product is validated by 9 experts using Formula Aiken. A criterion that is used is if the index bigger or equal with 0, 74 the development step can be continued.

3. RESULTS AND DISCUSSION

3.1 Research and Information collecting

Activity in doing need analysis was field study which involved distributing questioner to the students and teachers toward the need of learning media, learning condition in the classroom, the existence of instructional material, interviewing to the students and teachers as well as analyzing the last three years of national examination's result and the result of interim test.

Preliminary research obtained that natural science is assumed as difficult subject. There were some materials that are difficult to be understood and teacher has limited time to provide more explanation to the students as well as teaching and learning is conducting using teacher center. Preliminary survey to the students revealed that materials

that are considered as difficult materials are materials that need analyzation toward pictures and cycles such as digestive system.

3.2 Planning

The activity conducted throughout this step was designing android multimedia-based students' work sheet by using augmented reality based on school-based curriculum.

3.3 Develop prototype of product

Students' worksheet was designed for the fifth grade students of elementary school in human digestive system. Furthermore, the augmented reality application was combined with students' worksheet.

Furthermore, nine experts such as experts of material development, learning media, learning experts, and language experts validated prototype. Education practitioners also validates this prototype, they are five elementary school teachers. The validation obtained that prototype of developed students' worksheet is valid with 0.9528 based on Aiken validity score.

Figure 1. Appearance of Students' Worksheet

Figure 2. Appearance of Augmented Reality in Android

3.4 Preliminary field-testing

Prototype was distributed to ten students and on teacher to evaluate its quality. This limited scale test was aimed to investigate the readability of students' worksheet. This activity was conducted by distributing questioner to the students and teacher. Preliminary field-testing revealed that the quality of students' worksheet is good. Mean percentage of students' evaluation toward quality of students' worksheet is 85.8, meanwhile percentage of teacher's evaluation is 87.5.

Table 1. The Result of students' and teacher's evaluation in preliminary field- testing

No	Component of Quality Evaluation	Students		Teacher	
		Persentage (%)	Category	Persentage (%)	Category
1.	Content	86.4	Good	89.6	Good
2.	Language	85.1	Good	85.5	Good
3.	Organization	86.4	Good	89.6	Good
4.	Layout	85.3	Good	85,3	Good
	Mean	85.8	Good	87.5	Good

3.5 Prototype Revision

The activities conducted in main product revision step were revising prototype based on notes provided by students and teacher from preliminary field-testing. The activities include editing sensitivity maker in augmented reality and adding explanation of anus' functions in digestive process.

3.6 Main Field Testing

Throughout this step, students' worksheet was tested into larger scale is that one class covers 25 students and 2 teachers. The result of test shows that the quality of students' worksheet is good. Mean percentage of students' evaluation toward quality of students' worksheet is 85.8, meanwhile percentage of teachers' evaluation is 89.6.

Table 1. The Result of students' and teachers' evaluation in preliminary field- testing

No	Component of Quality Evaluation	Students		Teachers	
		Persentage (%)	Category	Persentage (%)	Category
1.	Content	86.4	Good	89.6	Good
2.	Language	85.1	Good	85.5	Good
3.	Organization	86.4	Good	89.6	Good
4.	Layout	85.3	Good	85,3	Good
	Mean	85.8	Good	89.6	Good

3.7 Operational Product Revision

Operational product revision was conducted by revising and adding students' worksheet based the data from main field-testing. Addition was also done by inputting application of augmented reality digestive system into play store, so that the user can access it easily.

3.8 Operational Field Testing

Operational field-testing was conducted to examine the quality of students' worksheet into larger scale, six classes. Furthermore, these six classes were divided into three classes for experimental class and three classes for control class. These classes were selected from high, average, and low school category. Students of control class were not taught using the prototype. Teacher taught using commonly teaching model and students' worksheet. On the other hand, students of experimental class were taught using prototype. This treatment was conducted through four meeting. The aim of the large-scale test is to examine the effectiveness of students' worksheet.

The result of data analysis showed that students' worksheet that is used to teach experimental class is effective. Students score in experimental class also higher than students in control class who are not taught using students' worksheet multimedia android based. In addition, students' worksheet multimedia android based also can improve students' score in learning human digestive system, natural science for the fifth grade of elementary school.

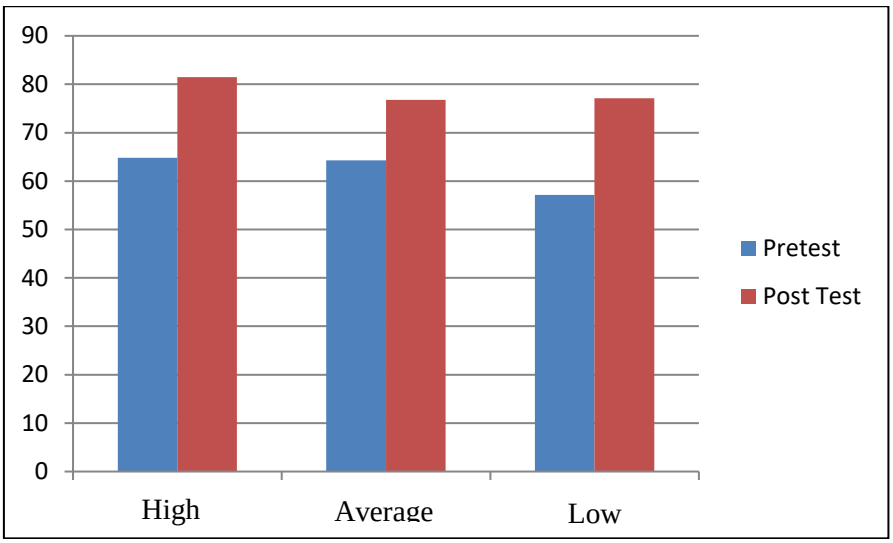

Figure 1. The Result of Pretest and Posttest in Experimental Class

3.9 Final Product Revision

This step was not neglected because students' worksheet is good and effective to be implemented in learning digestive system to the fifth grade students of elementary school.

3.10 Dissemination and Implementation

Students' worksheet multimedia android-based using augmented reality was disseminated though various ways, directly and through online media as well. Direct dissemination means that the product directly distributed to elementary schools in Surakarta, meanwhile online dissemination was done by publishing the product in website, social media, and play store.

4. CONCLUSIONS

1. The assignment worksheet has been developed by using 10 Borg and Gall stages. Based on discussion, the result show that the quality development has been very good.
2. Worksheet augmented reality base has been validated, the percentage of validity is >85.1% with good category
3. Worksheet augmented reality base is effective for Science Learning usage in Elementary School, especially digestion system material on human being. The effectiveness is showed by the average of result study on experimental class which is higher than control class.

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