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PROCEEDING

The 3rd International Seminar on Chemistry 2014

Innovation and Advances in Chemistry for The 21st Century Challenges

Organized by:
Department of Chemistry
Faculty of Mathematics and Natural Sciences
Padjadjaran University

In cooperation with Indonesian Chemical Society

Welcoming Address by the Organizing Committee

Honorable Rector of Universitas Padjadjaran
Distinguished invited speakers, participants, ladies and gentlemen,

It is our great pleasure to welcome all the honorable guests, the distinguished guest and all the participants to the 3rdtriennial International Seminar on Chemistry (ISC) 2014, here in Jatinangor, the small town you might never heard before. We are particularly happy that this seminar has been organized to celebrate the 57th anniversary of Universitas Padjadjaran and as a continuation of the previous ISC in 2008 and ISC 2011, we are organizing a seminar with invited speaker from various international scientific institutes. We believe that the 3rdISC 2014 meeting will be a place where the recent 21stcentury advances and innovations in chemistry can be discussed and shared, not to mention issues emerged can be brought to light so all participants will be concerned and can contribute to solve the issues. We hope this seminar will bring communication and cooperation in educational and research activities with the support and collaboration of Indonesian Chemical Society, nongovernmental organizations, research institutions and scientific associations both in Indonesia and overseas.

This event will offer a comprehensive understanding on how to optimize the utilization of natural resources through the knowledge of chemistry. It will cover topics, among others, not only associated with innovation in analytical methods, techniques and instrumentations, innovation in material chemistry and nanotechnology, natural product chemistry and organic synthesis, biomolecular sciences and biotechnology, information technology in chemical sciences and computational chemistry, environmental and green chemistry, and chemical education.

As a chairperson, we are very proud to report that today, the international seminar is attended by more than 300 participants, come from all overAustralia, Indonesia, Japan and Malaysia. We invite 2 Indonesian invited speakers, and 7 foreign invited speakers come from Australia, Germany, Japan, Malaysia, The Netherlands, and USA. There are 99 papers to be presented orally and 87 papers presented by poster covering wide-variety subjects of chemistry and its application. We hope this event will open scientific discussions of the new findings particularly toward "the role of chemistry in the utilization of natural resources" and followed by better understandings, formal and non-formal cooperation and also opportunities to explore further collaboration among participants.

We would like to express our appreciation to all invited speakers, and contributed lecturer who are willing to share their knowledge, experiences, and advices in this event. We thank our sponsor for their support to this event. Finally, we would also like to thank Rector of Universitas Padjadjaran for his support to this seminar.

We hope you will enjoy a pleasant and valuable seminar at Universitas Padjadjaran.

Dr. rer.nat. Iwan Hastiawan

Remarks by Head of Indonesian Chemical Society

Distinguished guests, all invited speakers, participants, ladies and gentlemen,

It is a great pleasure for me on behalf of the Indonesian Chemical Society to welcome you at the 3rd International Seminar on Chemistry 2014 this morning here at the Auditorium of the Center for Japanese Language Study, Jatinangor Campus, Padjadjaran University. Chemistry as a science discipline is rapidly growing and embracing other sciences. This means that chemists should always update their recent chemical knowledge and information they acquired. Therefore, this International Seminar on Chemistry has a special importance for us, particularly for Indonesian chemical society, primarily as it shows our endeavour in chemical research and development, appropriate for our unique demands, and secondly as it provides means to exchange information and experiences, particularly with regard to the chemistry progress and other related matters including international cooperation.

Distinguished guests, ladies and gentlemen,
I hope that you will gain a lot of benefits from the fruitful discussion during the seminar. I would like to express my expression to the Organizing Committee of the seminar. I am indeed delighted and thank you for all your effort and hard working for making this seminar happened.

To all distinguished guests from abroad and from other cities in Indonesia, enjoy your stay in Jatinangor and have a nice time.

Head,
Indonesian Chemical Society

Dr. Muhamad Abdulkadir Martoprawiro

Opening remarks by Rector of Universitas Padjadjaran

Honorable invited speakers, and all participants of the 3rd International Seminar on Chemistry 2014.

First of all, on behalf of Universitas Padjadjaran, please allow me to extend my warmest welcome. I am much honored to be the host of this event which is organized as one of activities in celebrating the 57th anniversary of Universitas Padjadjaran.

This seminar will cover topic "Innovations and Advances In Chemistry for The 21st Century Challenges". As we all aware, chemistry is the science that studies atoms and molecules along with their properties. All matter is composed of atoms and molecules, so chemistry is all encompassing and is referred to as the central science because all other scientific fields use its discoveries.

We all know that investigating materials and devices at the nanoscale level has become the topic of discussion in our daily life even at the dinner table. The behavior of nanoscale materials is close to atomic behavior rather than that of bulk materials. This leads to vivid properties and well-defined concepts, even though the description of these properties can be understood in terms of quantum mechanics, which provides only a fuzzy picture.

On the other hand medicinal chemistry has evolved from the chemistry of bioactive compounds in early days to works at the interface of chemistry and biology nowadays. Medicinal chemistry of bioactive natural products spans a wide range of fields, including isolation and characterization of bioactive compounds from natural sources, structure modification for optimization of their activity and other physical properties, and total and semi-synthesis for a thorough scrutiny of structure activity relationship. In addition, synthesis of natural products also provides a powerful means in solving supply problems in clinical trails and marketing of the drug, for obtaining natural products in bulk amounts is often very difficult.

Knowing that committee has selected outstanding speakers from various prestigious institutions, I believe that all participants will enjoy the discussion of the issues covered by the topic of this seminar. I hope this meeting will serve as an excellent opportunity for all of us to discuss views, trends, exchange knowledge, establish links and lead us to the right direction while stepping in the next future with green chemistry.

I would like to express my appreciation to all invited speakers and participants, and last but not least my deep thanks to the organizing committee for their efforts to make this seminar happened which will enrich Basic Scientific Pattern of Universitas Padjadjaran i.e. "Nurturing Law and Environment".

I wish all of you have a nice stay and inspiring meeting in our university.

Rector,

Prof. Ganjar Kurnia

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Invited Speaker

From Donor-Acceptor Complexes to Gallium Nitride Nanorods

Henry F Schaefer

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In this lecture we outline theoretical advances in modeling gas phase reactions leading to the formation of 13-15 binary materials from donor-acceptor complexes. Recent theoretical studies of the structures, stabilities, and reactivities of 13-15 donoracceptor complexes, amido and imido compounds, as well as large 13-15 clusters and nanotubes are highlighted. Of particular interest here are the gallium nitride systems. Use of 13-15 compounds as single-source precursors to 13-15 alloys is discussed. Also presented are directions for further theoretical and experimental studies in this area.

Roles of TMEPAI/PMEPA1 in Cancer Progression

Mitsuyasu Kato[1]*, Yukihide Watanabe[1], Thanh Thao Vo Nguyen[1], Riezki Amalia[12]

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TMEPAI/PMEPA1 is a direct target gene of transforming growth factor-β (TGF-β)/Smad signaling and participates in negative feedback control of the duration and intensity of TGF-β/Smad signaling. TMEPAI is constitutively expressed in many types of cancer cells and is associated with poor prognosis of the patients. We found that TMEPAI is highly expressed in the lung adenocarcinoma cell lines. Expression of TMEPAI in these cells is activated by TGF-□ and EGFR/RAS/MAPK signaling pathways. Knockdown of TMEPAI in lung adenocarcinoma cell lines enhanced levels of Smad2 phosphorylation and significantly suppressed cell proliferation in the presence of TGF-β, indicating that highly expressed TMEPAI suppresses levels of Smad phosphorylation and reduces the growth inhibitory effects of TGF-β/Smad signaling. Furthermore, knockdown of TMEPAI in lung cancer cells suppressed sphere formation *in vitro* and tumor formation *in vivo* both in subcutaneous tissues and in lungs after tail vein injection in NOD-SCID mice. Together, these experiments indicate that TMEPAI promotes tumorigenic activities in lung cancer cells. Recently, we performed *Ab initio* prediction of TMEPAI structure by I-TASSER server (Zhang Lab.) to examine further functional roles of TMEPAI in cancer formation. All models had low C-score lower than -3 (Range of -5, 2) but predicted the presence of interesting domains. We are currently trying to elucidate the functional significance of these predicted domains and will report our preliminary data indicating that the usefulness of these low score prediction to guide the experimental designing.

Keywords: TMEPAI, Cancer, TGF-□, EGF, Wnt

Selective tumour-targeting with biomolecule–chemotherapeutics conjugates

Stefan Richter[1], Wilma Neumann[1], Menyhárt B. Sárosi[1], Imola Sárosi[1], Verena Ahrens[2], David Böhme[2], Annette G. Beck-Sickinger[2], Evamarie Hey-Hawkins[1]*

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Many metal compounds are widely used in medicine as diagnostic and therapeutic agents (e.g., as contrast agents, radiopharmaceuticals, antitumour drugs, etc.).[1]Cisplatin is one of the most widely used drugs in the treatment of cancer; other complexes are also being studied in clinical trials as potential cytostatics. However, conventional platinum-based chemotherapy is often associated with strong side effects, and the efficacy of anti-tumour drugs is strongly limited by intrinsic and treatment-induced resistance of tumour cells.

One of our strategies for overcoming resistance and for improving the selectivity of antitumour drugs involves the conjugation of cisplatin analogues, ruthenium(II) compounds or other antitumour agents with highly selective and biologically active biomolecules, such as modified neuropeptide Y ([Phe^7,Pro^{34}]-NPY), which displays a high selectivity towards the human Y_1 receptor subtype (Figure 1). We expect that the cytotoxic drug will be selectively transported into breast tumour cells with the help of [Phe^7,Pro^{34}]-NPY.

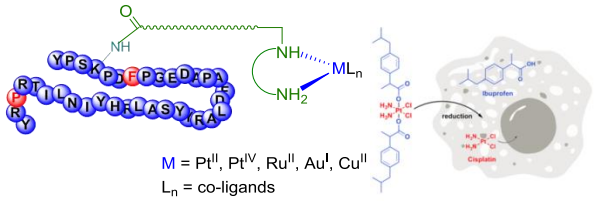

Figure 1

Figure 2

Another strategy involves an enzyme which is overexpressed in several tumour tissues and is also involved in tumour onset and progression, i.e. COX-2, an isoform of cyclooxygenase (COX).[2] Clinical studies revealed that targeting the COX-2 pathway is a promising strategy for the prevention and treatment of tumours. Furthermore, promising results were obtained with combinatorial treatments with chemotherapeutic agents, such as cisplatin, and COX inhibitors. These observed synergistic effects could possibly be improved by combining both molecules in one compound (Figure 2).[3]

Financial support from the the Fonds der Chemischen Industrie (scholarship for W.N.),the European Union and the Free State of Saxony, the Graduate School BuildMoNa (funded by the DFG) as well as donation of chemicals by Umicore AG & Co. KG are gratefully acknowledged

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Keywords: biomolecule–chemotherapeutics conjugates, cancer, tumour-targeting

Transaminases as tools for the synthesis of chiral amines

A.C. Terwisscha van Scheltinga, C.C. van Oosterwijk& B.W. Dijkstra

Laboratory of Biophysical Chemistry, University of Groningen, The Netherlands

Transaminases reversibly exchange an amino group on one molecule with a carbonyl oxygen atom on another molecule. They are predominantly enzymes that depend on pyridoxal-5'-phosphate (PLP; Vitamin B_6) as a cofactor, and are classified according to the position of the amino group that is transferred. Whereas α-and β-transaminasesperformtheir reactions at the α-or β-carbon atom of an amino acid, respectively, ω-transaminasesmodify their substrate at a non-α position. Transaminases can catalyse the synthesis of chiral amines. Theseform an important class of chemical building blocks, which can contribute a stereogenic centre to pharmaceutically active substances, agrochemicals and other fine chemicals.They can be obtained from asymmetric synthesis with transaminases, but this process is not straightforward and requires substantial further development. In addition to improving protein stability, improved enantioselectivity and co-substrate regeneration are important challenges for the biotechnological optimization of these enzyme systems. Therefore, research on enantioselective transaminases has intensified in recent years.

Making these enzymes suitable for large-scale application in industry requires optimisation of their enantioselectivity for their particular substrate, as well as their adaptation to the temperature, pH, and presence of (organic) solvents during the reaction. Availability of 3D structures of the enzymes of interest would facilitate rational engineering of the enzymes to improve their enantioselectivity and other kinetic and thermodynamic properties. We will discus several aspects of ω-transaminases. X-ray structures are used to describe the substrate-binding site, the arginine switch, their catalytic mechanism and the structural determinants governing enantioselectivity.

Strategies in Bacterial Production of Recombinant Human Thrombinfor Its Use as a Fibrin Sealant Component

Toto Subroto[1], Iman Permana Maksum[1], Saronom Silaban[2], Khomaini Hasan[3],Shabarni-Gaffar[1], Sutarya Enus[4], Soetijoso Soemitro[1]

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Fibrin sealant (also called fibrin glue) has been used in creating fibrin clot, a way to heal wounded tissue via the action of thrombin that converts fibrinogen into fibrin monomer. The sealant mixture also contains factor XIII, an enzyme that stabilizes fibrin upon circulation in the blood. This autologous fibrin sealant can replace the current suture techniques, which is difficult and may cause discomfort for the patient. Fibrinogen and factor XIII can be obtained easily and quickly from the blood plasma of the patient. However, obtaining the thrombin is far more difficult and requires highly skilled person. Therefore, commercially available thrombin is commonly employed. Commercial thrombin is derived from the blood plasma of either human or cow. Whichever the source is, this commercial preparation is highly exposed to contamination and may also allergenic, as well as expensive. Therefore, recombinant human thrombin has been developed. The recombinant protein can be produced by various microorganisms, in which *Escherichia coli* is the most preferred host for easy culturing, short fermentation time, well-known genetics, and easy to manipulate. However, heterologous gene expression in *E. coli* is often hampered by the formation of non functional protein, mostly due to the formation of insoluble aggregates. At this moment, this issue has been solved in individual cases, thus no universal strategy.In this presentation the strategies for production of soluble and functional recombinant human thrombin in *E. coli* is reviewed. At least five strategies are available to achieve that aim, which are (1) optimization of the gene sequences (2) production of protein fusion partners/affinity tag, (3) change of host, (4) chaperonco-expression, and (5) induction using either thermal or chemical means. Modification at gene level also allows inclusion of protein tag, which is useful for the purification but may need to be removed at the final purification step. Tag removal by means of proteolytic digestion results in contamination of the product by both the fragments and protease employed. In our case, this issue was solved by the use of self cleavable intein system. This review thereby highlights problems encountered and troubleshoots in bacterial production of soluble and biological function recombinant protein.

Keywords:*E. coli*, Recombinant protein expression, Thrombin, Fibrin sealant, Intein system

Deproteinized Natural Rubber (DPNR) ProductionFrom The Latex Of *Hevea brasiliensis* MUELL ARG With Enzymatic Method

R. Ukun MS Soedjanaatmadja[1*], Dewi Astrid[1], Inky Febrianty[1], Ade Sholeh Hidayat[2], Ace Tatang Hidayat[1], Iman Permana Maksum[1], Iman Rahayu[1], Saadah Diana Rachman[1], Safri Ishmayana[1]

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Indonesia is the one of the biggest rubber producer, next to Thailand. Considering to the production of natural rubber, the culture of the plant *Hevea brasiliensis* is economically most important to tropical countries that produce rubber. One of the opportunities in latex production from rubber trees is by producing low density natural rubber with low protein content, as the raw material for making sponge of floating pipe, rubber gloves, contraception's tool, and so on. The objective of this research was to produce low density of natural rubber with has low protein content through enzymatic process, by using papain, bromelain and the mixture of protein denaturant. The method of this research beginning with taking sample of the fresh latex of the *Hevea brasiliensis* PR-255 clone, and then following by the separation of the latex into three main fractions (rubber fraction, C-serum and bottom fraction), using centrifugation method with the variation of speed of 19,000 (60 minutes) and 4,000 rpm (180 minutes). Then, the rubber fraction was taken and it was treated by adding the proteolytic enzymes (papain and bromelain), followed by adding protein denaturant, and incubated in variation of time; 48 and 96 hours. The density and protein (total nitrogen) content before and after enzymatic process were measured. The results of research showed that the proteolytic enzymes and protein denaturant could decrease the density and total nitrogen content of the natural rubber, in incubation time of 96 hours. The mixture of papain and protein denaturant could decrease total nitrogen content of the rubber fraction from the initial of 0.40% to 0.06%, and the density of DPNR decreased from 0.9516 to 0.8092, respectively. While, the mixture of bromelain and protein denaturant could decreased total nitrogen content of the natural rubber in optimal condition, from 0.40% to 0.03%, and the density of DPNR decreased from 0.9516 to 0.7965, respectively.

Keywords: DPNR, Hevea brasiliensis, proteolityc enzyme, protein denaturant.

The Utilization of Unlimited Sunlight Energy to Sustain Our Better and Safe Environment by the Hybridization of Artificial Photosynthesis of Ti-oxide Based Photocatalysts and Photosynthesis of Green Vegetables in the Plant Factory

Masakazu ANPO

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Director of R&D Center for the Plant Factory of OPU
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The lecture is consisted of the following three research topics. The first one is (a) Invesitigation of highly active Ti-oxide based photo-functional materials from molecular level to bulk semiconductor thin films. Environmentally harmonious, clean and safe scientific technologies to address energy as well as pollution and climatic change are the subject of much research and discussion, especially, since March 11, 2011. Photocatalysis, in which the abundant and clean energy of sunlight could be harnessed, would be a major advance in the development of sustainable, non-hazardous and economic technologies. We have successfully developed Ti-oxide photocatalysts which enable the absorption of visible light (longer than 400 nm) and operate as efficient and effective environmentally-friendly photocatalytic materials. The lecture presents the results obtained in the highly dispersed Ti-oxide single site photocatalysts prepared within zeolite frameworks for the reduction of CO2 with H2O (artificial photosynthesis) and visible light-responsive TiO2 thin film photocatalysts for the decomposition of H2O with a separate evolution of H2 and O2 under sunlight irradiation.1-4). The second one is (b) Introduction of R&D Center for Plant Factory. The artificial-light plant factory of OPU using fluorescent lights and LED lights for the cultivation of various fresh and safe vegetables, in much shorter period as compared with outside fields, in clean rooms will be introduced, focusing on various facilities and reserach activities. Such plant factory is surely known as an important business target nowadays and the plant factory is a new agricultural system that can supply safe, good and nutritious produce regardless of any natural or manmade phenomena such as global warming, climatic change, pollution or any damage and destruction of agricultural fields.4,5). At the end, the efficient photocatalytic H2 and CO2 production from H2O involving various biomass as a sacrificial reagent will be discussed in connection with the hybridization of the artificial photosynthesis (photocatalytic decomposition of H2O) and photosynthesis of green vegetables in the plant factory in order to construct a clean and sustainable chemical system to utilize the unlimited clean sunlight energy.

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Novel and Bioactive Chemicals from Malaysian Plants

Khalijah Awang

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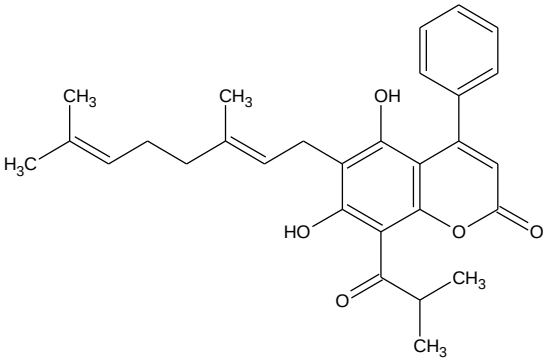
Malaysian flora is one of the oldest and most diverse floras in the world. It houses more than 15,000 plant species in which about 3000 are tree species. Therefore, it is not surprising that Malaysia is one of the potential mega producers of natural chemical entities. Today, natural products have again attracted the interest of many medicinal scientists as the source of lead compounds in attempts to discover more efficient and cost saving drugs. Our laboratory has been involved in the search of chemical compounds from the forest plants notably from species of Annonaceae, Apocynaceae, Lauraceae and Meliaceae. In the presentation, the compounds discovered shall be presented and the focus will be on some novel structures and bioactive molecules; malayanine A & B (*Chisocheton erythrocarpus* - limonoids), kingianines (*Endiandra kingiana* - polyketides), subditine (*Nauclea subdita* - indole alkaloid) and mesuagenines (*Mesua kunstleri*- prenylated coumarin).In addition, Malaysia is fortunate to inherit knowledge on traditional medicinal practices from three very rich cultures; Malay (*jamu* and *ubat periuk*), Chinese (sensei) and Indian (ayurvedic). Hence, our findings from the studies of selected medicinal plants shall be presented; *Alpinia conchigera* - antifungal, *Curcuma zedoaria* - anticancer and *Goniothalamus* species – anti cancer. An example of a structure activity relationship (SAR study) from a series of synthesized amido stilbenes will also be briefly discussed.

Keywords: Malaysian plants, Annonaceae, Apocynaceae, Lauraceae and Meliaceae.



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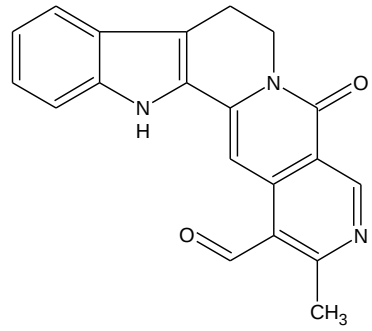

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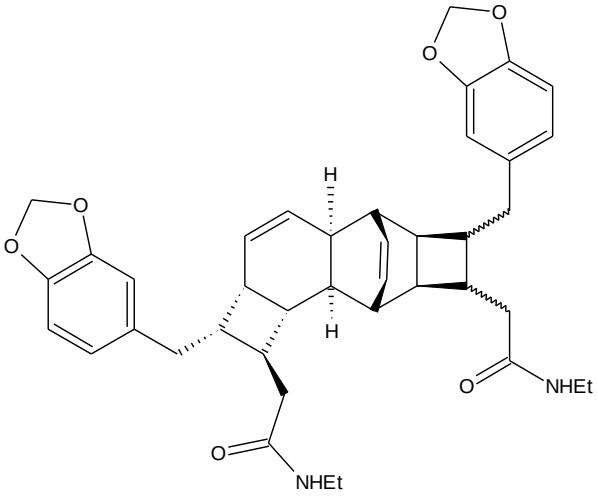

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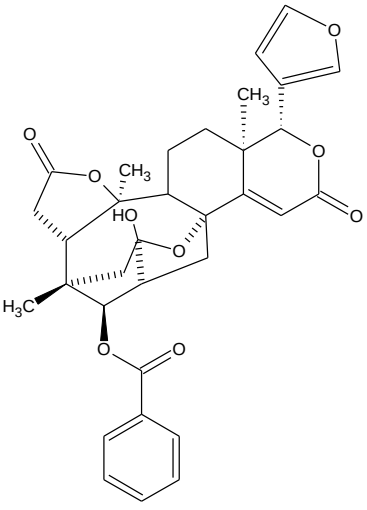
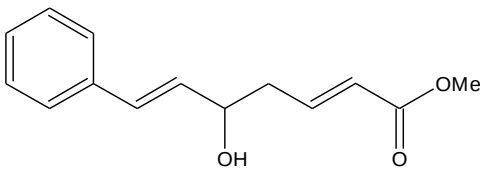


Malayanine B



Goniomicin B

Small molecule fluorine chemistry: applications in organic synthesis, specialised materials and medicinal chemistry

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Fluorine can have a profound effect on the physical, chemical and biological properties of otherwise normal organic molecules. Advantage can be taken of these unique properties in many different applications. For example, selective fluorination influences the reactivity of neighbouring groups, moderates conformation, and serves to inhibit biological processes. In this paper, another application of monofluorination will be illustrated from our work in the design of molecules as candidates for non-invasive, radiolabelled diagnostic agents for the study of age related diseases and psychiatric disorders. Polyfluorinated molecules can also adopt extreme properties that characterise them as 'fluorous'. When regular organic molecules are substituted by polyfluoroalkyl substituents they can adopt some of these (fluorous) characteristics while retaining some or all of their inherent, organic molecular properties. In this sense the fluorous tagged molecules can then adopt the added function of the taggant in the organic molecule. As a result, the molecules can take on unique aggregation properties or phase transition properties that have applications in complex systems, efficient organic synthesis strategies, and techniques for separations. This lecture will draw upon examples from our research in which polyfluoroalkyl substituents have been used in association with the synthesis of compounds with biomedical interest and materials applications.

Keywords: acetal; fluorinated amphiphile; fluoroorganic; fluorosurfactant; receptor ligand; self-assembly; 1,2,3-triazole

Contributed Paper

Modification of Natural Silica with PEG 4000 as The Stationary Phase for The Analysis Of Caffeine by HPLC

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Abstract

In previous research has been conducted on the activation of the natural silica using 1 M HCl and was applied to column chromatography as a polar column for the analysis of n-hexane. However, to improve the usability of the natural silica were modified by using PEG 4000 on natural silica which has been activated it. This study has been carried out activation, modification and characterization of the natural silica so that the natural silica has to be non-polar and can be used as a filler column (stationary phase) on HPLC. Characterization is done by using XRD, XRF, and FTIR. The column was used for the analysis of caffeine using HPLC and the results obtained have been compared with ODS C18 column.

Keywords: HPLC, modification, natural silica, caffeine, PEG 4000

1. Introduction

Silica is a compound result of polymerization of silicate acid, which is composed of a chain of SiO_4 tetrahedral units with the general formula SiO_2. In nature silica compound found in some natural materials, such as sand, quartz, glass, and so on. Silica as a natural compound found in the crystalline structure, while the synthetic compound is amorphous. In synthetic compounds can be made of silica or a silicate solution of silane reagent[1].

Silica is one of the inorganic oxide material with a very wide utilization, both for the manufacture of various types of silica-based materials, such as ceramics, zeolite synthesis, and organic-inorganic composite as well as directly, for example in vegetable oil refining, pharmaceutical products, detergents, adhesives, fillers (stationary phase) column chromatography, and polymer fillers[2].

Properties of silica are very supportive in its use as a stationary phase in chromatography, which is a combination of the solid structure, the framework substance, and the system sockets. The nature of the silica is in accordance with the basis of chromatography[3]. Stationary phase can be made and treated in various ways to change the nature and capacity, as well as a number of attempts have been made to control the equivalent stationary phase. Surface activity of each stationary phase is different on one side to the other side. Pretreatment in a manner prescribed can eliminate the activity difference[4].

Silica is often modified with certain organic groups to improve the adsorption ability. This is because silica has some unique properties not shared by other inorganic compounds, such as inert, adsorption and ion exchange properties of a good, easily modified by certain chemical compounds to improve its performance, high mechanical and thermal stability, and can be used for preconcentration or separation analytes due to analyte binding process on the silica surface is reversible. Natural silica modification done to get the shape and composition of cations of different frameworks. Chemical surface modification is usually done through binding organosilan in accordance with the binding of the desired end functional groups[5]. From previous studies it is known that the polymer surface modification of inorganic oxides such as silica can be done with the help of coupling agent[2].

In this study, silica will be modified with surfactants for changing the properties of silica are polar to non-polar. Surfactant used is Poly Ethylene Glycol 4000 Surfactants or surface active agent is an organic molecule that has a part or group liofilik (like solvent) and group liofobik (not like solvents)[6]. Poly Ethylene Glycol (PEG) also known as macrogol, a synthetic polymer of oksietilen the structural formula $H(OCH_2CH_2)_nOH$, where n is the average number of clusters oksietilen. PEG generally have a molecular weight between 200 – 300000. The naming of PEG is generally determined by the number that shows the average molecular weight. Consistency is strongly influenced by the molecular weight. PEG 3000-20000 or more in the form of semi-crystalline solids[7].

β-carotene is used for the determination of the absorption of silica modified with PEG 4000 β-carotene is one of about 500 carotenoids that exist in nature and has the highest vitamin A activity[8]. The presence of double bonds causing beta-carotene is sensitive to oxidation. Oxidation of β-carotene may be faster in the presence of light, and metal catalysts, particularly copper, iron and manganese[9]. Silica modified subsequently

incorporated into the chromatography column as the stationary phase to test the ability of the modified silica as a non-polar column. Substances that will be analyzed for the test is caffeine, subsequent test results compared to the standard ODS C18 column.

Caffeine is one of the many types of alkaloids contained in the leaves of tea (Camellia sinensis), coffee beans (Coffea arabica), and cocoa beans (Theobroma cacao). Caffeine has beneficial pharmacological effects clinically, such as stimulating the central nervous system, especially smooth muscle relaxation of bronchial smooth muscle, and heart muscle stimulation. Based on the pharmacological effects of caffeine are often added in a certain amount to drink supplements. The side effects of excessive use of caffeine may cause nervousness, restlessness, tremor, insomnia, hyperesthesia, nausea, and seizures[10].

Chromatographic determination of caffeine levels using a technique developed in analytical chemistry. Several studies using HPLC have been reported by several investigators[11-14]. The use of natural silica which has been modified for the determination of caffeine by HPLC very precise because HPLC analysis with fast, good separation power, sensitive, easy sample preparation, and can be connected with an appropriate detector[15].

2. Experimental

2.1. Instrument

FTIR (Perkin Elmer tipe: FTIR Spektrometer Frontier), XRD (X'Pert Powder PANAlytical Pw 90/60), Spectrofotometer Genesys 20, HPLC Agilent 1120

2.2. Materials

Poly Ethylene Glycol (PEG) 4000, NaOH, Petroleum Benzene, β Carotene, caffeine, HCl. (All materials from Merck)

2.3. Synthesis of Sodium Silicate

Weighed 1 gram of NaOH and then diluted with 10 mL of distilled water and placed in a beaker. After NaOH and distilled water was added 0.6 grams of reacted silica which has been activated with 1 M HCl, and then the solution was stirred with heating until the reaction between NaOH and silica. Finally, the silica becomes soluble. Sodium Silicate formed tested by XRD.[16, 17]

2.4. Modification of natural silica in the form of sodium silicate with PEG 4000

Silica which has been treated with NaOH was added 1.5 grams of PEG 4000. Then, the solution was stirred for 2 hours without heating. Then filtered and heated at a temperature of 110 ^{o}C. Perform characterization by XRD and FTIR.

2.5. Determination of the absorption of ß-carotene on silica before modified with PEG 4000

Provides erlenmayer 100 ml, 5 pieces. Then enter the natural silica before modified PEG 4000 as much as ± 1 gram. Then added standard solution of ß-carotene by 25 mL with a concentration of 2; 4; 6; 8; 10mg / L in each erlenmeyer. After it was stirred with a shaker with a contact time of 2 hours at a speed of 150 rpm. Then the solution was filtered using whatman No. 1 filter paper obtained a clear solution. The solution is to be measured with Spectrophotometer to determine the ability of the silica absorption. Silica absorption is determined by using the Langmuir isotherm equation.

2.6. Determination of the absorption of ß-carotene on silica after modified with PEG 4000

Provides erlenmayer 100 ml, 5 pieces. Then enter the natural silica before modified PEG 4000 as much as ± 1 gram. Then added standard solution of ß-carotene by 25 mL with a concentration of 2; 4; 6; 8; 10mg / L in each erlenmeyer. After it was stirred with a shaker with a contact time of 2 hours at a speed of 150 rpm. Then the solution was filtered using whatman No. 1 filter paper obtained a clear solution. The solution is to be measured with Spectrophotometer to determine the ability of the silica absorption. Silica absorption is determined by using the Langmuir isotherm equation.

2.7. Caffeine analysis by HPLC using a modified silica as the stationary phase

Natural silica which has been modified subsequently incorporated into the HPLC column 150 × 4.6 mm id. Tests carried out using a mobile phase of methanol and caffeine samples with UV-Vis detector at λ 273 nm, the results obtained were compared with commercial ODS C18 HPLC column same size as the natural silica columns were refilled.

3. Results and Discussion

3.1. Natural silica modification with PEG 4000

Modification aims to change the nature of the polar silica into non-polar. First, silica was activated with 1 M HCl. Furthermore, silica is reacted with NaOH to include the sodium group before modified with PEG 4000.

$$SiO_{2(s)} + 2NaOH_{(aq)} \longrightarrow Na_2SiO_{3(aq)} + H_2O_{(l)}$$

XRD test was used to determine the level of purity of precipitated silica which has been merged with NaOH. XRD test results can be seen in Figure 1.

From Figure 1.a can be seen that there is a highest peak at 2θ positions ; 29.356 which is a crystalline peak Na_2SiO_3 International Centre for Diffraction Data of Inorganic Crystal Structure Database (ICSD-ICDD).

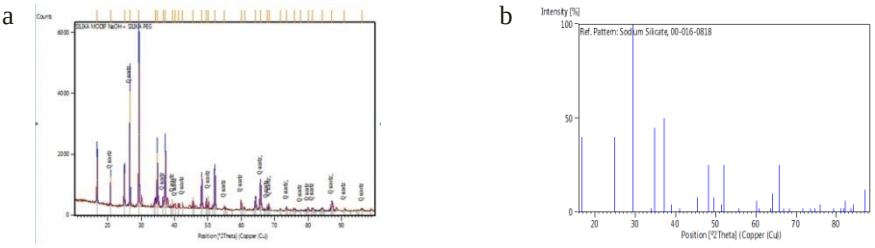

Fig. 1. Spektra XRD; (a) silica is reacted with NaOH; (b) Sodium silicate.

Liaison compound, sodium silicate reacts with PEG that will allow chemically modified silica.

$$Na_2SiO_{3(aq)} + 2PEG_{(s)} \longrightarrow PEG\text{-}SiO_{3(s)}$$

Characterization by FTIR shows the wave number of 2881.91 cm^{-1} is characteristic of PEG 4000. Where, the wave number of 2881.91 $^{cm-1}$ is the CH stretch of PEG. Modification with PEG, the peak seen in the wave number of 2881.91 cm^{-1}. Spectra of PEG shown in figure 2.

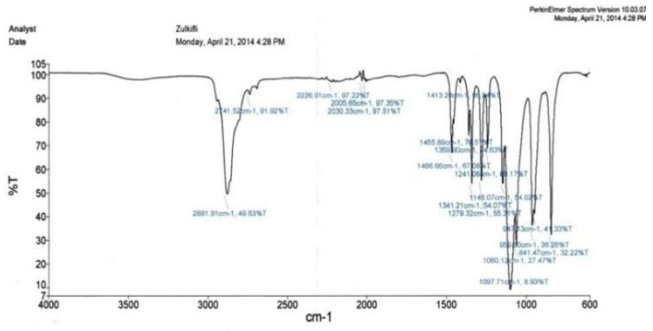

Fig. 2. FTIR spectra of PEG 4000

Furthermore, natural silica modified with PEG 4000 can be seen in Figure 3 where obtained by testing with FTIR most excellent conditions on the addition of 1.5 g of PEG 4000. On the wave number 2884.49 cm^{-1} appeared peaks of PEG 4000.

3.2. Silica absorption test with β carotene before and after modification with PEG 4000

To test the absorption of natural silica used a solution of β Carotene is non-polar and the concentration determined using a formula that is absorbed by the regression and the determination of the absorption capacity of natural silica. Based on the Langmuir isotherm formula, obtained maximum absorption capacity of natural silica before modification is 0.026364355 mg / g. Of the curve C / m to C can be seen that the resulting graph is not a straight line and random spreading. This proves that the natural silica before modification of the Langmuir isotherm equation has not met. Based on the Langmuir isotherm formula, obtained maximum absorption capacity of natural silica after modification is 0.1253604mg / g. Of the curve C / m to C can be seen that the resulting graph is almost a straight evidenced by the regression value 0.946 and this proves that the natural silica after modification meets the Langmuir isotherm equation.

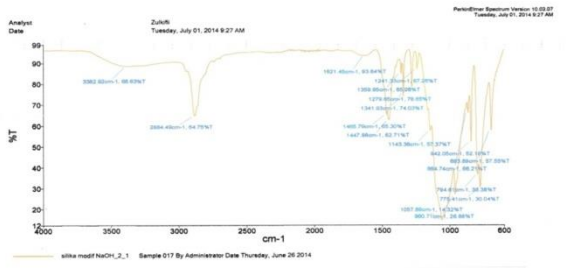

Fig. 3. FTIR spectra of silica modified with PEG 4000

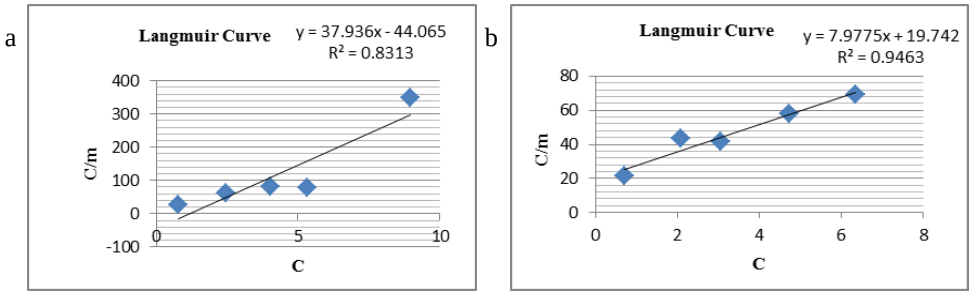

Fig. 4. (a) Langmuir isoterm curve for natural silica before modified with PEG 4000 (b) After modified.

Based on the Langmuir isotherm formula, obtained maximum absorption capacity of natural silica before modification is 0.026364355 mg / g. While obtained after modification of maximum absorption capacity of natural silica is 0.1253604 mg / g. Of the curve C / m to C can be seen that the resulting graph is almost a straight evidenced by the regression value 0.946 and this proves that the natural silica after modification meets the Langmuir isotherm equation.

3.3. Application of natural silica modification on the determination of caffeine by HPLC

Natural silica which has been modified by using PEG 4000 was added to the HPLC column as the stationary phase. Natural silica transformed into a non-polar because the binding properties of PEG 4000 which has been proven to increase the adsorption of β Carotene on natural silica which has been modified, meaning columns formed can function as a chromatographic system in Reversed Phase HPLC, the stationary phase is non-polar while the mobile phase is more polar.

In this application to the analysis of caffeine using a mobile phase of methanol with a flowrate of 0.5 ml / min and UV-Vis detector at a wavelength of 273 nm. The results of the analysis compared to the ODS C18 column chromatography on commercially. The results of the HPLC analysis of the both column can be seen in Figure 5 below.

The analysis showed that the natural silica column made yet gives pretty good results. The peak of 200 ppm caffeine on modified natural silica column width is still 11 minutes compared with the peak of 50 ppm caffeine on ODS C18 for 2 minutes. However, the peak of caffeine in natural silica column has a longer retention time, peak out at 9 minutes compared to the peak of ODS C18 column which came out at minute 3 This suggests that the natural silica modified more non-polar than C18 ODS column. Peak broadening will result in peaks that appear to overlap with peaks of other compounds when the system is used for the

chromatographic separation of a mixture sample. It will be necessary for further research to obtain better results.

4. Conclusion

Based on the research that has been done can be concluded that the natural silica can be modified by using the agent for NaOH to form sodium silicate. The resulting sodium silicate reacted with PEG 4000 to produce silica non-polar. Tests showed that the absorption of beta-carotene can be absorbed 5 times more than before the modification. Modification of natural silica has been used as a filler column chromatography for analysis in reversed-phase systems, but the results are not maximized because of the resulting peak is still wide but is more non-polar than ODS C18 column.

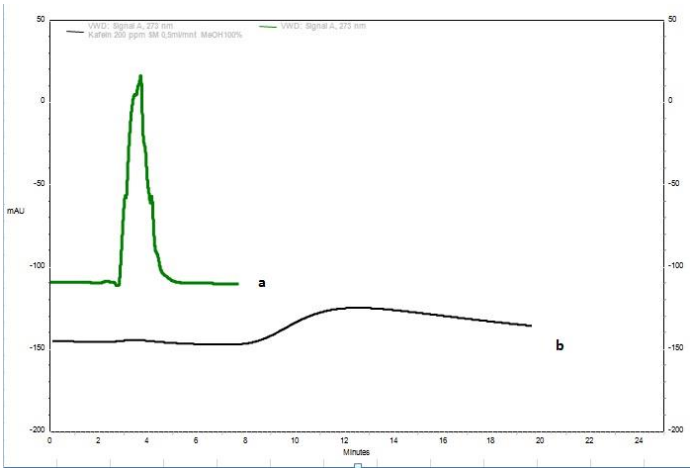

Fig. 5. The results of the analysis of caffeine using HPLC at λ 273 nm, flowrate 0.5 ml/min, mobile phase methanol 100% (a) Using ODS C18 column (b) Using modifed silica column.

Acknowledgements

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Chitin Extraction From Shrimp Shells By Deproteinization Using Papain

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Abstract

Chitin extraction from shrimp shells using papain from *Carica papaya* latex were done. Papain have potentially for catalysis on deproteinization chitin from crustacean shells. Papain were collected form *Carica papaya* latex. Crude papain from *Carica papaya* were mixed with solution of 0.1% v/v ($NaHSO_3$: NaCl, 1:1). Papain were added 100 mL of phosphate buffer pH of 7. The mixture were stirred and cooled for 1 hour at 4°C. Papain were separated by centrifuse at 1500 rpm for 20 minutes. Supernatant were stored at 4°C and papain were obtained from *Carica papaya* latex. Deproteinization of chitin were done by 10 to 50 % of papain supernatant on phosphate buffer solution at pH 7. Deproteinization of chitin were conducted for 3 to 5 hours at 50ºC. Demineralized with lactic acid for 3 hours at 40°C. Depigmentation of chitin were used chloroform, methanol and water (1: 2: 4, v / v). Chitin obtained were filtered and washed using distilled water. Chitin were dried for 12 hours at 65°C. Chitin extraction by papain have more higher deacetylation degree and more higher relative crystallinity than chitin extraction by *Lactobacillus plantarum*. Deacetylation degree of chitin from papain increase significantly up to 55 %. The results showed that papain have potentially for hydrolysis of protein to provided chitin. Deproteinization of chitin by 30 % of papain have more higher physicochemical properties than 10 % and 50 % of papain.

Keywords: papain, chitin, deproteinization, physicochemical properties

1. Introduction

Chitin extraction from shrimp shells by enzymatic process be a concern on some study. Traditionally chitin extraction by chemical treatment using NaOH for deproteinization and HCl for demineralization is more expensive and environmentally unfriendly[1]. Chitin from enzymatic and biological process have more higher quality than chemical process and have homogeneousness structure. Chitin can be used for medical and pharmaceutical applications. Chitin have some biological properties such as biocompatibility, biodegradability, non toxicity, and analgesic, antitumor, hemostatic, hypocholesterolemic, antimicrobian, and antioxidant[2].

Papain have potentially for catalysis on deproteinization chitin from crustacean shells. Papain form *Carica papaya* latex contain protease enzymes. Catalytic activity of protease enzyme due to sulfihydryl groups. Papain form *Carica papaya* latex have 0.76 mol sulfihydryl groups per mol papain[3]. The study reported chitin have produced by deproteinization by 2 to 6 % of papain[4]. Deacetyllation degree of chitin from papain more higher than chemical method. Hydrolysis of protein on chitin structure need optimum concentration for losing protein content. Papain as protease enzyme for hydrolysis amino acid to produce chitin.

This research study on chitin extraction from shrimp shells by deproteinization using 30 to 50 % of papain and study of physicochemical properties. Preparation of protease enzyme with procedures for extraction crude papain[5]. Deproteinization of chitin using papain supernatant on phosphate buffer solution at pH 7. Demineralized of chitin by lactic acid[6] and depigmentation of chitin using organic solvent[1]. The control activity of papain with biological method using *Lactobacillus plantarum* on liquid MRS-B medium[7,8].

The physicochemical properties study by infrared spectrum for characterization and determine deacetyllation degree of chitin using baseline method with Baxter equation[9,10]. Determination of molecular weight using viscometer[10,11,12.] Deacetyllation degree and molecular weight as important parameter that affect properties and application. The success of extraction is influenced by the parameters. Crystallinity of chitin determine by X-ray diffraction[2,13] and moisture and ash content by gravimetric method and also protein content by titration method. This research is important to be an alternative method for extraction of chitin by enzymatic methods to reduce the use chemicals.

2. Materials and Methods

Shrimp shells

The shrimp shells obtained from shrimp suppliers in Yogyakarta, Indonesia. The shells were separated from the head, legs and other residual. Shrimp shells are washed with water and dried at 65°C for 48 hours. The shells milled to obtain a powder of 100 mesh sieve.

Preparation of papain from *Carica papaya* latex

Papain from papaya (*Carica papaya*) was obtained from the fruit sap. Preparation of papain follow procedures[5]. Papaya latex that has been was obtained is mixed with a solution of 0.1% v / v ($NaHSO_3$: NaCl, 1:1). The mixture was stirred until smooth and added 100 mL of phosphate buffer solution at pH of 7. The mixture cooled at 4 °C for 1 hour and then centrifuged at 1500 rpm for 20 minutes. The supernatant of papain was collected and stored at 4 °C.

Deproteinization of chitin by papain

The 25 g of shell powder put into erlenmeyer flask and added 50 mL of supernatant solution on phosphate buffer solution at pH of 7. Deproteinization of chitin was carried out by 10, 30 and 50 % of papain supernatant on phosphate buffer solution at pH 7. Deproteinization of chitin were conducted for 3 to 5 hours at 50ºC. After that, continued heater for 30 minutes at 80ºC. Filtered the results of chitin, washed using distilled water and dried for 12 hours at 65°C. Demineralized of chitin by lactic acid for 3 hours at 40°C[6]. Filtered chitin, washed and dried for 12 hours at 65°C. Depigmentation of chitin were used chloroform, methanol and water[1]. Filtered, washed using distilled water and dried for 12 hours at 65°C.

Preparation of inoculation *Lactobacillus plantarum*

Lactobacillus plantarum culture was purchased from Microbiology Laboratory PAU Gadjah Mada University. Inoculation of *Lactobacillus plantarum* on liquid MRS-B medium[7,8]. The de Mann Rogose Sharpe-Broth (MRs-B) medium was used for growing *Lactobacillus plantarum.* The medium containing 1 % of MRS-B and 2 % of glucose. The medium was autoclaved at 121°C and 101 kPa for 20 minutes. The inoculated culture was cultivated in incubator shaker.

Deproteinization of chitin by *Lactobacillus plantarum*

The procedur of deproteinization of chitin by *Lactobacillus plantarum* followed Khorami et al. (2012). The 20 g of shell powder put into fermentation flask and 10 g of glucose, 10 mL of phosphate buffer solution at pH of 7 and 500 mL of distilled water. Autoclaved at 121°C and 101 kPa for 20 minutes. Cooled and added culture of *Lactobacillus plantarum*. Inoculated in incubator shaker for 6 day at 30°C. Filter dan washed using distilled water. Dried chitin for 12 hours at 65°C.

The study of physicochemical properties

Characterization of chitin carried out with infrared spectrophotometer. Infrared spectrum of chitin were measured from KBr pellets by a Shimadzu FT-IR/8201 PC spectrophotometer. Deacetylation degree of chitin was determined by infrared spectrum at wave number between 4000 to 400 cm^{-1}. Deacetylation degree of chitin calculated by baseline method using Baxter equation[9,10].

Determination of molecular weight using viscometer[9,11,12]. Dissolved chitin on 0.1 M of acetic acid and 0.2 M of sodium chloride. Chitin solution put into Ostwald viscometer. Determined intrinsic viscosity for 0.025 to 0.200 % of chitin solution. Calculated molecular weight of chitin by Huggins and Mark-Houwink equation. Other physicochemical properties determined by X-ray diffraction for study increased crystallinity of chitin[2,13]. Determined of moisture and ash content by gravimetric method and protein content by titration method.

3. Results and Discussions

Deproteinization of chitin by papain

Papain have potentially for hydrolysis of protein from chitin structure. Sulfihydryl groups from papain[3] have active sites for hydrolysis of protein. Chitin extraction from shrimp shells by deproteinization using papain were done. Figure 1. shows specific infra red spectrum of chitin from deproteinization by papain. Infra red spectrum of chitin from 10 %, 30 % and 50 % of papain supernatant have characteristic spectrum for chitin. Base on Figure 1. vibration at 3446 cm^{-1}, characteristic of aliphatic –OH on glucosamine ring[9]. The weak band at 2983 cm^{-1} indicates stretching vibration of C-H. The strong band at 1643 cm^{-1} due to C=O stretching vibration and also peak at 1126 cm^{-1} shows C-O stretching vibration, supported peak at 3271, 3109 and 1627 cm^{-1} shows bending vibration N-H for N-acetyl[10].

Infra red absorption band of chitin describe similar peak of chitin extraction from biological method by *Lactobacillus plantarum*. Characteristic peak at 3448 cm^{-1} indicates stretching vibration-OH. Band at 2931 cm^{-1} shows symmetrical stretching of C-H, peak at 1658 cm^{-1} indicates amides group. Spectrum at 1558 and 1026 cm^{-1} show bending vibration in amino group and C-O group in amide[7].

Deproteinization of chitin by 30 % of papain gives intensity of the absorption approach biological method. Absorption strength of chitin at 3446 cm^{-1} for aliphatic –OH on glucosamine ring shows optimum concentration for deproteinization of chitin. Hydrolysis of protein on chitin structure optimum by 30 % of papain. Deproteinization of chitin have infra red spectrum approach infra red spectrum of chitin by *Lactobacillus plantarum.*

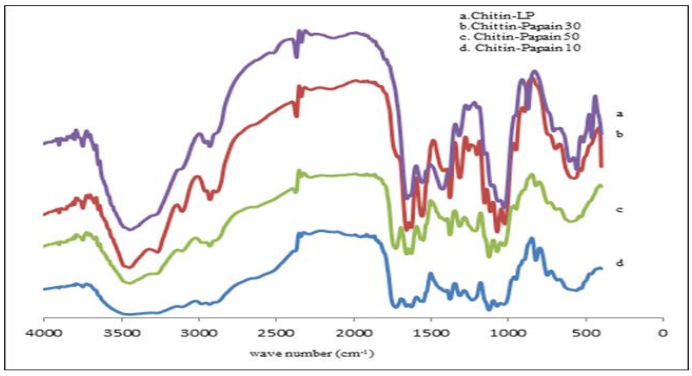

Figure 1. Infra red spectrum of deproteinization chitin by (a) Lactobacillus plantarum (b) 30 % of papain (c) 50 % of papain (d) 10 % of papain

The study of physicochemical properties

Table 1. show physicochemical properties of chitin. Deproteinization of chitin by 30 % of papain have higher physicochemical properties than deproteinization of chitin by 10 % and 50 % of papain. Deproteinization of chitin by 30 % of papain have 3 deacetylation degree of 12.61 %. Deacetylation degree of chitin form papain more higher than biological method. Table 1. deacetylation degree of chitin by *Lactobacillus plantarum* under bellow 10 %. The researcher previously reported that deacetylation degree of chitin by *Lactobacillus plantarum* until 45 %[7]. Biological method for chitin extraction depending pH, substrate utilization, cell growth, time, and temperature. Physicochemical properties from *Lactobacillus plantarum* more lower than previous research[7]. The data as a control for comparation deprotenization by papain.

The moisture content for 30 % of papain under bellow 10 % and 1 %. Difference from 10 % and 50 % of papain, its have moisture content just over 10 %. Deproteinization chitin by papain have low ash and protein contents. Molecular weight of chitin optimum for deproteinization by 30 % of papain, more higher than 10 % and 50 % of papain or *Lactobacillus plantarum.* Deproteinization using 30 % of papain be able to produced which high molecular weight of 975.99 Da. The investigation of physicochemical properties give information that chitin extraction was done by deproteinization with papain as protease enzyme. Sulfihydryl groups from papain[3] have active sites for hydrolysis of protein to provide chitin with more higher physicochemical properties than biological method.

Table 1. Physicochemical properties of chitin

Parameters	Deproteinization of Chitin by			
	Lactobacillus plantarum	10% of Papain	30% of Papain	50% of Papain
Moisture (%)	3.30	28.2	5.42	16.56
Ash (%)	26.29	2.13	0.83	1.9
Protein (%)	22.28	3.95	3.51	2.3
Deacetylation Degree (%)	6.94	10.88	12.61	8.4
Moleculer Weight (Da)	174.02	185.21	975.99	352.87

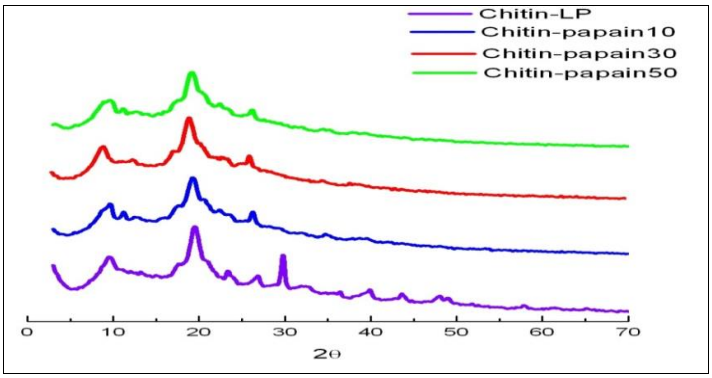

Figure 2. XRD Pattern of deproteinization chitin by (a) *Lactobacillus plantarum* (b) 30 % of papain (c) 50 % of papain (d) 10 % of papain

Figure 2. shows XRD pattern of chitin from biological deproteinization method by *Lactobacillus plantarum* and enzymatic deproteinization method by 10 %, 30 % and 50 % of papain. The WRD pattern of chitin from deproteinization by 30 % of papain have peak similar to chitin from *Lactobacillus plantarum.* The XRD pattern of chitin from 30 % of papain describes the significant characteristic peaks at $2\theta = 19.08^{o}$ (d = 4.6460 Å), $2\theta = 8.88^{o}$ (d = 9.9455 Å), $2\theta = 20.34^{o}$ (d = 4.3626 Å), $2\theta = 26.11^{o}$ (d = 3.4100 Å), and $2\theta = 17.22^{o}$ (d = 5.1455 Å). The relative peak intensity indicates increase relative crystallinity of chitin. The 30 % of papain have high physicochemical properties, crystallinity of chitin more lower than deproteinization by 10 % and 50 %. However, 30 % of papain have more higher relative crystallinity than *Lactobacillus plantarum*. Hydrolysis of protein by papain give rise relative crystallinity of chitin, it is optimum at 10 % of papain. Therefore need further experience to obtain optimum results. Control were used wherever possible using optimum conditions to obtain data which similar with previous research.

Conclusion

Papain have potentially for deproteinization of chitin from shrimp shells. The results give physicochemical properties more higher than biological method using *Lactobacillus plantarum*. Chitin extraction by papain have deacetylation degree and molecular weight at using 30 % of papain. Deproteinization of chitin by papain have more higher relative crystallinity than deproteinization by *Lactobacillus plantarum*.

Acknowledgements

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Uncertainty Measurement in the Analysis of Wastewater Chemical Oxygen Demand at Integrated Laboratory of Islamic University of Indonesia

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Abstract

The Uncertainty of Chemical Oxygen Demand (COD) in wastewater from integrated laboratory of Islamic University of Indonesia has been conducted. Wastewater derived from lecturer or student research and practice activity at laboratory. COD is one of parameters which is calculated for evaluation prior to waste disposal. Standard method of SNI 6989.2:2009 was used to COD determination with analysis principle is organic compound oxidized by $K_2Cr_2O_7$ with making of Cr^{3+} green color. Stages for calculating uncertainty is determining standard uncertainty, combined uncertainty and expanded uncertainty. The results of COD concentration in wastewater was 267.5 mg/L with an uncertainty of 37.35 mg /L. Analytical reports for COD analysis was 267.5 ± 37.45 mg/L.

Keywords: Uncertainty, COD, wastewater, organic compound, SNI

1. Introduction

Wastewater is a mixture of water with pollutants carried by water, either dissolved or suspended in a state of waste from domestic sources (office buildings, housing, and trading), industrial sources, and at some time will be mixed with ground water, surface water or rain water. The quality of the wastewater showed the specification of the amount of waste that measured pollutant content in wastewater. The content of pollutants in the wastewater consists of several parameters. Oxygen demand is the need molecular oxygen to meet thee needs of biological and chemical processes in water or wastewater[1]. Chemical Oxygen Demand (COD) parameters were most often tested to determining wastewaters quality and it is commonly used to inderectly used measure the amount of organic compounds in water.

The chemical oxygen demand (COD) determination provides a measure of the oxygen equivalent of that portion of the organic matter that is suscepible to oxidation by a strong chemical oxidant[2]. It is the oxidation of organic matter or organic compounds by potassium dichromate as chemical oxidant[2,3]. The COD determination was conducted using a closed reflux by UV-Vis spectrophotometer[3,4]. Oxidation of organic compounds using potassium dichromate can be presented the following:

$$C_aH_bO_c + Cr_2O_7^{2-} + H^+ \rightarrow CO_2 + H_2O + Cr^{3+}$$

The green color of Cr^{3+} ions is then measured absorbance using UV-Vis spectrophotometer in the visible region. These reactions need to the heating and also the addition of silver sulphate catalyst to accelerate the reaction. Chloride can interfere because it contributes oxidized by potassium bichromate solution according to the following reaction:

$$6Cl^- + Cr_2O_7^{2-} + 14H^+ \rightarrow 3\,Cl_2 + 2\,Cr^{3+} + 7H_2O$$

Addition of mercury chloride sulfate can reducing interference fit of the following chemical reactions:

$$Hg^{2+} + 2\,Cl^- \rightarrow HgCl_2$$

One of the most important things in the determination COD which measures the value of uncertainty. Uncertainty measurement is parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand[5].
Structure

2. Material and Method

Wastewater samples was taken from wastewater reservoirs in the Integrated Laboratory of Islamic University of Indonesia. Standard solution to determination of COD used pottasium hydrogen phtalat with

concentration variations were 100 until 900 mg l^{-1}. Wastewater samples and standard solutions were digested by potassium dichromate ($K_2Cr_2O_7$) anhydrous and then added silver sulfate (Ag_2SO_4) anhydrous and mercury sulfate ($HgSO_4$) as catalisators. Standard solution and the wastewater samples was measured with UV Vis spectrophotometer. There are three parameters that determine as % recovery, precision and uncertainty.

3. Result and Discussion

Initial stages of the COD determination in water samples using UV Vis spectrophotometer is by making standard solution to create calibration curve with concentrations 100 until 900 mg/L respectively. Standard solution was measured using UV Vis spectrophotometer which has been optimized at 603 nm. The result of standard solution absorbance can be seen in Table 1.

Table 1. Absorbance of KHP standard solution

No	Solution standard oh KHP (mg/L)	Absorbance
1	0	0,000
2	99,96	0,032
3	199,92	0,057
4	299,88	0,086
5	399,84	0,115
6	499,8	0,149
7	599,76	0,177
8	699,72	0,200
9	799,68	0,231
10	899,64	0,263

Based on Table 1 shows that there is a correlation between the concentration and the absorbance of COD standard solutions. This is evidenced by the higher concentration of the standard solution is also higher absorbance. This correlation can be seen obviously when the relationship between concentration and absorbance of lead standard solutions was made in the graph absorbance vs. concentration, where the concentration of the standard solution as the x-axis and the absorbance of standard solutions of lead as the y-axis. The following is a graph of absorbance vs. concentration relationship of COD standard solution.

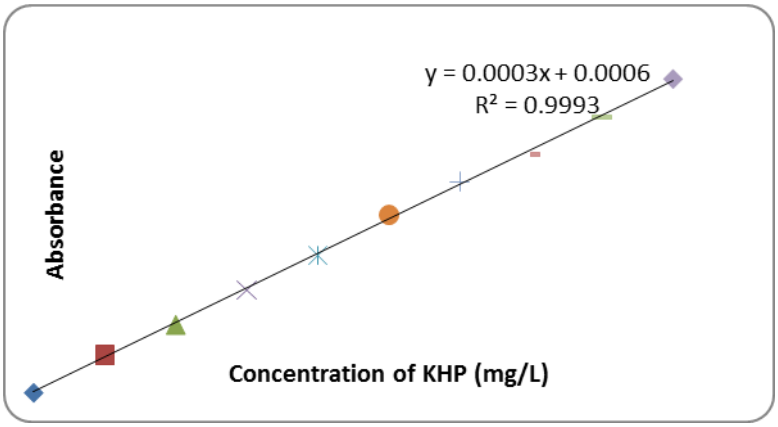

Figure 1. Relationship between absorbance and KHP concentrations

Linear equation of the calibration curve used to determine the concentration of lead in filter layer water and has a correlation coefficient (r) of 0.999. Correlation coefficient (r) is close to 1 on the calibration curve showed a correlation between concentration and absorbance. This is in accordance with the Lambert - Beer laws namely A = abc, where the value of absorbance (A) is proportional to the concentration (c)[6]. Calibration data also useful to obtain an estimate of the uncertainty associated with the predicted concentration values for test samples[7]. Absorbance measurements also done on wastewater samples with the same treatment as the standard solution and the absorbance data obtained as shown in Table 2.

The average of COD measurement result at Integrated Laboratory wastewater of Islamic University of Indonesia was 267,5 mg/L. While for the results % recovery was 97.775%. Precision obtained from eight

(8) samples with the same treatment. Final data from these observations expressed in terms of concentration (mg / L) so that the value of precision described in terms of% CV Horwitz obtained by the formula:
% CV Horwitz = $2^{1-0,5\log}$

Table 2. sample absorbance at a wavelength of 603 nm

No	Sample	Absorbance
1	Sample 1	0,079
2	Sample 2	0,081
3	Sample 3	0,082
4	Sample 4	0,078
5	Sample 5	0,079
6	Sample 6	0,080
7	Sample 7	0,082
8	Sample 8	0,081

C

Determination of Measurement Uncertainty

There are 4 steps at the Process of measurement uncertainty estimation .The steps involved are specify measurand, identify uncertainty sources, quantify uncertainty components and calculate combined uncertainty[8]. To simplify the determine the uncertainty created schema analysis of COD at wastewater samples as shown in Fig 2.

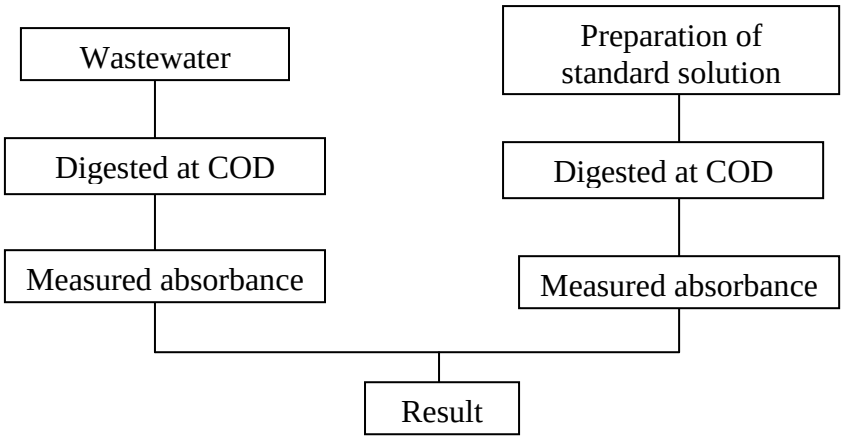

Fig 2. Scheme analysis of COD at wastewater

Determination of the COD at wastewater refers to the Lambert -Beer Law A = abc where a and b is a constant so that the absorbance value (A) is proportional to the concentration (C). If made in a linear line so obtained equation y = bx ± c, where y and x, respectively absorbance and concentration, while b and c are the slope and intercept values. So that the formula used to determine the concentration of COD in the sample can use the equation x '= x. df, so from the equation shows the concentration as a source of uncertainty. The other components is the source of uncertainty is the mass, volume, COD reactor and purity of KHP. The sources of uncertainty of these measurements can be made in a fishbone diagram as shown in figure 3

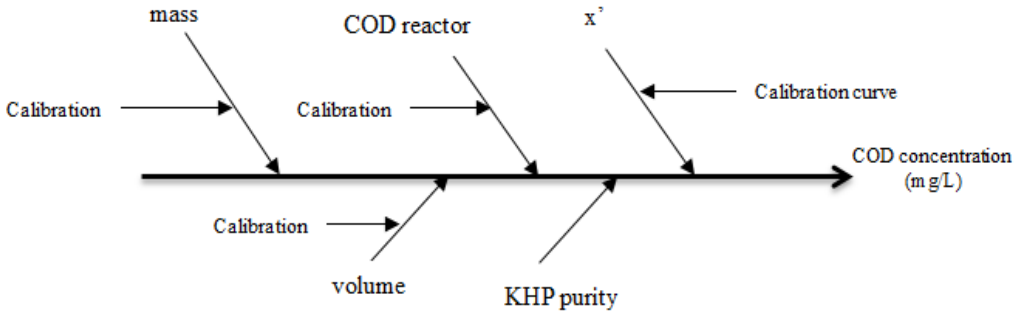

Fig 3. Fishbone diagram to determination of COD concentration

Determination of standard uncertainty

a. Volume uncertainty (μ_V)

The uncertainty of the volume is influenced by several sources that flask 10 mL, 50 mL, and 100 mL. Factors affecting the uncertainty of which flask 10 mL, 50 mL, and 100 mL as of the calibration, the effects of expansion, and the pipette used in each flask. Estimation of uncertainty flask 10 mL, 50 mL, and 100 mL was 0.0173 mL, 0.1733 mL, and 0.109 mL. The uncertainty of the volume is obtained by combining the uncertainties of each flask and found at 0.205 mL.

b. Mass uncertainty (μ_m)

Analytical balance was used for weighing 204 Mettler Toledo AL type that has been calibrated. Analytical balance calibration results provide information which of value uncertainty of limit of performance (LOP) and sensitivity whose value is 0.0036 grams and 0.0001 grams respectively. The calculation of the uncertainty of balance is calculated by dividing the value of the LOP and sensitivity uncertainties by coverage factor of is 2 with which 95% confidence interval. The results obtained from the mass uncertainty value is 0.0018 grams

c. COD reactor uncertainty (μ_T)

COD reactor used was WTW CR 2200 type has been calibrated. Results COD reactor calibration uncertainty provide value of 1.611 ^oC. Estimation of uncertainty sourced from COD reactor was calculated by dividing the value of the calibration certificate uncertainty by coverage factor of 2 with a 95% confidence interval. The result of uncertainty of COD reactor obtained at 0.8055 ^oC.

d. Sample concentration uncertainty (μ_x)

To determine the concentration (x') uncertainty was calculated from the calibration curve ($S_{y/x}$) by the formula (2) and calculation of the concentration uncertainties ($S_{x)}$ determined by the formula (3)

(2) $$S_{y/x} = \sqrt{\frac{\sum(Y-Yi)^2}{}}$$

(3) $$Sx = \frac{Sy/x}{b}\sqrt{\frac{n-2}{1+\frac{1}{n}+\frac{(Y-Yi)}{b^2\,\Sigma(x-xi)}}}$$

Uncertainty of sample concentration value found for 18.718 mg / L.

e. HP purity uncertainty (μ_p)

Information of KHP purity obtanined from label of material container. Uncertainty estimation of KHP purity refers to rectangular distribution with degrees of freedom $\sqrt{3}$ and the resulting in value by 0.000288.

Determination of combined and expanded uncertainty

The combined uncertainty is the overall combined standard uncertainty. Unit standard uncertainty is different so the combined uncertainty is determined by using the formula (4).

$$\frac{\mu_C}{} = \sqrt{\left(\frac{\mu_m}{}\right)^2 + \left(\frac{\mu_V}{}\right)^2 + \left(\frac{\mu_x}{}\right)^2 + \left(\frac{\mu_T}{}\right)^2 + \left(\frac{\mu_P}{}\right)^2} \quad (4)$$

The result of combined uncertainty and expanded uncertainty by coverage factor of 2 with a 95% confidence interval are 18,725 mg/L and and 37, 45 mg/L respectively.

Conclusion

The results of the determination of Chemical Oxygen Demand (COD) with closed reflux[9] at Integrated Laboratory wastewater tank of Islamic University of Indonesia obtained concentration,% recovery and the measurement uncertainty are 267.5 mg / L, 97.775% and 37.45 mg / L respectively. Precision determination of COD is expressed good because the value of the % CV analysis is smaller than the % CV Horwitz.

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Preparation of Composite Apatite $La_{9.33}Si_6O_{26}$ (LSO) - $Zr_{0.85}Y_{0.15}O_{1.925}$ (YSZ)

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Abstract

A great challenge to reduce high operating temperature of SOFC to intermediae temperature, IT (500-750 ºC), is the development of solid electrolyte materials with high ionic conductivity IT range. In response to this challenge, here we report a novel composite material $La_{9.33}Si_6O_{26}$ (LSO)-$Zr_{0.85}Y_{0.15}O_{1.925}$ (YSZ). LSO-YSZ composite synthesis was carried out by combining LSO with commercial YSZ (9:1, 8:2, 7:3) using hydrothermal method. In order to get dense pellet, all of the product were sintered at 1450 °C for 3 hours. X-ray diffraction of the entire pellets show typical both of LSO and YSZ pattern which indicate that the composite was succesfully prepared.

Keywords: composites, hydrothermal, IT-SOFC, LSO, YSZ

1. Introduction

The increasing energy demand is one of the motivations to find an alternative energy sources. In addition, global warming and pollution caused by the exhaust gas from fossil fuels combustion has driven the research and development of solid oxide fuel cells (SOFCs) during the last three decade [1]. SOFCs are now of great potential applications as an alternative for internal combustion engines due to their high efficiency and environmentally friendly [2].The advantages of lowering the operation temperature of SOFCs have attracted great interest worldwide. The importance one is to find a new electrolyte materials [3].

In general, there are two possible ways to improve the conductivity of the electrolyte for intermediate temperature SOFC applications. The first is to find a new type of material of oxygen-ion conductor and the second is to modify the microstructure of oxygen ion conductor material that already exists. The first way is certainly more difficult to perform, because it is not easy to get a new compound that has a high and stable conductivity values at intermediate operating temperatures (500-700 °C) [1].

Apatite-type lanthanum silicate oxide materials general formula is $La_{9.33+x}(SiO_4)_6O_{2+3x/2}$ and consist of an isolated SiO_4 tetrahedral. The extra oxygen atoms occupy channels running through the structure that are responsible for the high oxygen ion conduction [4]. In one of our previous studies [5] we have successfully synthesized the oxide apatite silicate type ($La_{9,33}Si_6O_{26}$ / LSO) using hydrothermal method with La_2O_3 and Na_2SiO_3 as reactant in 3 M NaOH solution at 240°C for 72 hours. In the study, LSO were sintered at 1600°C and 500°C operating temperature and the conductivity value reached 2.95 x 10^{-4} Scm^{-1}. The conductivity value was still lower than the requirement for an electrolyte that can operate on intermediate temperature. In addition, the nature of the apatite is unstable and furthermore the homogeneity and density was not solid membrane, and therefore it is not appropriate for solid electrolyte fuel cells. YSZ electrolyte is also an electrolyte with high conductivity, good mechanical properties, and has a conductivity of 0.16 S cm^{-1} at 1000°C or 0.027 S cm^{-1} at 800 °C[1]. Oxide material that is well known to have a good oxide ion conductor is the YSZ, but only at high temperature. At lower temperatures, the ionic conductivity of YSZ is much lower than that of ceria-based electrolytes such as gadolinia-doped ceria (GDC) or lanthanum gallate-based electrolytes [6].

One of the efforts to obtain highly conductive electrolyte material is to synthesize composites, such as performed by previous researchers that synthesize a composite between the YSZ and the LSO, with the former being higher in proportion [1]. Composition, dopant concentration, and processing conditions can change the properties of grains and grain boundaries and also related to an electrical properties [6].Therefore, this study focused on the formation of the composite electrolyte with composition variation of LSO to YSZ (9: 1, 8: 2, 7: 3). In addition, based on literatures, preparation of LSO-YSZ composite with ratio LSO to YSZ used in the present study have never been reported. This modification efforts, is expected to improve the quality of the electrolyte for SOFC applications.

2. Experimental

Synthesis of apatite-phase $La_{9.33}Si_6O_{26}$ (LSO)

The compound of apatite-phase $La_{9.33}Si_6O_{26}$ was prepared by hydrothermal method. The starting materials were high purity La_2O_3 (Aldrich, 99.999%), and Na_2SiO_3 (Sigma, 97%) powders. The La_2O_3 powder was precalcinated at 1100ºC for 10 hours in order to achieve complete decarbonation and dehydroxylation. The starting materials were weighed in the appropriate ratios (0.8197 g of La_2O_3 and 0.1953 g of Na_2SiO_3) were mixed and added to the NaOH solution, after which the mixtures were transferred into a stainless steel autoclave (302 AC Bombs Parts 105 mL) until 60 % volume of the autoclave was occupied. After being heated at 240 °C for 3 days, the precipitate was collected by filtration and washed with demineralized water. The resulting powder was dried at 120°C.

Synthesis of LSO-YSZ composite

To prepare the composite materials, three batches with the LSO to YSZ (YSZ; TZ-8YS TOSOH) weight ratios of 90 : 10, 80 : 20 and 70 : 30 were designed (recorded as YSZ-9LSO, YSZ-8LSO andYSZ-7LSO, respectively). According to the weight ratios, LSO powder and YSZ powder were weighted and mixed using by a ball mill for 60 minutes. Three batches mixture powders were pressed under 50 kg/m^{2}to get green pellets with 2 mm in diameter and about 2 mm thickness. The pellets (YSZ-9LSO, YSZ-8LSO andYSZ-7LSO) were sintered at 1450 ºC for 3 hour. The densities of the sintered pellets were calculated using ratio of weight and volume (based on theirs dimension).

Characterization of LSO-YSZ composite

Phase identification was performed by using X-ray diffraction (Bruker D8 Advance using Cu-Ka radiation (λ=1.5406 Å)) at room temperature. Morphology of the calcined powders and sintered pellets were investigated by using scanning electron microscopy (SEM JEOL, JED 2200 series).

3. Results and Discussion

Fig. 1 shows the XRD patterns of the as-calcined powders of YSZ and LSO. Significant peaks for LSO appeared at 2θ = 21-22 º, 31º, and 45º, meanwhile for YSZ at 2θ = 30 º, 35º, 46º, 51º and 60º. These diffraction peaks indicate that YSZ has cubic structure while LSO has hexagonal apatite-type structure. Each of the YSZ and LSO powders shows a pure single phase. These suggest that the composite preparation process was successfully performed.

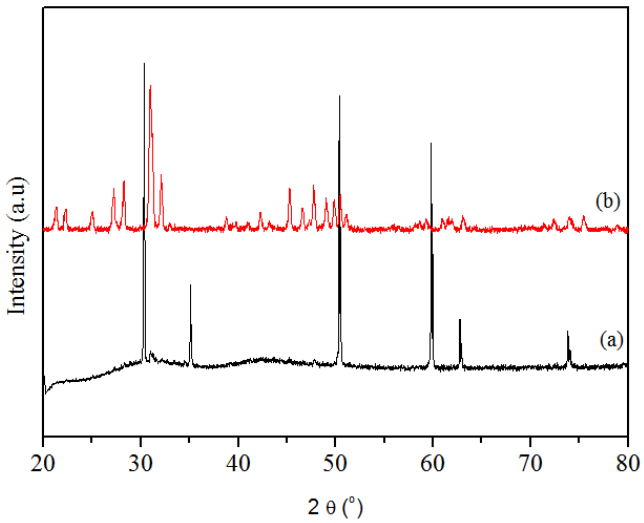

Fig. 1 Diffraction pattern of (a) YSZand (b) LSO resulted by hydrothermal at 230 °C for 3 days.

The diffraction peaks of three sintered ceramics (1450 ºC for 3 hours) of YSZ-9LSO, YSZ-8LSO andYSZ-7LSO exhibit typical YSZ and LSO phases (Fig. 2). The result indicate that YSZ-9LSO, YSZ-8LSO, andYSZ-7LSO composites have been successfully prepared. The structure of the LSO powder, obtained through a hydrothermal process has hexagonal structure and adopts the *P 63/m* space group.

It is easily found that the main phase of each ceramics are hexagonal structure, indeed for the YSZ-9LSO ceramic (small amount of YSZ), the diffraction peaks from the YSZ phase was not clearly observed compare to the other peaks. However, in contrast to the previous report of YSZ-LSO ceramic (small amount of LSO, ratio 96 to 4), diffraction peaks of LSO phase, was not clearly detected [1].

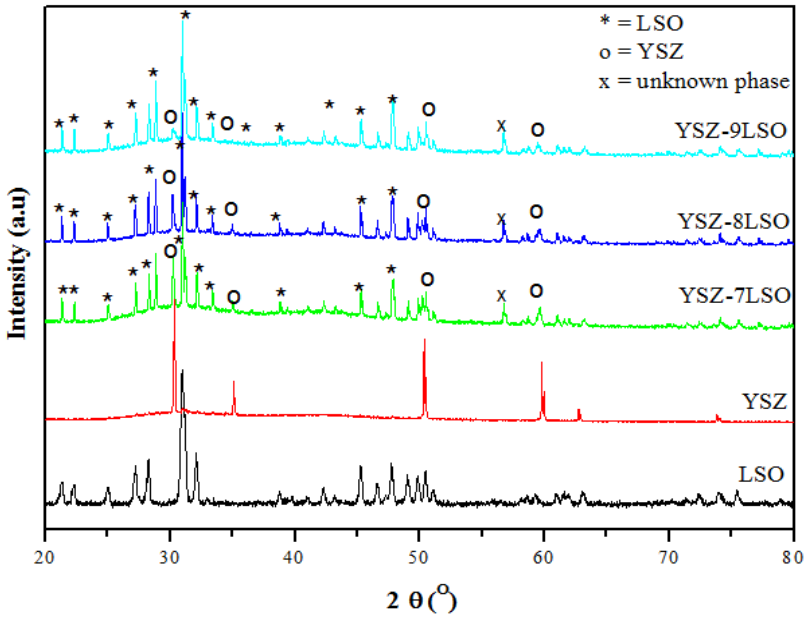

Fig. 2 Diffraction pattern of of LSO, YSZ, three sintered ceramics (1450 ºC for 3 hours) of YSZ-9LSO, YSZ-8LSO and YSZ-7LSO composites, (*) = LSO, (o) = YSZ, and x = unkown phase.

The SEM photograph of sintered YSZ-7LSO and YSZ is presented in Fig. 3. As previously mentioned, the density of composite influence its conductivity. The size of grains was in the range of 2 to 5μm and each grain consists of many sub-grains. The ceramics have two types of grains in different colors, most of them were grey while the rest were white. The chemical composition of the white grains were analyzed by using an energy dispersive X-ray spectrometer (EDS). The EDS combined with the XRD results, suggested that the white grains is mostly belong to the YSZ phase. The YSZ phase locates not only at the grain boundaries but also at grains.

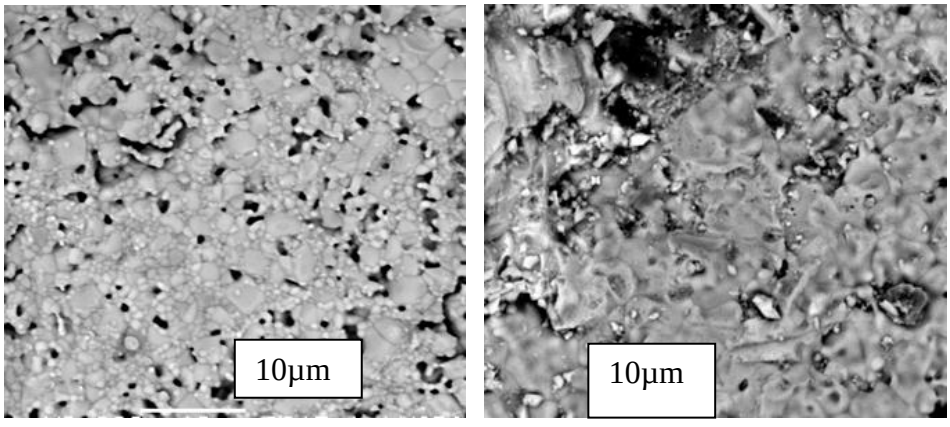

Fig. 3 Microstructure observations of the samples SEM image of the as-sintered (a) YSZ-7LSO and (b) YSZ.

EDS analysis give La/Si ratio of 2.27 and Zr/Y ratio of 8.27, respectively. This ratio is relate to the formula of LSO and YSZ ($La_{9,33}Si_6O_{26}$ - $Zr_{0.85}Y_{0.15}O_{1.925}$), listed in Table 1. It also concluded that LSO-YSZ composite was formed with the composition ratio of the atoms corresponding to the target compound and showed no other atoms that appear significantly in comparison composition element.

Table. 1 Composition of YSZ-7LSO composite

Element	Composition (%)
oxygen	68.804
silicon	6.013
Yttrium	1.238
Zirconium	10.241
Lanthanum	13.704

4. Conclusion

LSO-YSZ composite with composition (9: 1, 8: 2, and 7: 3) was successfully synthesized by using solid state reaction at 1450 °C for 3 hours. The size of grains were in the range of 2 to 5μm and each grain consists of many sub-grains.

Acknowledgements

The authors would like to thank PUPT Project no. 2002/UN6.RKT/KU/2013 (DIKTI) for financial support.

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Characterization of Nickel(II) Coordination Polymers with 2,4,5-triphenyl-1H-imidazole Ligand

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Abstract

In this study, research has been done on polymer complex Ni(II) synthesized using 2,4,5-triphenyl-1H-imidazole ligand together with mono-anionic NCS^- ion ligand. This study aims to obtain polymer complex compound $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$. The process of formation of the polymer complex compound using solvothermal method. The synthesis result obtained solid green color. The synthesized compounds were analyzed using spectroscopic characterization of UV-Vis and Fourier Transform Infra Red (FT-IR), thermal analyses and crystal morphology. From the results of spectroscopic analysis of UV-Vis obtained that the maximum wavelength value was 329 nm. IR spectrum of this compound showed absorption typical vibration metal-ligand appeared in the 320-380 cm^{-1} region: the vibrations of $Ni\text{-}N_{(imidazole)}$ appears at 378 cm^{-1}. Thermal stability of the polymer complex can be achieved up to 300 °C. The resulting crystal imaging using scanning electron microscopy showed reasonably homogeneous.

Keywords: characterization; nickel(II) complex; 2,4,5-triphenyl-1H-imidazole; coordination polymer.

1. Introduction

Over the last decades of investigation efforts were made in the porous solids become a present day challenge, especially in the category of polymer complexes or coordination polymers (metal-organic frameworks) [1]. Properties of polymer complexes can be applied as selective gas storage [2], separation [3], and catalysis [4] or promising magnetic materials. Topologies of coordination polymer are determined by geometry of metal ions, coordination geometries and flexibility of organic ligands, and counter anions [5]. Central atom of Ni (II) has coordination number if it is bonded covalently with ligand. Counter anion, such as NCS^-, NCO^-, and N_3^- in polymer complex can modify form, geometry, stereochemistry of obtained compound. Variation of ligand (for example: Imidazole, pyrazole, triazole and its derivative) can be changed in the synthesis for using in various application [6].

Many researches about coordination polymer have been already advanced, such as polymer complex used as material of hydrogen storage [7]. Polymer complex compound has function for processing of hydrogen adsorption as active site in interaction with hydrogen [8]. IRMOF-3-AMPh is material of hydrogen storage which has big capacity because it has many more phenyl group [9]. Therefore, 2,4,5-triphenyl-1H- imidazole ligand which has three phenyl group can adsorb many more hydrogen. Nickel (II) phosphate has coordinately access site unsaturated in Ni^{2+} which can interact highly with H_2 [10].

Polymer complex compound which can become material of hydrogen storage have micropores characteristic (~2 nm). Besides that, polymer complex has space in nanometer scale and it can form framework. In this research, polymer complex $[Ni(NCS)_2(2,4,5\text{-triphenyl-1H-imidazole})_4]_n$ using ratio molar 1:2:4 was synthesized and it can form coordination compound such as $[Ni(NCS)_2(2\text{-methylpyrazine})_4]_n$ [11 12]. Application of this research, this coordination complex can be used as material of hydrogen storage. Besides that, Ni (II) complex form octahedral compound, in order that it can be characterized easily.

2. Experimentals

2.1. Materials and general methods

2,4,5-triphenyl-1H-imidazole ligand was purchased from Sigma Aldrich; $NiCl_2.6H_2O$, NH_4SCN, DMF were obtained from Merck and all other chemicals used in this experiment were chemical grade. Infrared spectra (4000-375 cm^{-1}) were recorded from KBr disks with a 8400S Shimadzu FT-IR spectrometer. UV-Vis spectra (500-200 nm) were recorded on a Thermo Electron Sci. Inst. UV-Vis Spectrophotometer 335908P-000. Thermal analysis was performed on a Mettler Toledo TGA/DSC 1 Star System under dynamic nitrogen. A heating rate of 10 °C min^{-1} in the range of 30-500 °C was used. For Scanning Electron Microscopy imaging (SEM), the sample was coated with palladium in a nitrogen atmosphere before imaging. Images were taken on an EVO MA10 SEM-Zeiss.

2.2. Preparation of [Ni(NCS)(2,4,5-triphenyl-1H-imidazole)]$_n$

$NiCl_2.6H_2O$, NH_4SCN, and 2,4,5-triphenyl-1*H*-imidazole were diluted in 15 mL DMF using reflux at 70 °C for 3 hours then put in oven at 120°C for 2 day using autoclave bottle. Subsequently, sample was evaporated in vacuum condition so that it can be formed precipitation. The precipitation was washed using methanol and DMF and dried in nitrogen condition.

3. Result and discussion

3.1. Synthesis

Polymer complex compound $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$ were prepared by using solvothermal method in a DMF solution as a solvent which have been reported for $\{[Ni_2(NCS)_4(azpy)_4].EtOH\}_n$ [13], $[Ni_2(NCS)_4(pyridazine)_2(CH_3OH)]_n$ [11], trans-$[Ni(NCS)_4(PpzH)_2]$ [14], and $[Ni(NCS)_2(pyridazine)]_n$ [15]. The compound is relatively unstable (hygroscopic) and can be stored in a closed condition. This polymer complex is insoluble in common organic solvents such as water, methanol and ethanol. Evaporation treatment on a synthesis is not a general method for the preparation of this polymer complex, as it leads to the crystals precipitate obtained after heating in an oven. In this method a DMF solvent is utilized to dissolve the reactants polar and non-polar. When these reactants diffuse into the solvent and meet with each other.

3.2. UV-vis spectrum

UV-visible analysis of polymer complex uses wavelength at 500-200 nm. The green colour of polymer complex is caused electronic transition between energy level which is difference. That transition is appropriate with wavelength of visible ray. Value of $\lambda_{maks\ of}$ polymer complex compound is determinate with dissolving $NiCl_2.6H_2O$; 2,4,5-triphenyl-1H-imidazole and NH_4SCN in DMF. The maximum wavelength of $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$ polymer complex is obtained 334 nm, see Fig.1.

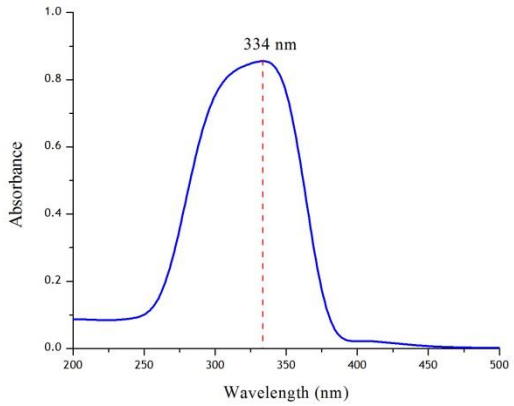

Fig. 1. The maximum wavelength $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$.

Value λ_{maks} of polymer complex compound become a criterion for synthesized complex. Wavelength determination of $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$ polymer complex is done by dissolving polymer complex and $NiCl_2.6H_2O$ in DMF at same concentration then measured at 300-500 nm. Figure 2 shows that maximum wavelength of $NiCl_2.6H_2O$ solution is 420 nm whereas maximum wavelength of solution of $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$ polymer complex is 329 nm.

Shift of maximum wavelength between $NiCl_2.6H_2O$ and $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$ shows that it has already happened polymer complex between Ni(II), NCS^- and 2,4,5-triphenyl-1H-imidazole. The difference of wavelength is caused capacity transfer from ligand to metal so that occurring an electron transition from 2,4,5-triphenyl-1H-imidazole which has higher character of ligand to molecule orbital of Ni(II) which has higher character of metal.

3.3. FT-IR spectroscopy

Used wave number of FT-IR analysis of polymer complex compound is 4000-375 cm^{-1}. The result is used to know function groups through vibration bonding and analyse the interaction between metal and ligand at finger print area under 1400 cm^{-1}. Spectra of 2,4,5-triphenyl-1H-imidazole ligand shows absorption at 1510 cm^{-1} which is related with typical peak of C=N stretching, vibration of wave number at 3434 cm^{-1} for

N-H stretching and vibration of wave number at 2993, 2470, 1638 cm^{-1} for aromatic stretching [16]. Compared with polymer complex of imidazole which has vibration C=N and N-H at 1504, 3398 cm^{-1} respectively whereas vibration of C=C aromatic at 3037, 2449, 1649 cm^{-1}. Thiocyanate (SCN^-) ligand has spectrum vibration of C-N at 840 cm^{-1}, SCN^- bonding coordinated between N atom and Ni at 493 cm^{-1}. Bonding of vibration $Ni\text{-}N_{(imidazol)}$ is around 320-380 cm^{-1} [17], and 400-230 cm^{-1} [18]. FT-IR spectra of $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$ polymer complex shows that vibration Ni-N is at 378 cm^{-1}. The shift and separation of wave number shows that there are group coordination of imidazole and thiocyanate in a complex [18]. FT-IR spectra of this research are shown in Figure 3.

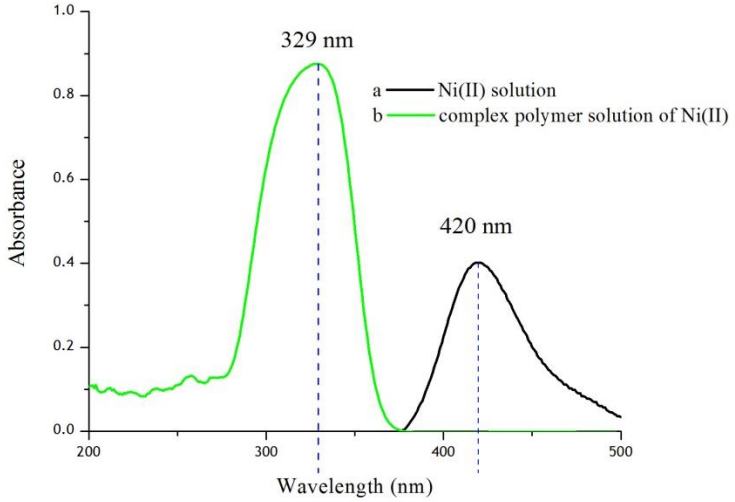

Fig. 2. UV-Vis Spectra: (a) $NiCl_2.6H_2O$ solution; (b) $[Ni(SCN)(2,4,5\text{-trifenil-1}H\text{-imidazol})]_n$ solution.

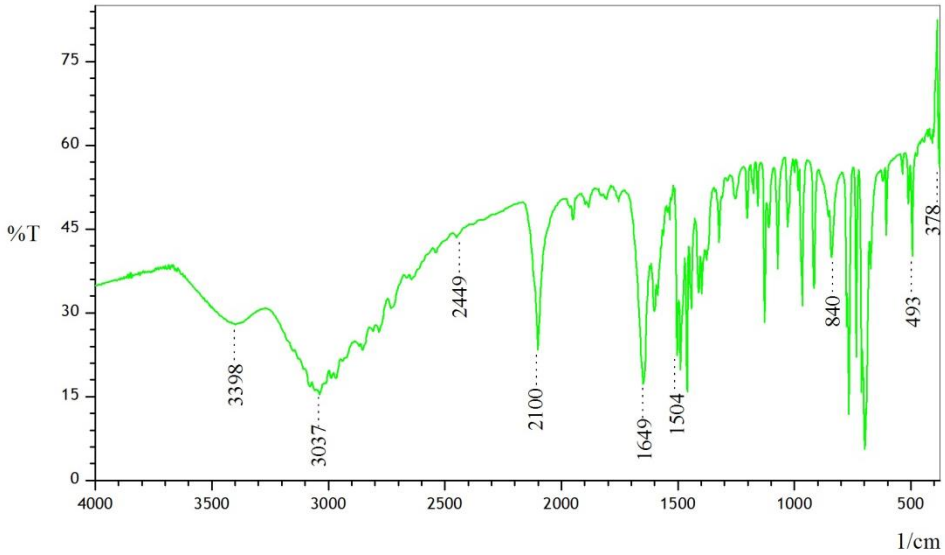

Fig. 3. FT-IR Spectra of $[Ni(NCS)(2,4,5\text{-triphenyl-1H-imidazole})]_n$.

3.4. Thermal analysis properties

Thermal stabilities of $[Ni(SCN)(2,4,5\text{-triphenyl-1}H\text{-imidazole})]_n$ polymer complex used temperature around 500 °C and warming rate around 10 °C/min in nitrogen condition, see Figure 4.

From figure 4, there were 3 peaks of exotermic 50-500 ^oC. First step, weight of sample which decreased around 50-100 ^oC was 37.55%. This step lost crystal water, because it has hygroscopic characteristic and also contained methanol significantly. In the second step, weight of sample decreased 4.75% at 269 ^oC. This step lost DMF molecules. And the third step, weight sample decreased significantly 50.07% at 294-347 ^oC whereby 2,4,5-triphenyl-1*H*-imidazole and organic compound have lost. This polymer complex left NiC at 500 ^oC around 7,62 % (theory calculation is around 5.49%).

3.5. Microstructure imaging

Scanning electron microscopy (SEM) of [Ni(SCN)(2,4,5-triphenyl-1*H*-imidazole)]$_n$ polymer complex used coating with Pd. Figure 5 showed average particle size was 10 μm. Particle size is around 7-12 μm. From that figure, side of each crystal was reasonably homogeneous.

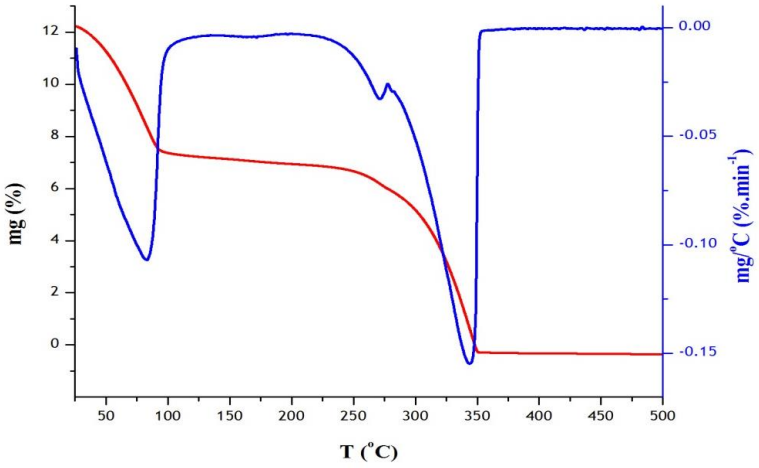

Fig. 4. Curve of thermogravimetry/differential thermal gravimetric (TG-DTG).

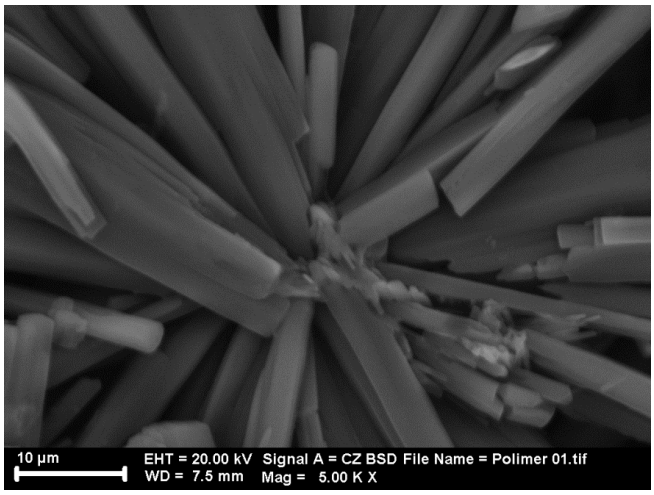

Fig. 5. SEM of [Ni(SCN)(2,4,5-triphenyl-1*H*-imidazole)]$_n$ polymer complex.

4. Conclusion

Polymer complex of the 2,4,5-triphenyl-1H-imidazole ligand with nickel(II) ion have been successfully synthesized and characterization by UV-visible absorption, infrared spectroscopy, thermal analysis and scanning electron microscopy. Comparison of molar ratio Ni(II) : 2,4,5-triphenyl-1H-imidazole : NH_4SCN = 1 : 4 : 2 result λ_{maks} 329 nm whereas value of λ_{maks} Ni(II) 420 nm which indicated, polymer complex have formed. Result of FT-IR analysis can prove that vibration of nickel metal to 2,4,5-triphenyl-1H-imidazole ligand. Thermal stability of anhydrous polymer complex is achieved up to 300 °C.

Acknowledgements

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Kinetics of Dehydrochlorination Process Reaction Between Dichloropropanol and Sodium Hydroxide

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Abstract

Conventionally epichlorohydrin (EPCH) is made by chlorohydrination of allyl chloride obtained from high-temperature chlorination of propylene. Unfortunately, the method produces a large amount of chlorinated by-products and consumes loads of energy due to high operating temperature. An alternative approach for the production of epichlorohydrin would be to use glycerol from biodiesel production plant. Epichlorohydrin is made by dehydrochlorination of 1,3-dichloropropanol (1,3-DCP) obtained from hydrochlorination of glycerol with aqueous hydrogen chloride. Experimental study on dehydrochlorination reaction was carried out under operating temperatures ranged from 50 to 80^{o}C and reactant molar ratio from 1:1 to 1:12. The optimal reaction conditions were: temperature, 70^{o}C; reactant molar ratio, 1:5 and duration, 10 minutes. Kinetics of dehydrochlorination was then studied in the presence of excess sodium hydroxide solution. The reaction kinetics conformed to pseudo first order with respect to dichloropropanol concentration. The activation energy of the reaction was determined at 38.8 kJ/mol and the pre-exponential factor A was at 1.62 x 10^{7} sec^{-1}. Quantitative analyses of the reaction products were performed using GC-MS.

Keywords: Epichlorohydrin, Dichloropropanol, Dehydrochlorination, Kinetics study, Muriatic acid

1. Introduction

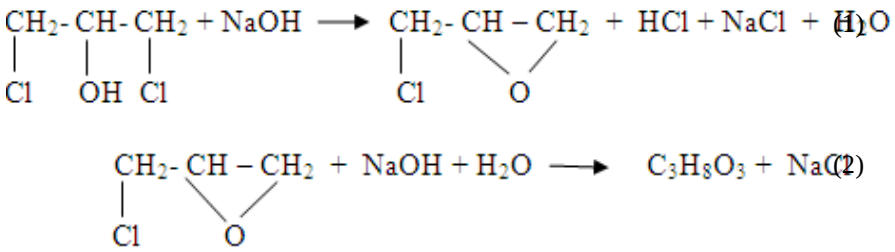
Epichlorohydrin, an organochlorine compound and an epoxide, is commonly used as a raw material in producing several synthetic materials, including epoxy, phenoxy, and polyamide resins, polyether rubber, synthetic glycerin, glycidyl ethers, polythiols, elastomers, surfactants, plasticizers, dyestuffs, pharmaceutical products, oil emulsifiers, lubricants, and adhesives. Other applications of epichlorohydrin are solvent for resins, gums, cellulose, esters, paints, and lacquers. Epichlorohydrin is also widely used as a stabilizer in chlorine-containing substances such as rubber, pesticide formulations, and solvents [1]. Conventionally [2], epichlorohydrin is made by chlorohydrination of allyl chloride, which is obtained by high-temperature chlorination of propylene [3]. Unfortunately, the method has some drawbacks such as formation of a large amount of chlorinated by-product and high-energy consumption because of high operating temperature [4]. Today, the glycerol is in abundance as by-product of biodiesel, thus has provided an opportunity to synthesize epichlorohydrin from glycerol by adding some basic solution. The two-step process, which utilizes glycerol as a feedstock was firstly developed by Solvay's, called Epicerol® technology [5-6]. The first step is the direct hydrochlorination process between glycerol and hydrochloric acid to produce dichloropropanol. The second step is the dehydrochlorination of dichloropropanol obtained from the first step with basic solution, generating the final product, epichlorohydrin [7]. Glycerol used in the Solvay process was the byproduct from biodiesel production from rapeseed oil.

Kinetics parameters for the reactions using sodium hydroxide is still lacking but crucial for the design of dehydrochlorination reactor. However, Carra et al. [8] reported the dehydrochlorination kinetics using calcium hydroxide while Ma et al. [9] focused on the kinetics of the side reaction of epichlorohydrin i.e. hydrolysis. In this study, we focus on the second step, the dehydrochlorination reaction between dichloropropanol and sodium hydroxide (Scheme 1) [8-9,10-12]. Our earlier study on Aspen Plus simulation investigated the effects of process parameters such as temperature and reactant molar ratio on the reaction [13]. The study reported that the second reaction was very fast and could be completed in approximately 4 minutes. The simulation also generated some interesting results for setting the experimental design, pressure, temperature range, molar ratio of reactants, and duration of reaction. The parameters were used in simulation can be seen in Table 1.

2. Materials and methods

2.1 Chemicals

Commercially available sodium hydroxide and 1,3-dichloropropanol were purchased from Merck Chemical Co. While 1,3-dichloropropanol for GC standard calibration was obtained from Sigma Aldrich Co. Singapore.



Scheme 1

Table 1: Required Parameters used in the Simulation of dehydrochlorination Reaction [13]

Parameter	Information
Reactor Block	RBatch
Base Method	Wilson
Input Variable	
Temperature (°C)	20 to 60
Pressure (bar)	1.05
Chemical reactions	Scheme 1
Kinetics data	
A, s^{-1}	10^7
E_a, kJ/mole	49.21
Feed of Reactor	
Reactan mol ratio (1,3-DCP to NaOH))	1:1; 1:1.5; 1:2.3; 1:4; 1: 9

2.2 Isothermal Experiment

The above-mentioned reactions were carried out in a three-neck flask (500-ml), which was immersed in oil bath, equipped with a thermometer, a sampling port and a condenser. The condenser was connected to an accumulator. A temperature controlled oil bath was needed to control the reactor temperature and the mixture inside the reactor was stirred by the magnetic stirred vigorously. The reactor was fed with the base solution, which contains certain amount of sodium hydroxide then the temperature was increased to the desired temperature. When the intended reaction temperature was reached, a known fixed amount of the organic reagent, dichloropropanol, was poured into the reactor-containing base solution. The analysis of the reaction products was performed using GC-MS. HP-WAX capillary column with a dimension of 25 meter, 0.25 mm and a film thickness of 0.25 μm was used. The prepared gas chromatographic column was able to separate the reaction products: EPCH and 1,3-DCP. In addition, titration method was used to analyze moles of OH^- during the reaction [14].

The five different reaction temperatures were applied namely 50, 60, 70, and 80°C to investigate the effect of temperature on the reaction rate constants. While 5 different molar ratios 1:1, 1:2, 1:4, 1:6, 1:9 were chosen to investigate the effects of excess NaOH on reaction conversion and yield. The stoichiometric ratio between 1,3 DCP and NaOH is 1:1. Samples were taken at certain time intervals for analysis. Each sample was collected in a small vial, capped and kept in an ice water bath, in order to stop reaction then the hydrolysis of epichlorohydrin can be prevented. Samples were analyzed for 1,3-dichloropropanol, 1,2-dichloropropanol, and epichlorohydrin by Gas chromatography (GC). Carra et al [8] and the earliest study by Zhang et al. [15] also used GC method to monitor rate of the reaction. Equations 3 and 4 were used to calculate the conversion of 1,3-DCP and yield of EPCH respectively:

$$\text{Conversion of glycerol (\%)} = \frac{\text{Moles of 1,3–DCP reacted}}{\text{Moles of 1,3–DCP supplied}} \text{ x } 100 \qquad (3)$$

$$\text{Yield for EPCH (\%)} = \frac{\text{Moles of EPCH produced}}{\text{Moles of 1,3–DCP supplied}} \text{ x } 100 \qquad (4)$$

2.3 Model development

Kinetics of dehydrochlorination was studied in the presence of sodium hydroxide solution at various temperatures. According to Carra et al. [8], Ma et al. [9], and Zhang et al. [15] dehydrochlorination of 1,3-

DCP in aqueous basic solution is a fast occurring reaction. However, the competing hydrolysis reaction may have taken place, as shown in Equation 2, when the operating temperature reaction was higher than 80°C, presence of excessive base solution and longer reaction time [8-9]. The experiments were conducted to determine the kinetics parameters such as reaction rate constants and activation energies for the reactions as shown in Scheme 1. The stoichiometric molar ratio between the dichloropropanol and sodium hydroxide is 1:1. In the analysis of kinetic study, molar ratio of 1:9 was used throughout the experiments. This is to ensure that the rate of reaction was not influenced by the sodium hydroxide concentration [16]. The effects of molar ratio dichloropropanol:NaOH on the conversion to epichlorohydrin was conducted at 60°C.

According to Ma et al. [9], dehydrochlorination of dichloropropanol with sodium hydroxide is a second order irreversible reaction then the rate equation can be written as follows:

$$-r_{epy} = -\frac{d[DCP]}{dt} = k[DCP]^{\propto}[OH^-]^{\beta} \quad (5)$$

where [1,3-DCP] and [OH^-] are concentration of 1,3-DCP and OH^-, respectively.

In this study, we did not consider the formation of 2,3-DCP as an isomer of 1,3-DCP because pure 1,3-DCP (99.9%) was used [17]. Moreover, based on observation studied by Ma et al. [9], the reactivity of 1,3-DCP is much higher than the reactivity of its isomer 2,3-DCP due to the inductive effects and space effect. Both halogenoalkyls in 1,3-DCP increased the chlorine mobility or the negative charge on the oxygen [18]. Thus, the hydroxyl could attack 1-C and 3-C, but only one halogenoalkyl in 2,3-DCP could increase the negative charge on the oxygen, and the hydroxyl could only attack 2-C. at the same time [19]. Furthermore, it is harder for the hydroxyl to attack 2-C in 2,3-DCP due to the space effect [9], which does not even exist in 1,3-DCP. In order to determine the kinetic parameters, the concentration of OH^- used was used in large excess, 10 times the initial concentration of 1,3-DCP. Thus, [OH] can be assumed zero order, and the rate law can be described by a pseudo-first-order kinetic model [16,20]:

$$-r_{epy} = -\frac{d[DCP]}{dt} = k[DCP] \quad (6)$$

3. Results and discussion

3.1 Effect of molar ratio

Following the Le'Chatelier principle, one of the methods to promote a forward reaction as depicted in Equation 1 is by using one of the reactants in excess [16,20]. Since NaOH is relatively cheaper compared to organic compound dichloropropanol, the reaction was subjected to excess sodium hydroxide. The stoichiometric molar ratio between the dichloropropanol and sodium hydroxide was 1:1.

The effects of molar ratio dichloropropanol:NaOH on the conversion moles of DCP consumed over moles of 1,3-DCP fed to epichlorohydrin was conducted at 60°C and is shown in Fig. 1 and 2. These two figures showed that increasing molar ratio 1,3-DCP:NaOH from stoichiometric to 1:6 can improve the conversion of the reaction. After that there was no benefit of increasing molar ratio where conversion nearly remained constant since the reaction has reached its chemical equilibrium. This result also compared well with the simulation analysis using Aspen Plus as shown in Fig. 2.

Based on the results obtained from the Aspen Plus simulation, the optimal molar ratio of dichloropropanol to basic solution was found to be at 1:6 in terms of conversion of limiting reactant 1,3-DCP. However, in terms of the yield of epichlorohydrin, the optimum molar ratio was found to be at stoichiometric molar ratio. Excessive presence of solution sodium hydroxide, particularly at both high temperature (above 70°C) and longer reaction time, can lower yield of product epichlorohydrin due to competing reaction of hydrolysis of epichlorohydrin to glycerol [8-9]. Ma et al. [9] used a stoichiometric mol ratio between 1.3-DCP and NaOH. In another study, Carra et al. [8] used molar ratio 1,3-DCP : $Ca(OH)_2$ at 1:10 .

The effect of molar ratio on yield of epichlorohydrin is shown in Fig. 3 and 4. The results clearly indicate that excessive use of base solution in the dehydrochlorination reaction enhances the hydrolysis of epichlorohydrin, thus lower the yield of epichlorohydrin significantly. The results were also in good agreement with the Aspen Plus simulation results as depicted in Fig. 4.

3.2 Effect of temperature

The effect of temperature on the conversion of 1,3-DCP within the range of 50 – 80°C is shown in Fig. 5. Fig. 6 appraised the effect of temperature on the conversion after 5 minutes of reaction time. Under the operating conditions employed in this study, we can see that the reaction rate was slightly improved as the temperature was increased to 70°C. Carra et al. [8] found the optimum operating temperature at 60°C and 0.5 bar pressure. On the contrary, Ma et al. [9] did not find any marked effect of temperature on process performance. Figure 6 also shows that there is no marked improvement on conversion by increasing the temperature above 70°C.

The occurrence of hydrolysis reaction of epichlorohydrin to glycerol may have taken place at temperature above 70°C, thus lowered the yield of epichlorohydrin. The simulation study also confirmed that conducting the reaction above 70°C (343 °K) should be avoided due to the hydrolysis reaction. This finding was in good agreement with the reported data by Ma et al. [9]. They concluded that at temperature above 70°C, the hydrolysis reaction may have taken place of which would lower the yield of epichlorohydrin. According to Carra et al. [8], the hydrolysis reaction rate would be dominating at temperatures above 80°C and prolonged reaction time. Therefore, a suitable operating temperature and short contact time should be considered in order to reduce the probability of hydrolysis reaction. Carra et al [8] reported that 98% total conversion to dichloropropanol was achieved at 60°C and 0.5 bar. This study compared well with our earlier simulation study as shown in Fig. 6.

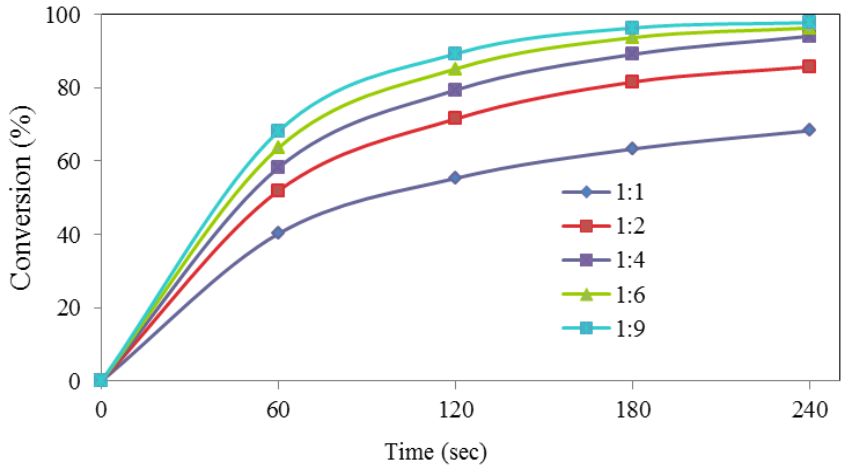


Fig. 1. Trend of conversion vs. time for the dehydrochlorination reaction of 1,3-DCP at different molar ratio

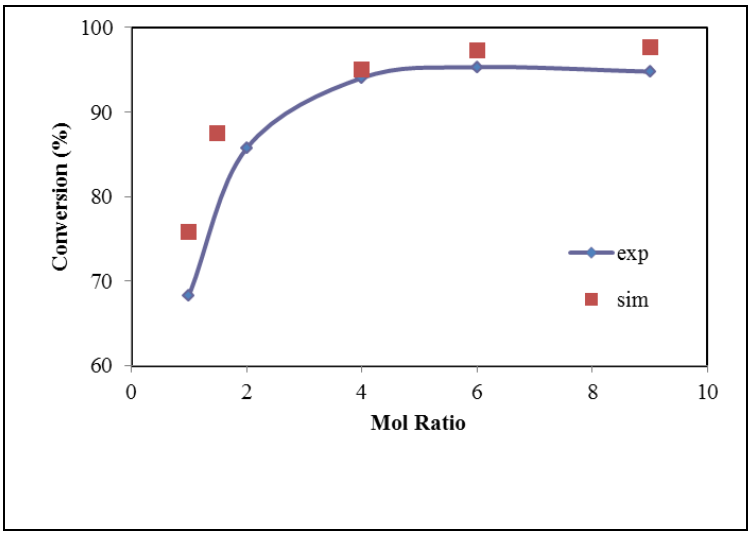


Fig. 2. Effect of mol ratio on conversion of 1,3-DCP: comparison between experimental and simulation

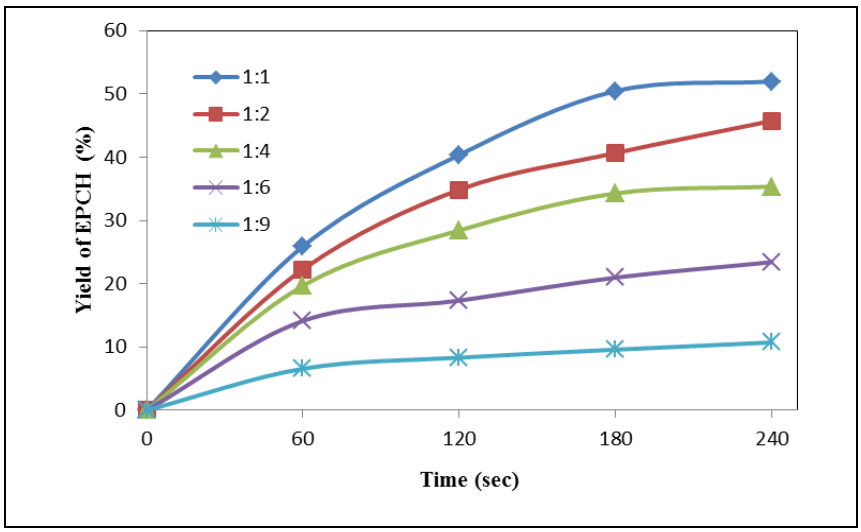


Fig. 3. Trend of yield of EPCH vs. time for the dehydrochlorination reaction of 1,3-DCP at different molar ratio

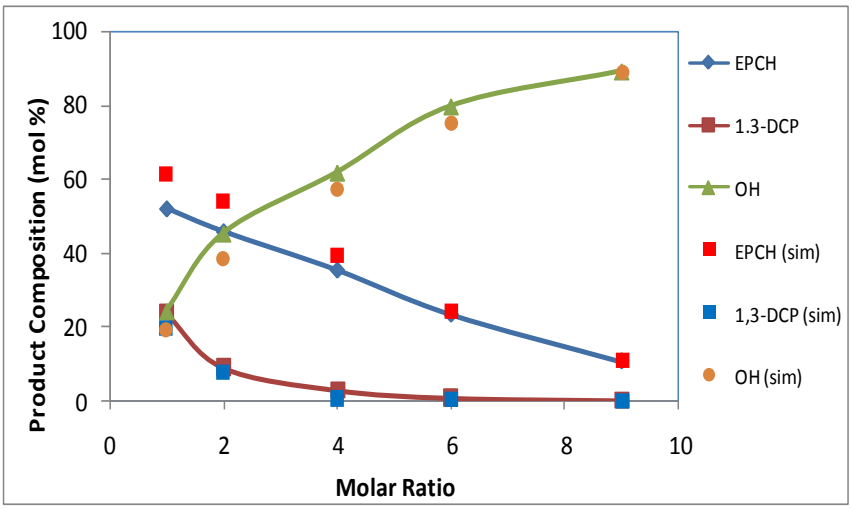


Fig. 4. Effect of mole ratio on product composition: Comparison between experimental data and simulation

Furthermore, Fig. 7 and 8 illustrated the influence of temperature on the yield of epichlorohydrin at end of reaction. Again, it exhibited similar behavior as the conversion described earlier. These figures showed that the yield of epichlorohydrin was relatively low at 50°C and then increased with temperature up to 70°C. However at temperature above 70°C, the side hydrolysis reaction of epichlorohydrin to glycerol would have been occurred. The insights into the occurrence of this hydrolysis reaction would be further exemplified in the forthcoming kinetics study. However, the peak in the GC chromatogram confirmed the formation of glycerol from the hydrolysis described above as shown in Figure 8.

The results were in good agreement with the Aspen Plus simulation data as can be seen in Fig. 9. Since both Carra et al. [8] and Ma et al. [9] did not study the effect of temperature on product composition, but this study has provided new insights on the effect of temperature on dehydrochlorination of 1,3-DCP to epichlorohydrin.

3.3 Reaction kinetics

Determination of rate constant highly depends on the order of the reaction. Using the experimental data, the correct order would be determined by which function of rate equation best fit the linear requirement. To analyze the kinetics of dehydrochlorination of 1,3-DCP with sodium hydroxide, two kinetics models including the pseudo-first-order and second-order were used. Linear equations of these models can be seen in Eq. (7) and (8). The parameter, reaction rate constants, in these models (pseudo first and second-order) were estimated from the slope of the linear plot [20].

$$\ln[1,3-DCP]_o - \ln[1,3-DCP] = kt \quad (7)$$

$$1/[1,3-DCP] - 1/[DCP]_o = kt \quad (8)$$

A straight-line plot, as shown in Fig. 10, supports our hypothesis that the dehydrochlorination of 1,3-DCP and sodium hydroxide follows the first order kinetics model with higher value correlation coefficient (R^2>0.99), compared to the second order model, (R^2<0.90). The reaction rate constants, k at various temperatures, 50 to 80°C, were obtained from the slopes of these lines and tabulated in Table 2. The indicator which also was used to determine the goodness of fit kinetic models was total sum squared error (SSE) [21]. In this study we found that SSE for the pseudo first order kinetic at 50 – 80°C were 0.00319, 0.00243, 0.00619 and 0.00511 for the total 0.0169 while the SSE for the second order kinetic were 0.1005, 0.0334, 0.401 and 0.249 for the total 0.783. The comparisons of SSE confirm that dehydrochlorination of 1,3-DCP with sodium hydroxide to produce epichlorohydrin fits well with pseudo first order kinetic model.

Table 1 explains the dependence of the rate constant, k, on temperature, T, for the dehydrochlorination of 1,3-DCP. The dependency of rate constant, k, on temperature follows the Arrhenius Equation [16]:

$$lnk = lnA - \frac{Ea}{RT} \qquad (9)$$

Where A is the frequency factor, Ea is the activation energy for the reaction, R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature expressed in K. From the slope of a plot of ln(k) versus 1/T x 1000 we can estimate the activation energy by multiply the negative slope with the universal gas constant. The frequency factor A was determined from the exponential value of y-intercept. Both A and Ea are very useful kinetic parameters to be known because the rate constants can be determined for any temperature once these two parameters are known. The plot of ln (k) versus 1/T x 1000 is shown in Fig. 11.

The activation energy Ea obtained from the plot was 38.8 kJ/moles and the frequency factor A was 1.62 x 10^7 sec^{-1}. Carra et al. [8] reported the activation energy for the reaction involved in the dehydrochlorination of dichloropropanol with $Ca(OH)_2$ of which the value of A and Ea are at 10^7 sec^{-1} and 49.18 kJ/mol respectively. Activation energy, E_a, can be thought of as the height of the potential barrier (sometimes called the energy barrier). A chemical reaction can be performed when an appreciable number of molecules with energy equal to or greater than the activation energy for a chemical reaction to proceed. Otherwise lower activation energy makes rate of reaction faster. The Arrhenius equation, $= Ae^{\frac{-Ea}{RT}}$, shows that k value will be higher when the activation energy is lower therefore the rate of reaction proceed faster. In this study, since the activation energy obtained was slightly lower compared to reporting by Carra et al. [8] then it may be a reason that time of our reaction to completion the reaction was slightly faster.

Once the value of E_a and A at the temperature range have been determined, we can then formulate that the dehydrochlorination reaction rate. The reaction rate in the presence of caustic soda within the selected temperature ranges can be expressed as follows:

$$r = 1.62\ x\ 10^7\ e^{-38.8/RT}[DCP] \qquad (10)$$

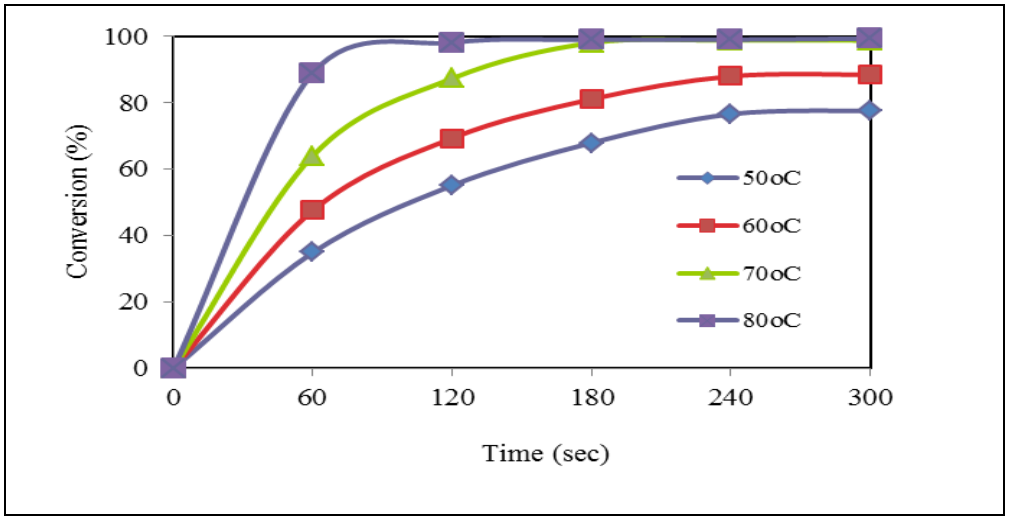


Fig. 5. Trend of conversion vs. time for the dehydrochlorination reaction of 1,3-DCP at different temperature

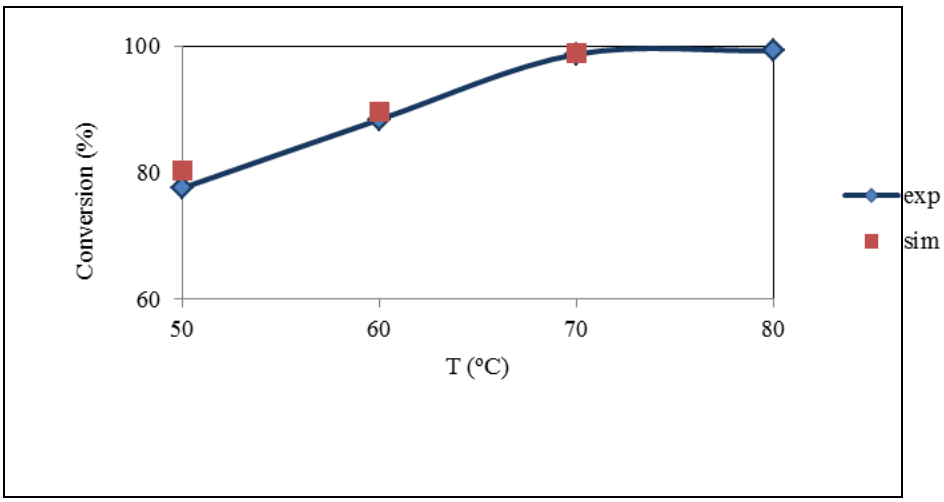


Fig. 6. Effect of temperature on conversion of 1,3-DCP: Comparison between experimental and simulation

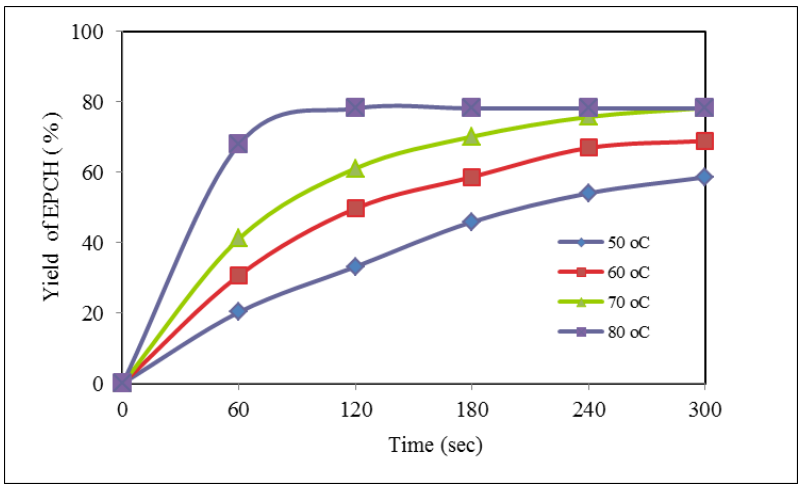


Fig. 7. Effect of temperature and time on yield of epichlorohydrin dehydrochlorination of 1,3-DCP with NaOH, mol ratio 1:10

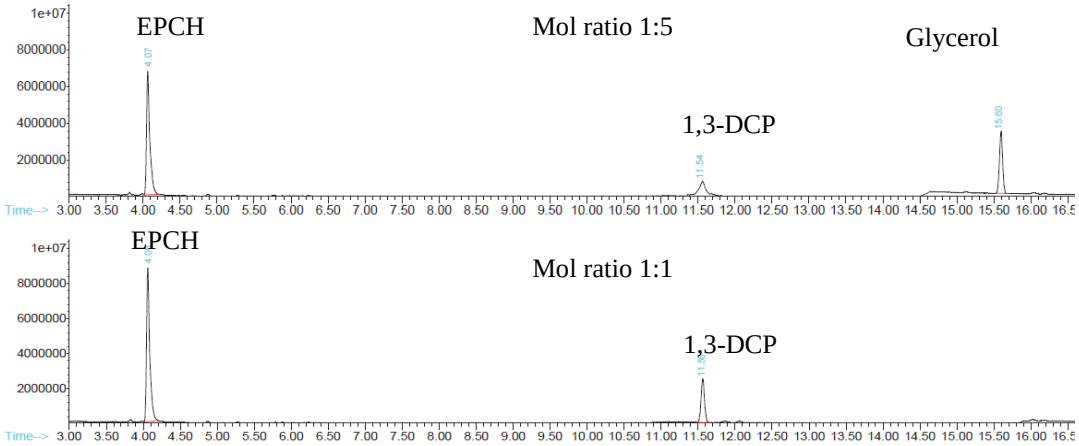


Fig. 8. GC Chromatogram (a) molar ratio 1:1 (b) ratio molar 1:5

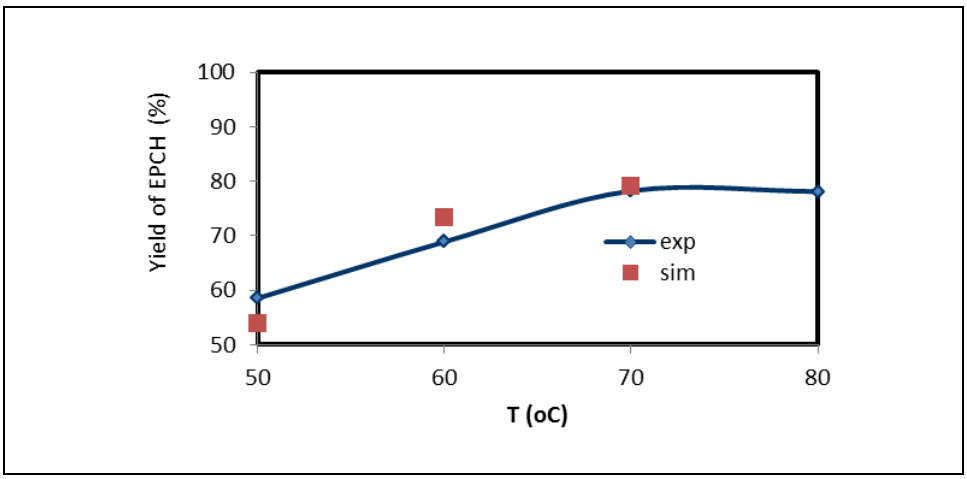


Fig. 9. Effect of temperature on yield of EPCH: Comparison between experimental and simulation using Aspen Plus

Table 2. Rate constant, k for reaction between 1,3-DCP and NaOH

Temperature (°C)	k (1st Order) sec^{-1}	k (2nd Order) $Lmol^{-1}sec^{-1}$
50	0.0056	0.026
60	0.008	0.058
70	0.012	0.126
80	0.021	1.243

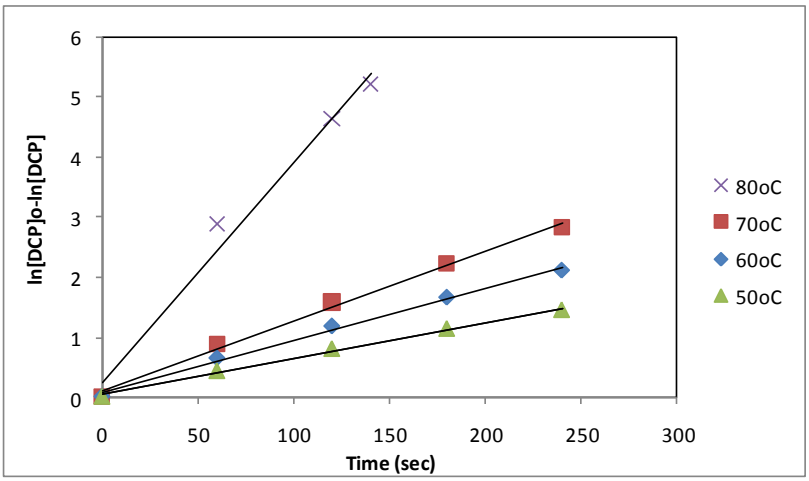


Fig. 10. Pseudo First-order kinetic model for dehydrochlorination of 1,3-DCP and NaOH

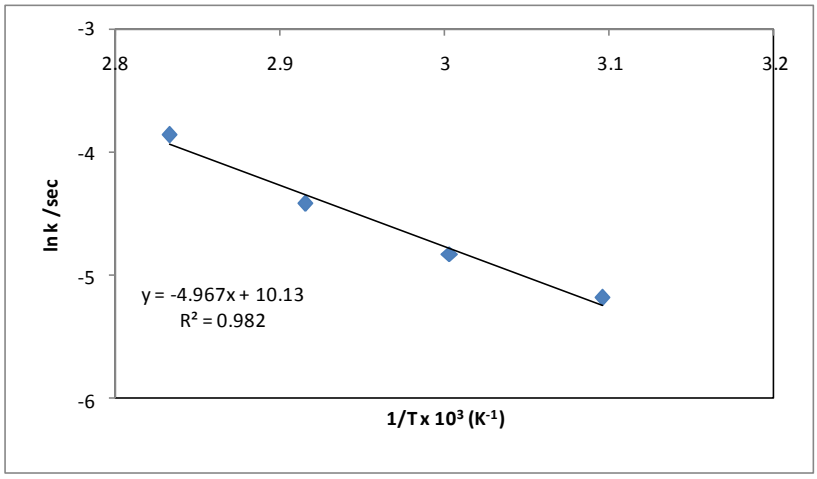


Fig. 11. Plot of ln (k) versus 1/T for the dehydrochlorination reaction

4. Conclusions

The reaction kinetics of dehydrochlorination of dichloropropanol and sodium hydroxide to epichlorohydrin was investigated. The effect of temperatures (50 to 80^{o}C) on such reaction was observed where the optimum temperature was found to be 70^{o}C. The effect of molar ratio 1,3-DCP:NaOH was also investigated where the best molar ratio in terms of conversion and in terms of yield were found at 1:5 and stoichiometric ratio, respectively. For the kinetic study we used sodium hydroxide in excess 1:10, then reaction rate was found to follow pseudo first order with respect to dichloropropanol concentration. The activation energy of the reaction was also determined at 38.8 kJ/mol and the pre-exponential factor A was at 1.62 x 10^{7} sec^{-1}. The reaction completion was achieved only after 2 minutes of reaction. Under these mild reaction conditions, the occurrence of the competing hydrolysis reaction can be suppressed.

Acknowledgements

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Study for Corrosion Inhibition of Mild Steel in Acid Media by Carboxymethyl Chitosan/Amylose Fraction Composite

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Abstract

The principle inhibition corrosion of Carboxymethyl chitosan (CMCh)/amylose fraction composite on the surface of mild steel in acid media has studied with the weight lost method and morphology analysis using SEM-EDS equipment. The results showed that the corrosion inhibition using composite effective CMCh/amylose is more effective as a corrosion inhibitor compared with no amylose. These research results showed that amylose molecules function as an adhesive by means of cross-linked and easy to absorb water so that the molecules CMCh directly contact the surface of steel in acid media. Layer formation on the surface of mild steel in acidic media studied by SEM photograph showing the morphology of the thin layer formed by the sharing of a lone pair of O atoms and N of CMCh is adsorbed on the steel surface to form coordinate covalent bonds. The EDS spectrum analysis results showed that mass of the O atoms increase are initially 9.05 % to 21.41 % and the atomic mass N of 1.04 % to 1.10 %. These results show that the surface layer formed mild steel cover prevents contact of aggressive ions directly on the steel surface in acid media.

Keywords: Amylose, Inhibitor, Composite, Carboxymethyl chitosan, Corrosion;

1. Introduction

Mild steel is widely used as construction material and chemical petrochemical industries due to its excellent mechanical properties that is easy to set up and low cost. The main problem of mild steel is its dissolution in acidic media that cause corrosion [1]. So that corrosion of steel is a major problem and this was due to serious corrosion problems associated with economic problems, safety, health and the environment. In terms of corrosion problems can be controlled by means of coating, painting, cathodic protection and the addition of the inhibitor to the corrosive medium. In this research to control corrosion used inhibitors derived from organic compounds, namely carboxymethyl chitosan (CMCh) were modified using amylose fraction. This compound is applied because it is biodegradability, bioactive, biocompatibility, cationic polyelectrolyte , high molecular weight, renewable and non-toxic [2].

Acid solutions are commonly used in the chemical industry to remove mill scales from the metallic surface. The addition of inhibitors effectively secures the metal against an acid attack. And many studies using CMCh inhibitors have been reported [3-5]. CMCh is of the mixed-type but acts as cathodic inhibitor for mild steel in 1 M HCl, this is because values of E_{corr} shift to the negative direction compared to uninhibited specimen. CMCh can be used as a corrosion inhibitor for mild steel in peat water. The inhibition of CMCh on the steel surface followed modified Langmuir isotherm equation and assumed to occur through chemical adsorption . Based on the chemical structure CMCh is contains active group hydroxyl, carboxyl and amine having electron pairs that can be attached directly to metal surface. CMCh is a good potential inhibitor need to be developed. Thus, for improved performance, certain physical and chemical modifications become necessary.

The aim of this study is to investigate inhibition of CMCh on mild steel in media sulfuric acid and hydrochloric acid has been modified with amylose fractions which form a composite, using weight loss measurements. Chemical preparation of water-soluble CMCh was according to experimental prosedure [6]. In this study amylose derived from cassava starch and separated from amylopectin by means of fractionation technique using n-buthanol. The formation of a layer on the steel surface before and after using CMCh inhibitors in acidic media is characterized using Scanning Electron Microscope SEM)- Energy Dispersive X-ray Spectroscopy (EDS) equipment.

2. Experimental section

2.1. Chemicals preparation

Test was performed on a freshly prepared sheet of mild steel of the following composition (wt.%): 0.16% C, 0.19% Si, 4.8% Mn, 0.16% P, 0.22% S, and the rest Fe) of the dimensions 2 cm x 1 cm x 0,1 cm.

The metal specimens were polished with emery papers (grade 100, 200 and 400), then degreased with acetone and washed with distilled water. The specimen were dried and kept in a desiccator.

The CMCh was prepared for chitosan with a degree of deacetylation of at least 85% was obtained from Sigma–Aldrich. Amylose derived from cassava starch and separated from amylopectin by means of fractionation technique using n-buthanol. The CMCh / amylose fraction was prepared by mixing CMCH with amylose fractions with a volume ratio.

The acid media solutions, 0,5 M H_2SO_4 and 1 M HCl were prepared by dilution of analytical grade 98% H_2SO_4 and HCl with distilled water. The concentration range of CMCh was 300 – 700 M.

2.2. Weight loss measurements

The mild steel specimen, in triplicate were weighed using Analitical Balance Ohaus PA 214 and immersed vertically in the acid media solution with and without addition of the inhibitor which maintained at room temperature. The difference in weight was measured once in every 48 hours for a period of 9 days. In each weight loss measurement the corroded samples were in chloroform, acetone and etanol, dried in an oven at a temperature of 40 ° C for 15 minutes and the weight was noted. The corrosion rate (R) in $mgdm^{-2}day^{-1}$ was calculated from the following equation:

$$R = \frac{W}{S.t} \qquad (1)$$

Where W is the average weight loss of three mild steel specimens, S the total area of one mild steel specimen, and t is the immersion time. With the calculated corrosion rate, the corrosion inhibition efficiency (E) was calculated as follows:

$$E = \frac{R_1 - R_2}{R_1} x100\% \qquad (2)$$

where *R1* and *R2* are the corrosion rates of the mild steel specimens in the absence and presence of inhibitor, respectively.

2.3. The surface morphology

The mild steel specimens in the acid media solutions with and without addition of inhibitor were examined the surface morphology using a JEOL-JSM-6510LV scanning electron microscope and Energy Dispersive X-ray Spectroscopy.

3. Results and Discussion

The corrosion inhibition efficiency test results of CMCh as inhibitor in H_2SO_4 and HCl media can be seen in Fig. 1. The corrosion inhibition efficiency of CMCh in H_2SO_4 media is lower than in HCl respectively 65.12% and 85.65% at a concentration of 0.25 M H_2SO_4 and HCl 1 M. This result occurs because H_2SO_4 is more corrosive than HCl so easily damaged CMCh compounds in H_2SO_4.

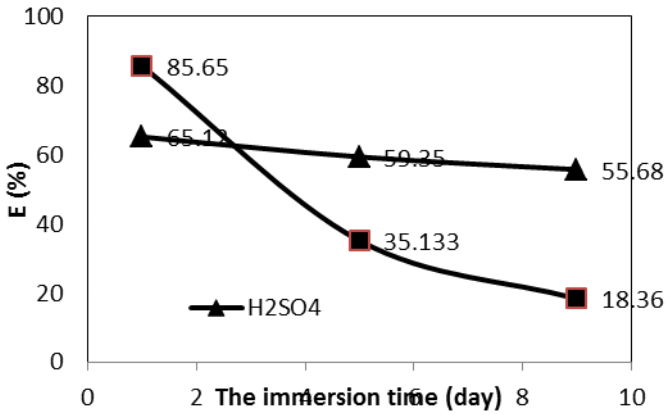

Fig. 1. The corrosion inhibition efficiency of CMCh on mild steel in H_2SO_4 and HCl media, at different immersion times

This result is supported by visualization photo of mild steel surface immersed in media acid, show in Fig. 2. The CMCh in HCl media is more effective than in H_2SO_4 to inhibit corrosion of mild steel. Mild steel in H_2SO_4 solutions causes the solution becomes brownish color using either inhibitors or with CMCh. While mild steel immersed in HCl solutions does not corrode is characterized solutions remain clear.

To analyze the shape of the steel surface after immersion in H_2SO_4 and HCl media used SEM-EDS equipment. Looks from Fig. 3, that the steel surface after immersion in H_2SO_4 more holes are formed due to the dissolution of iron in the steel surface than in HCl. This proves that H_2SO_4 media is more corrosive than HCl.

Form thin layer of the mild steel surfaces after immersed with CMCh inhibitor in acid media also analyzed by SEM equipment. The result can be seen in Fig. 4, it is seen that the surface of the mild steel using H_2SO_4 media, less homogeneous layer formed while using HCl media layer formed more homogeneous. This causes the inhibition efficiency of CMCh on mild steel in H_2SO_4 media to be low because not all surfaces are covered by CMCh molecules. It required research in order to modify the CMCh effective when used in acidic media both in H_2SO_4 and in HCl. In this study CMCh modified into a composite using amylose fraction isolated from cassava starch. Amylose is used because it is hydrophilic which may help the formation of a layer on the mild steel surfaces to be faster. The experimental results are shown in Table 1.

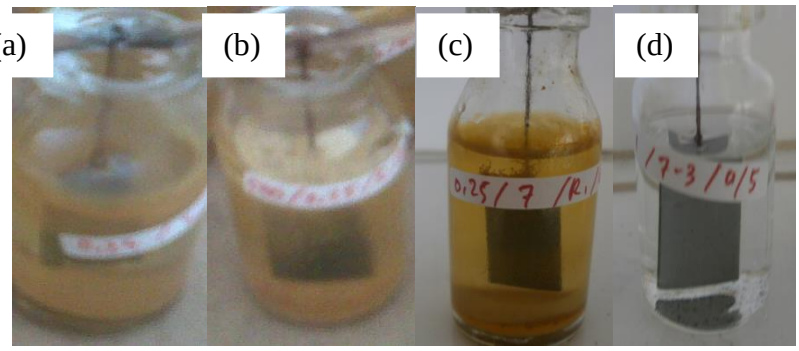

Fig. 2. The visualization Photos for mild steel immersed for 3 days in media: a) H_2SO_4 without CMCh, b) H_2SO_4 with CMCh, c) HCl without CMCh and (d) HCl with CMCh at room temperature

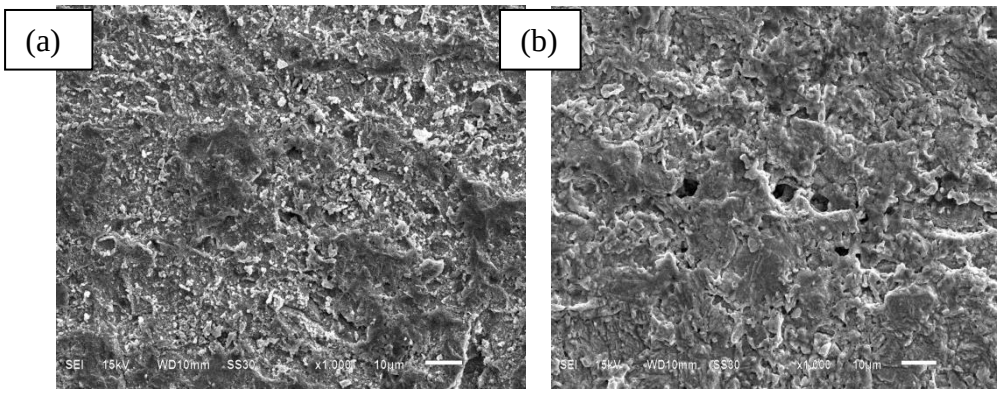

Fig. 3. The SEM images of the mild steel surface after immersed for 3 days in: (a) 0.25 M H_2SO_4 and (b) 1 M HCl at room temperature

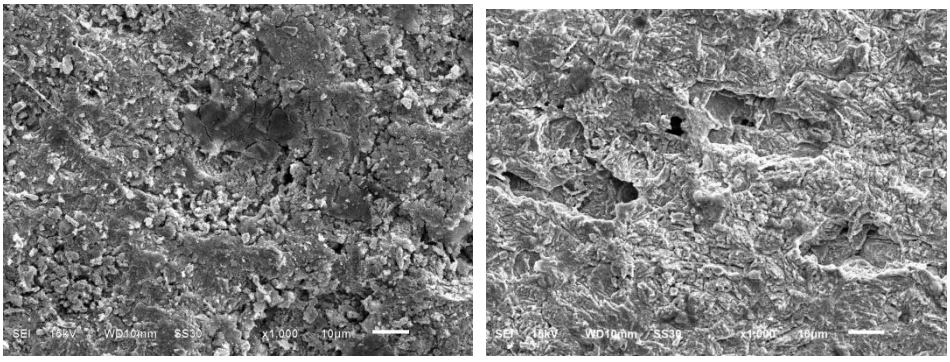

Fig. 4. The SEM images of the mild steel surfaces after immersed for 3 days in: (a) 0.25 M H_2SO_4 with 500 ppm CMCh and (b) 1 M HCl with 200 ppm CMCh at room temperature

Table 1. The corrosion inhibition efficiency of mild steel in 1 M HCl for 3 days used inhibitors of the CMCh/ amylose fraction composite

Rasio The CMCh/amylase fraction composit (V/V)	The corrosion inhibition efficiency, E (%)	
	H_2SO_4	HCl
6:4	89.95	88.56
7:3	90.3	91.46
8:2	88.7	88.94
9:2	84.5	87.208
10:0	65.12	80.53

Based on Table 1, it is seen that the amylose fraction can improve the corrosion inhibition efficiency of the mild steel in HCl and H_2SO_4. The experimental results showed that the corrosion inhibition efficiency increase in the volume ratio CMCh/ amylose fraction (7:3) reached 90.3% and 91.46%. This proves than the amylose molecules readily absorbs water molecules directly contact CMCh on the mild steel surface to form a thin layer. Amylose in a way serves as an adhesive molecule crosslinked with CMCh. According to [7] that adsorption of organic molecules on metal surfaces occurs, because the interaction energy between the metal surfaces with higher inhibitor of the interaction energy between the metal surface with water.

The layer formation on the mild steel surfaces in HCl media studied by EDS spectrum is shown in Fig. 5. The results of spectrum is show that a change in the composition of atomic mass constituent of mild steel before and after the use of inhibitors, the results of the analysis are shown in Table 2. Based on the results of the analysis of layer formation that occurs on the surface due to the use of mild steel with the lone pairs of O atoms and N of CMCh is adsorbed on the mild steel surface to form coordinate covalent bonds. This is evidenced by the increasing atomic mass of O is initially 9.05 % to 21.41 %, while the atomic mass of N from 1.04 % to 1.10 % after the addition of CMCh/ amylose fraction composit in HCl media . The presence of inhibitors led to reduced solubility of Fe in HCl media so that the atoms C and Fe which is the main composition of mild steel into a reduced percentage on the mild steel surfaces. EDS spectrum analysis results also showed that the surface layer formed mild steel cover can revent Cl^- ions are corrosive contacts directly on the mild steel, it is marked percentage decreases Cl atoms of 2.65 % to 0.63 %.

These results are in accordance with the statement of [8], that the corrosion inhibitors are generally formed layers adsorbed on metal surfaces to prevent metal contact with corrosive substances. The working principle is the inhibitor ions or molecules adsorbed on the metal surface is causes corrosion rate decreases, due to an increase or decrease in the anodic or cathodic reaction, a decrease in the rate of diffusion of the reactants to the metal surface and a decrease in the electrical resistance of the metal surface.

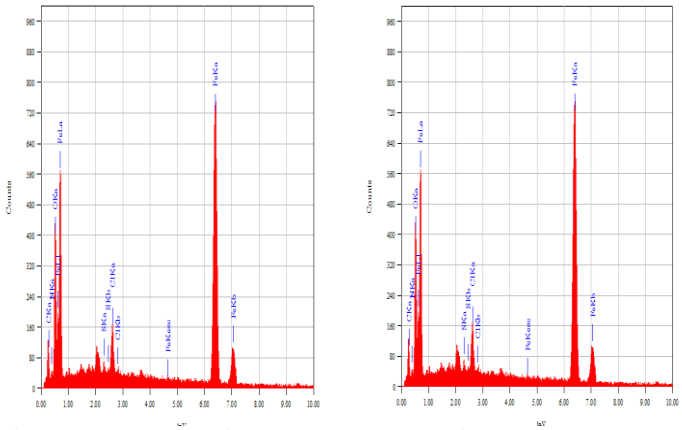

Fig. 5. The EDS spectrum from mild steel surface after immersion in 1 M HCl for 3 days: (a) without CMCh, and (b) with CMCh/ amylose fraction composite with a volume ratio of 7:3 at room temperature

Table 2. The results of the analysis of the elements on the mild steel surfaces immersed in 1 M HCl for 3 days at room temperature

Unsur	Mass elements on the mild steel surfaces after immersion in HCl (%)	
	Without CMCh	With CMCh/ amylose fraction composite
C	4.71	2.78
N	1.04	1.10
O	9.51	21.41
Cl	2.65	0.63
Fe	81.47	73.94

4.Conclusion

The CMCh performance as a corrosion inhibitor on mild steel in acid can be improved by modifying the CMCh using amylose fraction which can increase the corrosion inhibition efficiency of 65.12% H_2SO_4 media to 90.3% and from 85.65% in HCl to 91, 46%. The CMCh/amylose fraction composite worked as a corrosion inhibitor. Amylose molecules that easily absorbs water so as CMCh molecules is contact directly on the mild steel surface to form a thin layer. Based on the EDS spectrum of thin layers formed by sharing lone pairs of O atoms and N of CMCh is adsorbed on the steel surface to form coordinate covalent bonds.

Acknowledgements

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Synthesis $Mg_{3,1}Al_{0,45}Fe_{0,25}Ce_{0,3}$ Hydrotalcite Doped with Hydrothermal and Coprecipitation method : Different calcination temperature

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Abstract

In the present paper, we goal to the preparation of Mg/Al/Fe/Ce hydrotalcite catalyst are synthesed in varieties of catalyst mol ratio dopants Fe^{3+}/Ce^{3+}, and calcination temperature have synthezised with coprecipitation and hydrothermal method. Mg/Al/Fe/Ce hydrotalcite is layered double hydroxides (LDH) compounds and have a *brucite* structure in both cation and anion. This cation consist of ion +2 and +3 valences that are Mg^{2+} and Al^{3+}. These layers are composed from different composition in accordance with the balance of charge Moreover, in order to understand its layered double hydroxides, hydrotalcite is doped with Fe^{3+} and Ce^{3+} with varied mol rasio dopants, and calcination temperature synthesist of Mg/Al/Fe/Ce. They are to be characterised by X-ray difraction (XRD) and FTIR spectroscopy, Inductive Coupled Plasma (ICP), Brunauer Emmett Teller (BET), and Scanning Electron Microscope with energy dispersive X-ray spectroscopy (*SEM*/*EDX*) and The results show that $Mg_{3,1}Al_{0,45}$ $Fe_{0,25}$ $Ce_{0,3}$ is a hydrotalcite-like compound with the octahedral cations largely ordered with BET is 104,597 m^2/g at temperature calcination optimum 650 ^oC.

Keywords: Calcination temperature; Hydrotalcite-type precursor; Hydrothermal.

1. Introduction

Hydrotalcites are fundamentally anionic clays. The structure of hydrotalcite can be derived from a brucite structure ($Mg(OH)_2$) .This substitution creates a positive layer charge on the hydroxide layers, which is compensated by interlayer anions or anionic complexes [1, 2]. In hydrotalcites a broad range of compositions are possible of the type $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+}(A^{n-}_{x/n})\cdot mH_2O$, where M^{2+} and M^{3+} are $x = \frac{M^{III}}{M^{II}+M^{III}} = 3/1$, the di- and trivalent cations in the octahedral positions within the hydroxide layers with x normally between 0.10 and 0.31. A^{n-} is an exchangeable interlayer anion , m is water, n is oxidation number.

Doping with certain foreign ions[19], like Fe_2O_3 and CeO_2 effected measurable changes in the surface [21], and catalytic properties of a large variety of catalysts [13], morpologhy increase [17] , Loading cerium oxide on to an hydrotalcite surface led to the formation of thermally stable solids [18] **and surface increase [7]**. Base increase and for example, doping with CeO_3 of Mg/Al Hydrotalcite calcined at 650 °C resulted in an increase in the catalytic activity in Glyceril tributirate.

Traditional method, hydrotalcites are prepared by coprecipitation synthesis. In this report, the characterization of the hydrotalcites synthesized by an alternative method, the coprecipitation and by hydrothermal method, is presented.

By this method the Mg/Al ratio in the solid obtained is limited to the range 0.5-4.0, and it was then attempted to increase this ratio by the the coprecipitation and by hydrothermal method. The characterization techniques were X-ray diffraction, BET method, FTIR spectroscopy, TGA, Inductive Coupled Plasma (ICP), and Scanning Electron Microscope with energy dispersive X-ray spectroscopy (*SEM*/*EDX*).

2. Eksperimen

2.1. Material preparation

The First, preparated it were 60.963 gram $MgCl_2 \cdot .6H_2O$ (3 mol), 12.0665 gram $AlCl_3 \cdot 6H_2O$ (0.5 mol), 10.8655 gram $FeCl_3 \cdot 6H_2O$ (6. 0358 mol) dan 9.3146 gram $CeCl_3 \cdot 7H_2O$ (0.25 mol) in 30 mL aquades. *Second*, 1 mL Na_2CO_3 (1 mol) slowly dropped into a highly basic carbonate solution to $MgCl_2 \cdot .6H_2O$, $AlCl_3 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$ and $CeCl_2 \cdot 7H_2O$. Then, the sample was heated up to 60 ^oC temperature [23] and then sample mixed on the magnetic stirrer until reached the precipitate. NaOH 1M was slowly dropped into mixed sample until to reached optimum pH 12. Then solution do coprecipitation under magnetic stirrer for 7 h, 350 rpm. Eaches mixture heated up to 180^{o}C under magnetic stirring for 36 h in hydrothermal reactor, until precipite the performed.

The solid obtained was then filtered and washed with Na_2CO_3 (0.05 mol/375 mL), distilled water (373 K) until reaching pH 7. and this was dried at 7 h. The solid obtained calcination at 12 h at temperature 400^{o}C, 450^{o}C , 500^{o}C, 550^{o}C, 600^{o}C, 650^{o}C dan 700^{o}C until reaching amorf performed. The precipitates

were aged at 180 ^{o}C for 36 h by hydrothermal and then filtered, washed until the pH of the washing water reached 7 and air-dried [15].

2.2.Catalyst Characterization

The hydrotalcite Mg/Al/Fe/Ce composition of the synthesized sample was determined by X-ray powder diffraction patterns were recorded in a Rigaku X-Ray generator equipped with a graphite monochromator using CuKα radiation, 40 KV and 30 mA. Thermal decomposition of this sample was evaluated by TGA and DTA carried out in a Rigaku Thermobalance TAS 100 ^{o}C under a stream of air at 10 ^{o}C/min up to 1000 ^{o}C. The textural characteristics (BET specific surface area, microporous and mesoporous volumes) of some of the Mg/Al/Fe/Ce-mixed oxides obtained by calcining the parent hydrotalcite [3]. FT-IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR Spectrometer. The spectrum was recorded in the range of 4000-400 cm^{-1} [2]. ICP-AES was used for elemental analysis of the Mg, Al, Fe and Ce ions. A calibration series of standards for determining the relative amounts of Mg, Al, Fe and Ce.The relative amounts of each atom were recorded on Varian Liberty 2000 ICP – AES. [10]. Chemical compositions were investigated using both an energy-dispersed X-ray (EDX) unit attached to SEM and an X-ray photoelectron spectroscope (XPS; Esca3200, Shimazu, 8 kV, 30 mA; pass energy: 75 eV) [9].

3. Results and Discussion

3.1. XRD

The general formula for LDH is $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}(A^{n-}_{x/n})\cdot mH_2O$ and it indicates that it is possible to synthesize a number of compounds with different stoichiometries. In the natural hydrotalcites the value of *x* is generally equal to 0.246. Thus, the XRF results clearly confirmed that it is possible to synthesize LDH with the above formula having more than two metals [4].There are, however, many difficulties in determining the exact value of *x* in LDH. An elemental analysis of the metal content of a solid phase will not give correct values if the LDH is not monophasic, *i.e.*, mixed with $M^{2+}(OH)2$, $M^{3+}(OH)3/M^{3+}OOH$ or other phases which could segregate when the synthesis mixture contains either very high or very low M^{2+}/M^{III} ratios. However, more often these phases are amorphous and cannot be detected by XRD [11]. For examples in hydrotalcite the value of *x* are, 0.184, 0.181, and 0.269 at calcination temperature 450 ^{o}C, 600 ^{o}C , and 700 ^{o}C.

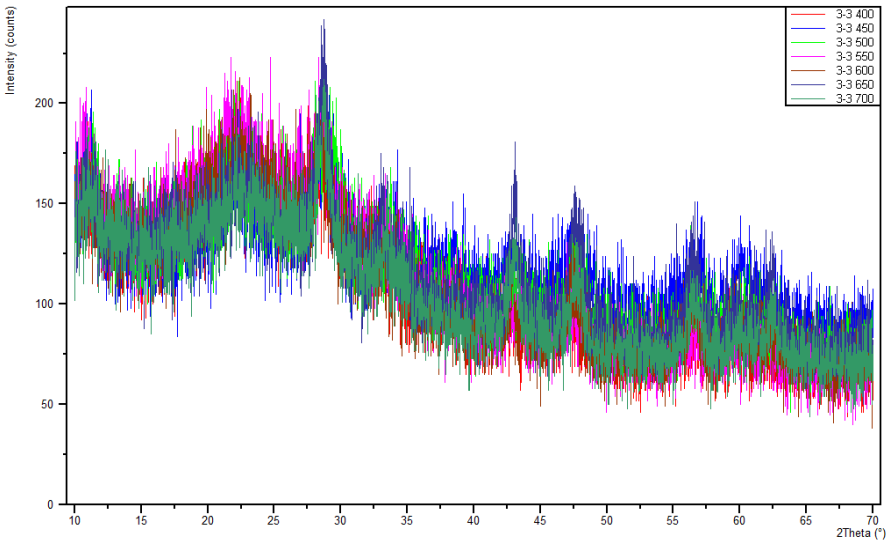

Figure 1. XRD of Mg/Al/Fe/Ce HTlcs synthesized with different calcination temperature.

X-ray powder diffractograms of pure and doped solids calcined in air at 400.450, 500, 550, 600, 650 and 700 ^{o}C were determined by using a diffractometer .

The XRD patterns in all the samples there is evidence of the presence of Ce oxides or hydroxides as separate phases, indicating that Ce atoms are randomly distributed inside the structure of the layers [1].

3.2. SEM/EDX

SEM/EDX images (Fig. 2) show that the samples were obtained in the form of homogeneous. Composition maps, obtained with coupled TEM-EDX analysis, showed that Mg, Al, Fe and Ce are homogeneously dispersed over the entire analyzed spot area, without creating single-metal domains, thus confirming the good distribution of the metals in the samples in their entirety. XRD analysis confirmed the absence of crystalline metallic Ce phases [8,1]. Catalytic activity of these materials depends on the presence of metallic Ce centres on the surface [1].

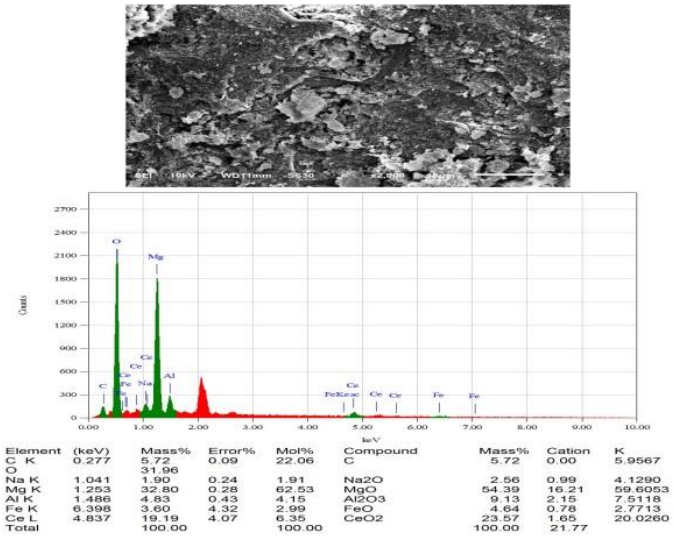

Figure 2. SEM/EDX of Mg/Al/Fe/Ce Hydrotalcite synthesized

3.3. ICP

ICP is an instrument that gives elemental analysis of sample. This technique of analysis is called Optical Emission Spectroscopy (OES). ICP is an energetic source, emitting many thousands of wavelengths, and producing a complex spectrum. When atoms are excited with sufficiently high energy they tend to emit characteristic radiation while returning to ground state. The ICP can analyze a whole spectrum of elements at a time by better technique and employing a SCD detector covering a range of 165nm to 403nm in UV and Visible range of spectra is 404 nm to 782 nm (Perkin Elmer, U.S.A. 2010).

Tabel 1. ICP of Mg/Al/Fe/Ce Hydrotalcite synthesized with different calcination temperature

Sample	Racio Mg/Al/Fe/Ce	**Unsure Analycis**				Final of racio characterize product ICP
		Ppm Mg	Ppm Al	Ppm Fe	Ppm Ce	
HTT1;650^{o}C	3,3/0,5/0,25/0,25	161872	25032	30804	97244	3,1/0,45/0,25/0,3
HTT1;700^{o}C	3,3/0,5/0,25/0,25	167034	35014	30996	96432	5,1/1,0/0,41/0,51
HTT1;550^{o}C	3,3/0,5/0,25/0,25	255481	32687	29140	91147	9,0/1,0/0,45/0,55
HTT1;600^{o}C	3,3/0,5/0,25/0,25	266793	32883	29719	94073	9,0/1,0/0,44/0,55

The elemental chemical analysis using ICP-OES is summarized in Table 1 where the results are shown in metal mole percent. For example hydrotalcite with temperature calcination 650^{o}C to have well crystalline Mg/Al/Fe/Ce hydrotalcite usually form in optimal pH = 12 condition. The presence of less quantity of aluminum cations in the solid solution can be explained as the trivalent aluminum used as support might not be precipitated fully during synthesis and the synthesis in strong basic condition at pH 12 is another cause in where the partial solubility of aluminum ions as aluminate species [16]. The higher content of Fe /Ce in Mg/Al/Fe/Ce compositional mixed oxides is due to the partial replacement of the aluminum by Fe and Ce cations, which often occur at high pH value [12].

3.4. BET

The specific surface areas of the catalysts are shown in Table 2. All catalysts exhibited a large surface area due to the porous structure derived from hydrotalcites as the precursors. An exceptionally small surface area of $Mg_{9.0}\,Al_{1.0}\,Fe_{0.45}\,Ce_{0.55}$ may be due to the formation of well crystallized CeO phase arising from phase separation of Ce(OH)3 from hydrotalcite during the preparation [8]. An increase surface area of $Mg_{3.1}\,Al_{0.45}\,Fe_{0.25}\,Ce_{0.30}$ This is also due to the formation of hydrotalcite, precursors which can accommodate all metal components in the structure after the coprecipitation- hydrotermal [8].

BET isotherm data shows a general increase in surface area upon calcination (Table 2), consistent with the formation of a porous mixed metal oxide on loss of CO2 and collapse of the HTC lattice. The doped tended to have larger surface areas than the undoped analogs. The highest surface areas were seen for the 3:1 Mg:(Al:Fe:Ce) [13]. HTCs, before and after calcination, so the present studies were confined to solids based on 3:1 Mg:(Al + dopant) ratios. Dopan same as Mg:(Al:Fe:Ce). Calcining at 550 ^{o}C resulted in slightly lower surface areas [13].

Tabel 2.BET of Mg/Al/Fe/Ce Hydrotalcite synthesized with different calcination temperature

No.	Calcination Temperature	BET (m^2/g)
1	400 °C	65,167
2	450 °C	79,500
3	500 °C	94,618
4	550 °C	21,993
5	600 °C	23,096
6	650 °C	104,597
7	700 °C	74,133

Tabel 3. ICP and BET of Mg/Al/Fe/Ce Hydrotalcite synthesized with different calcination temperature

Sample	Racio of Final Characterize Product of ICP.	Unsure Analycis				BET
		Ppm Mg	Ppm Al	Ppm Fe	Ppm Ce	
HTT1;650°C	3,1/0,45/0,25/0,3	161872	25032	30804	97244	104,597
HTT1;700°C	5,1/1,0/0,41/0,51	167034	35014	30996	96432	74,133
HTT1;550°C	9,0/1,0/0,45/0,55	255481	32687	29140	91147	21,993
HTT1;600°C	9,0/1,0/0,44/0,55	266793	32883	29719	94073	23,096

The surface metal compositions obtained by BET analyses are shown in Tabel 3. Mg/Al/Fe/Ce ratio was almost relative constant between 550 and 600 ºC, whereas Mg/Al/Fe/Ce ratio was almost constant up to 650 ºC and decreased at 600 ºC [3, 8].

3.5. TGA

The high resolution thermogravimetric analysis of carrboydite is shown in Figure 3. Five principal weight loss steps are observed at 305.34 and 705.34°C. Thermal decomposition causes the loss of water initially. The initial weight loss including the steps at 305.34 is around 13.49%. Calculations for the weight loss based upon the formula ($Mg_{3.1}$ $Al_{0.45}$ $Fe_{0.25}$ $Ce_{0.30}(CO_3)(OH)_{16}.4H_2O$) is 13.49 %. The broad weight loss step over the 100 to 250 °C is 6.5%. The weight loss step at 705.34 °C is 17.85%. These two steps are assigned to the dehydroxylation of the carrboydite. The experimentally determined weight loss for the carrboydite is 21.34 % [5, 8].

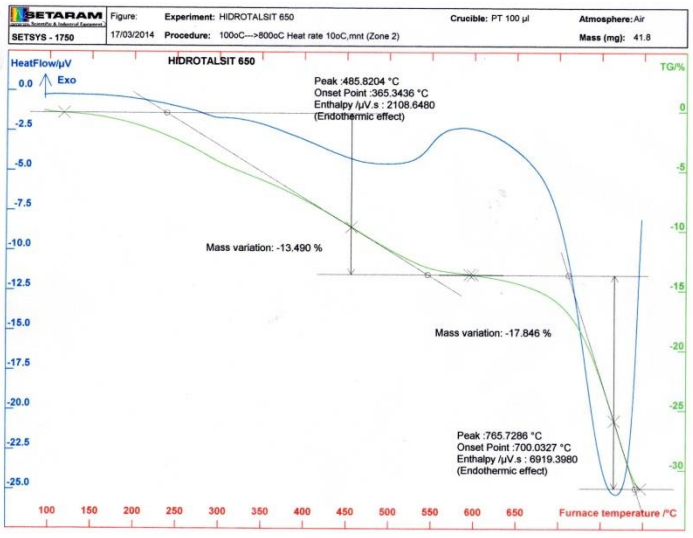

Figure 3 TGA-DTA of Mg/Al/Fe/Ce Hydrotalcite synthesized

Based on the formula $Mg_{3.1}$ $Al_{0.45}$ $Fe_{0.25}$ $Ce_{0.30}(CO_3)(OH)_{16}$.4H2O) the theoretical weight loss for dehydration is 13.15 % based upon 7 molecules of water in the formula. If there are four water molecules in the formula unit then the theoretical weight loss of 7.9 % is obtained. Thus the experimental result for dehydration is between that of honessite and hydrohonessite. Such a result is not unexpected as these minerals may dehydrate and rehydrate quite readily. Two weight loss steps are observed at 294 and 329 °C with a total weight loss of 10.5%. These two steps are attributed to dehydroxylation. The theoretical weight loss for dehydroxylation is 28.39 % based upon the hydrohonessite formula. There is an apparent large

difference between the experimentally determined and theoretical calculated results. This means that the number of hydroxyl units in the formula is not 16 but some number less. Two further weight loss steps are observed at 394 and 455 °C. The total for these two steps is 4.0 %. This weight loss step is attributed to the loss of carbonate. The final weight loss step is observed at 646 °C, and is attributed to loss of carbonate[8].

The complexity of the DTGA patterns is reflected in the mass spectrum of hydrohonessite. The mass spectrometric analyses together with the DTGA patterns of hydrohonessite are shown in Figure 4. The difference betweens the MS patterns of carrboydite and hydrotalcite [6,8].

3.6. FTIR

IR analysis can be useful to identify the presence of the foreign anions in the interlayer between the brucite-like sheets. In addition, the information about the type of bonds formed by the anions and their orientations can also be obtained1. It can be seen that the IR spectra of the samples obtained by low this methods are similar to each other.

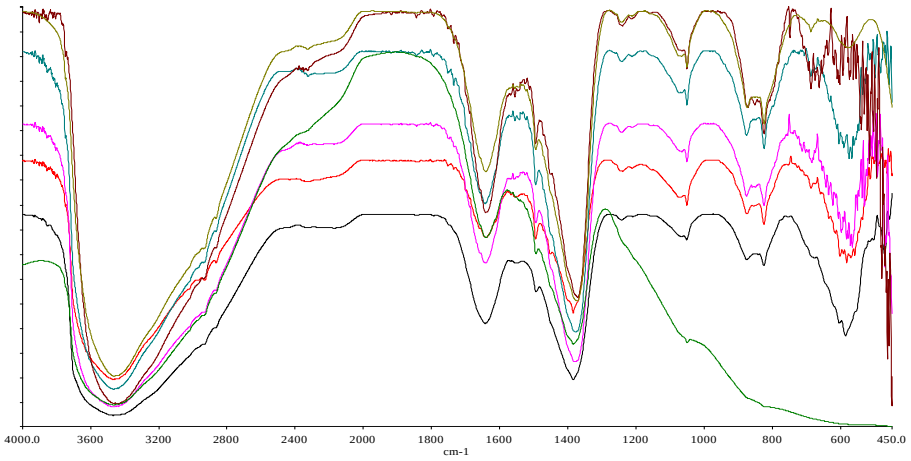

Figure 2 IR spectra of Mg/Al/Fe/Ce HTlcs synthesized with different method.

The split of the vibration at about 1380 cm^{-1} and a new peak at approximate 790 cm^{-1} appear especially in the spectrum of Mg/Al/Fe/Ce. These results indicate the difference of the gallery anions in the interlayer between the brucite-like sheets of the HTlcs synthesized with different temperature [20].

In general, the coprecipitation and hydrothermal method is an advanced pH control method. The crystallinity and the regular degrees in the structure of the products obtained with this method are clearly preferable to those synthesized with other methods [6, 20].

4. Conclution

This work has been to provide some rationalization of the observed differences in the activity as basic catalysts of hydrotalcites Mg/Al containing different types of cations and interlayer anions. In particular, we have tried to understand the reasons of the larger basicity of Mg/Al/Fe/Ce hydrotalcite [22].

This paper developed a new racio treatment for Mg/Al/Fe/Ce hydrotalcite, $Mg_{3,1}Al_{0,45}Fe_{0,25}Ce_{0,3}$ hydrotalcite compounds. $Mg_{3,1}Al_{0,45}Fe_{0,25}Ce_{0,3}$ hydrotalcite compounds (where x = 0, 246) were successfully synthesized [23] by a constant pH=12 coprecipitation-hydrotermal method. The derived oxide from hydrotalcites upon calcinations at 650°C for 18 hr is of hydrotalcite phase and crystalline hydrotalcite phase is detected. $Mg_{3,1}Al_{0,45}Fe_{0,25}Ce_{0,3}$ hydrotalcite compounds catalyst exhibits very good performance for methyl butirate is detected from transesterification reaction glyceril tributirate.

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Chemical Soil Characteristics Affected By Incubation Time Of Organic Hydrogel In Ultisols

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Abstract

Organic hydrogel is one of the soil conditioner which is easily decomposed and environmental friendly. This study investigates the appropriate type of organic hydrogel and the best incubation time with indicators of the characteristics of the chemical properties in soil. This research uses the randomized block design (RBD) which consists of treatments includes 0,10,20,30,40,50, and 60 days of incubation time between hydrogel and soil mixture (Ultisols) and repeated 4 times will be used. The results of this study showed that all the parameters indicating the difference between treatment (Organic C, Total N, CEC, pH,water content) and control are significant.

Keywords : Organic hydrogel; Soil Properties; Ultisols

1. Introduction

Chemical fertilizers in agricultural land in Indonesia is generally provided immediately (*instantaneous releases*) without adjusting with the needs of plants and soil behavior. Generally, the formulation of land management practices totally depends on the understanding relationship between the cause and effect of the response of the land to land manipulation. The dry land in Indonesia, based on the perspective classical literature soil sciences, has a low fertility. This phenomenon occurred due to the high leaching and content of iron oxide-oxide in the soil that makes the land management and cultivating become difficult (Rojanasoonthon and Kheoruenromne, 2002).

Providing chemical fertilizer level without adjusting nutrient needed by plants and soil behavior can pollute the environment in the long run. On the other hand, challenges for horticulture products in the era of globalization are more difficult because they have to be able to meet the need for product and also the production quality must be able to compete with the global market. Horticulture sector is one of the important developing sector in this country. In the future, this sector can be developed for the wealth of the nation. There are many challenges in the future that should be faced by this sector. The strategic role for increasing the agricultural production in quantity, quality, and high competitiveness should be sustainaible in order to secure the food and national self-sufficiency.

The agricultural land that is replaced for housing and industry with the rest of the land, having low quality of land quality in the negative impact to the environmental. Therefore, various strategies for securing agricultural production must be continued for giving efforts to expand the extensive planting in dry land, increasing of soil quality and intensify harvest as one of the security strategy agricultural production. Through this approach, the [1]ability of hydrogel *Super Absorbent Polymer* (SAP) is expected to breakthrough the technology that is adaptive and environmentally friendly as well as increasing the productivity crops. Hydrogel as soil conditioner has many benefits for the soil, one of them is the function to hold fertilizer and water for the plants.

Water is one of the factors that are important in plant growth. One of the agents play a role in the mechanism of entering the element into the plants. Water is also a vital part in supporting of plant growth. Plants with water shortages will cause water*stress*. Thus, it decreases the moisture land which will eventually affect to the plant growth. Water *stress* also can cause osmoregulation that caused by sugar contains, amino acids, and mineral in solution (Purbajanti, *et al*, 2005). In Sannino, *et.al*. 2009, hydrogel has more benefit in environment, especially to reduce water consumption and optimize water resources in agriculture and horticulture. The research about soil characteristics affected by organic hydrogel is still new in environmental topic. For that reason, this research can give more benefit especially in the use of organic hydrogel as a soil conditioner agent, especially in agriculture application.

Generally, this research is aim to investigate the ability of organic hydrogel superabsorban in fertilizer supply inorganic nitrogen and water availability with *slow release* for the plant. This treatments are conducted using the characteristics of the soil in dried land, and its application in increasing the production of the red chili crop in the early stages in Indonesia. In spite of this, this research only focuses on hydrogel organic characterization in the laboratory scale. Further, This research investigates the incubation time period in Utisols for some characteristics of chemical and physical soil properties.

2.2. Literature Review

The hydrogel superabsorben applications in a various fields have been done in recent years, like for waste treatment, planting, material to reduce friction in pipes, coating material with no leaking, protecting cable network under the ground, making packaging, a fire extinguisher, irrigation, and in the health industry (because non-biomaterial is usually a, biodegradabilitas and biocompabilitas) (Suwardi, et al 2008; Erizal, *et all* .2013). One of the factors that can cause polymers superabsorban is –COOH. It has an active role in the binding solution that was found in polymers superabsorben and has very important role in the binding of water molecules (Swantomo, 2008). The raw materials for making various hydrogel have been done by experts. In Swantomo (2008), *poliacrilamide* and zeolite are used for making good hydrogel by using radiation technique to acquire the physical and a good stability of polymer. In addition, absorption treatment on water from hydrogel using some methods has been done by Pourjavadi *et al .(*2010) to produce hydrogel with a good swelling capacity.

Hydrogel superabsorban can also be used as a tool to provide enough water needs for the plants. This hydrogel is early applied in many agricultural sectors from small-scale to large-scale agriculture such as plantation companies. The study of hydrogel as a tool to help water availability for the plant has been done by Chirino *et al.*(2010), which showed that the application of hydrogel can increase the capacity of the binding water by the root, a water to seed, as well as resistant seed in the field. Another Research done by Agaba *et al*. (2011) and Durovic *et al.*(2012) found that the use of hydrogel could improve the ability of *sandy soil*, sand, peat, and *chernozem* as the media on the ground binding and hold that the longer as matrix land for reducing water shortages.

The hydrogel decomposition in nature has also become interesting study to observe, because the decomposition is an important factor in its function as authorization of waters that has had a limitation time. A number of studies based on this study has been done including by Subagio, 2009, that shows the treatments gave significant influence on 6-8 WAP (*week after planting*) because water treatment using hydrogel by the indicator plant is jarak pagar, although the treatments was not give significant effect for agronomy indicators in general. This was hikes by the decomposition of hydrogel mentioned by microbes in their land. The decomposition of hydrogel in nature can happen, because of hydrogel that is used comes from natural materials has week binding. For that reasons, many researchers are now starting to move to the product that has giving more advantage like synthetic material than from hydrogel natural products. Hydrogel decomposition who came from synthetic materials can occur in two characteristics type of release, when we see from bonding that happened in hydrogel, in a low pH, dilution of hydrogel that is formed due to the chemical bond will be more difficult than hydrogel formed as a result from physical reaction (Mohadi, 2007). Based on that, using the organic hydrogel for the sustainability in environmental is important. Therefore, the hydrogel characteristic was as an agent that can play a role as a soil conditioner material which is an interesting topics to be investigated.

3. Material And Methods

Testing laboratory scale was done in a laboratory Faculty of Agriculture UNPAD and Indonesian Institute of Sciences (LIPI), while greenhouse experiments were carried out in the greenhouse Faculty of Agriculture UNPAD in Jatinangor. The materials used in this experiment were hydrogel organic (basic material from cellulose), as well as a chemical material used for analysis in the laboratory. Equipment that were used in this research, in the field, a laboratory as well as in the greenhouse effect are UV/VIS with wavelength (λ) 259.5 nm; sentrifuge, *Atomic Absorption Spectrophotometry* (AAS), Spektrofotometer, pH meters, and others.

The first stage is observing the characterization of the organic hydrogel appropriate in laboratory scale (LIPI, Soil Chemistry and Soil Fertility UNPAD). This stage is very important to be done because hydrogel that we choose must meet the criteria to maximize its application in the media. This phase will be done repeatedly, to know exactly the absorption, resistant to trace hydrogel material, etc. The aims of this research is to know the incubation time of hydrogel superabsorban that is used with indicators of several characteristics chemicals properties (pH, CEC (Cation Exchangeable Capacity), Organic-C, and total N). This experiment used Randomize Block Design (RBD) with various 7 treatments different incubation time for hydrogel in media. The organic hydrogel that we used is 5 gr/kg applied in a pot that contains as much as 4 kg of soil. The treatment consists of 7 levels treatments and repeated 4 times, so the total of treatments is 28 pots :

A = control (mixed hydrogel and soil with incubation time 0 days)
B = mixed hydrogel and soil with incubation time 10 days
C = mixed hydrogel and soil with incubation time 20 days
D = mixed hydrogel and soil with incubation time 30 day
E = mixed hydrogel and soil with incubation time 40 days

F = mixed hydrogel and soil with incubation time 50 days
G = mixed hydrogel and soil with incubation time 60 days

4. Results And Discussion

4.1. Characterization Organic Hydrogel in Laboratory scale

Figures.1. Type of organic hydrogel used

Organic hydrogel that is used in this research is a product from join research UNPAD with LIPI Bandung that was tested repeatedly with a modification to suit with the agriculture needs. The basic material organic hydrogel used in this research is cellulose. This product that we used is a second trial modification of the lab scale. This hydrogel is made of the process graft copolymerization batch system in reactor which includes several stages in the process. This hydrogel products from the copolymerization process which includes analysis further characterized as follows : 1) Swelling ratio to determine the ability of the WAC (Water Absorption Capacity) , 2) Fourier Transform Infra Red (FTIR) to determine the functional groups are formed , 3) Thermal Gravimetry Analyzer (TGA) to determine the stability of the hydrogel product weight to the effect of temperature , 4) Scanning Electron Micrograph (SEM) to determine the condition of homogeneity and morphology of hydrogel materials , and 5) High Performance Liquid Chromatography (HPLC) to determine the qualitative of chromatogram profile hydrogel material.

4.2. Incubation time of hydrogel against the characteristics of the chemical properties In Ultisols

4.2.1. Soil Analysis

Analysis of the Ultisols before studies show that this soil have texture clay dust, sand with the content of 4 percent, clay 30 percent, and the dust 66 percent. The chemical characteristics from this soil are acidity level pH H_2O 5.29, ; pH KCl 4.04 ; total N content 0.19 %; organic-C 1.47 %; C/N ratio 8 ; Available-P (Bray II) 7.16 ppm ; potential-K 8.84 mg 100 g^{-1}; and Strains Base 35.54 % include low, potential-P 37.86 mg 100 g^{-1} ; and CEC cmol.kg 19.81, the cations as the Ca 3.82 $cmol.kg^{-1}$; K 0.24 $cmol.kg^{-1}$; and Na 0.20 $cmol.kg^{-1}$, Mg 2.78 $cmol.kg^{-1}$ include than contains elements such as micro Mn 3.39 mg kg^{-1}. The description of chemical and physical characteristics above shows that the original Ultisols Jatinangor that we used in this research has a medium to low fertility. It is seen from the analysis of the early Ultisols Jatinangor that there were some problems like above. Therefore, there are needs to improve fertility level of Ultisols to be made as media for planting.

4.2.2. Cation Exchange Capacity Ability (CEC)

The parameter of Cation Exchangeable Capacity (CEC) showed that the incubation time of hydrogel against these parameters provided a significant result especially in treatment 10 days incubation time period (Tabel.2). This data gives the impression that incubation period of time can increase of CEC up to 75% from early time incubation (controls) but rapidly with CEC in soil decreasing when incubation above 10 days. Organic hydrogel in general can affect CEC in soil. This was described by the mechanisms giving natural ingredients (organic) into the media which give various benefits. It is because the organic matters can form a compound complex with metal ions such as Al, Fe and Mn. Besides that, the natural ingredients into the media can increase the phosphorus (P) and Al-dd along the use of material on humic acid in the various dosage fertilizer P on Ultisols (Herviyanti, *et al*. 2012).

Table 1 . The influence of the Cation Exchange Capacity (CEC) cmol/kg in Ultisols

CEC treatment		cmol/kg
A = control (mixed hydrogel and soil with incubation time 0 days)		27,7725 ab
B = mixed hydrogel and soil with incubation time 10 days		36,7925 c
C = mixed hydrogel and soil with incubation time	20 days	28,9620 b
D = mixed hydrogel and soil with incubation time	30 day	29,2250 b
E = mixed hydrogel and soil with incubation time	40 days	30,9100 b
F = mixed hydrogel and soil with incubation time	50 days	24,9450 a
G = mixed hydrogel and soil with incubation time	60 days	28,4500 b

Description : The average value of the same column marked with the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% citical level.

Soil conditioner material is solidifying aggregate soil for preventing soil erosion and pollution. This can change the hydrophobic or hydrophilic reaction, so this binding influence the soil capacity especially in water holding capacity which can improve cation exchangeable capacity (CEC) (Dariah, 2007). Hydrogel is one of the ingredients soil condisioner because they were able to expand and absorb water and nutrition, and release for longer periods. The hydrogel with good swelling will increase the pores in soil and provide more oxygen, that mechanism good for the development plant roots (Laila, 2010).

4.2.3. Acidity Level (pH)

Acidity level (pH) is one of the important chemical property in soil. The pH can give an influence on the chemical reaction in soil and overall mainly in the establishment mechanism of binding with elements. In this parameter, the data provides a significant result with control in all treatment incubation time period (10, 20, 30, 40, 50 and 60 days) (Tabel.3). In all treatments, incubation of hydrogel can increase acidity level in soil compare to control which ranged between 4% – 8%. This behavior showed that the longer hydrogel in soil can increase the acidity level.

Table 2 . The influence of organic Hydrogel to Acidity level soil (pH)

pH Treatment		
A = control (mixed hydrogel and soil with incubation time for 0 days)		5.36 a
B = mixed hydrogel and soil with incubation time 10 days		5,56 b
C = mixed hydrogel and soil with incubation time	20 days	5,74 cd
D = mixed hydrogel and soil with incubation time	30 day	5,58 bc
E = mixed hydrogel and soil with incubation time	40 days	5,76 d
F = mixed hydrogel and soil with incubation time	50 days	5,78 d
G = mixed hydrogel and soil with incubation time	60 days	5,79 d

Description : The average value of the same column marked with the same letter are not significantly different according toDuncan's Multiple Range Test at the 5% citical level.

Hydrogel has a hydrophilic, not dissolved in water and has a cluster functional that can cause to be able to absorb water and element in the soil to hydrogel. Negative (-) charge rises from dissociation H^+ of polymer hydrogel with other cation-cation including functional group to the surface to netralizing cation exchanges and cause increasing of pH in the soil (Ardiansyah, 2006). Strong cations-cation bases to the structure of the sorption of hydrogel would be easy for plant roots sorption at the time saturated water of hidrogel. The exchangeable cation-cation base with cation acid (H^+) absorption by roots of plants will cause the decrease of cation base and increase cation acid in the soil. This can cause the decrease of saturation base and the increase of soil acidity. This argument supported by research from Ardiansyah (2006), that hydrogel application on Inceptisol can increase the pH range from 5.96 to 6.90 - 7.03.

4.2.4. Organic-C

The influence of organic hydrogel in media can increase the organic C content and gave significant results in the incubation time period 30 dan 40 days, but decrease againt in 50 days (Tabel.3). In general, decomposition organic hydrogel into soil can add organic matter. This was strengthened in Wulansari (2009) which found that the decomposition hydrogel will disintegrate CO_2, nitrogen and water, so for that reason C content, N soil and water availabilities in the ground will increase.

Table 3 . The influence of organic Hydrogel The organic C Soil (%)

Treatment by C-organic		%
A = control (mixed hydrogel and soil with incubation time 0 days)		1,8975 a
B = mixed hydrogel and soil with incubation time 10 days		1,9225 a
C = mixed hydrogel and soil with incubation time	20 days	1,9400 a
D = mixed hydrogel and soil with incubation time	30 day	2,3350 b
E = mixed hydrogel and soil with incubation time	40 days	2,4125 b
F = mixed hydrogel and soil with incubation time	50 days	1,7875 a
G = mixed hydrogel and soil with incubation time	60 days	2,0200 a

Description : The average value of the same column marked with the same letter are not significantly different according toDuncan's Multiple Range Test at the 5% citical level.

According to Degaiorg (2002) in Barihi (2013), application of polymers superabsorben (hydrogel) had an influence on the increase of activities microorganisms on the soil and mikoriza. The increasing of the activities soil mikrorganism relevant with availability of carbon (C) due a source of energy/food for that organism. The results from Rahmatsyah (2010) gave information that the application of material soil condisioner liquid organic provided the increase of organic C in Ultisol Jatinangor from 1.04 percent to 2.07 percent. As a biologically system, organic matter is a source of energy and increases the populations of microorganisms. (Petchawee & Chaitep, 1995).

4.2.5. Total-N Soil

The effects of time incubation on total N gave interesting data. This treatment shows that the application of organic hidrogel significantly different between control (0 days) in treatment 10 days but not to others. (30, 40, 50 and 60) (Table.4). In this data, we can see that if we are doing incubation of hydrogel more than 10 days, total N in soil will decreasing. Nitrogen (N) is a mobil element with behaviour easily leaching and lost through evaporation. In this experiment, there is no additional water into the media at the time of the first time incubation period. For that reason, the leaching is not a main cause factor to be the decreasing of N in soil but evaporation process from media. Superabsorbent hydrogel or polymer composites (SAPC) is a three dimensional network of polymer chains with mild crosslinking that bring the ionic dissociation of functional groups such as carboxylic acids, carbocyamida, hydroxyl, amine, imide, and other groups.

Table 4 . The influence of organic Hydrogel Total-N Soil (%)

Treatment total N		%
A = control (mixed hydrogel and soil with incubation time 0 days)		0.2150 ab
B = mixed hydrogel and soil with incubation time 10 days		0.3300 c
C = mixed hydrogel and soil with incubation time	20 days	0.2000 a
D = mixed hydrogel and soil with incubation time	30 day	0.2575 b
E = mixed hydrogel and soil with incubation time	40 days	0.1850 a
F = mixed hydrogel and soil with incubation time	50 days	0.1825 a
G = mixed hydrogel and soil with incubation time	60 days	0.1725 a

Description : The average value of the same column marked with the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% citical level.

5. Conclusions

The application of hydrogel organics (cellulose based hydrogels) in soil are biocompatible and biodegradable materials which show promise in environmental issues especially in agricultural applications. Several chemical properties in soil (Ultisols) gave influence significant effect to control treatments consists of Cation Exchangeable Capacity (CEC), Total-N, organic-C and acidicy level in soil. Generally, the apllication of organic hydrogel in soil in a certain incubation time, can gave better influences to some chemical properties in soil.

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Synthesis and Characterization Of Nio Nanoparticles By Sol-Gel And Sonochemical Methods

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Abstract

Preparation of nickle oxide (NiO) nanoparticles by sol-gel process and sonochemical method have been studied. NiO nanopowders were obtained by sol-gel method and sonocemichal method by using nickle nitrate hexahydrate 2 M, methanol and sodium hidroxide 5 M were used as precursor, solvent and agent precipitator, respectively. The powders were formed by drying in the temperature of 110-110 ^{o}C and after heating at ± 450 ^{o}C for ± (1) one hour. The products were obtained black powders. The products were characterized by Energy Dispesive X-ray Fluorescence (ED-XRF), X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM). The ED-XRF pattern show that composition of NiO that produced was 96.9%. The XRD patterns showed NiO forms were produced generally. Crystallite sizes of NiO by sol-gel method was obtained in the range 72.16 nm and crystallite sizes of NiO by sonochemical method was 41 nm. SEM micrograph clearly showed that powder had a spheric with uniform distribution size is 0.1-1.0 μm approximately for sol-gel method and diameter size in the range 90-100 nm for sonochemical method. Sonochemical method is better for preparation of NiO nanopowder than sol-gel method by using the same precursor.

Keywords: nickle oxide, precursor, solvents, additive, nanopowder, sol-gel, sonochemical, spheric

1. Introduction

The study of nanomaterial has been an the subject of active in the last decade. Nanoparticles are classified as being materials in which at least one dimension of the material is less than 100 nanometers in diameter. Nanoparticles have unique properties, such as having increasing electrical conductivity, toughness, ductility and formability of ceramics.

Nickle oxide (NiO) is a p-type semoconductor that can be used in anti-ferro-magnetic film and optical device (Rasoul, et al, 2012), supermagnetic device, electronic devices, and catalyst (Mohammadyani, et al, 2012), supercapacitors (Junqing, et al, 2012), alkaline batteries cathode, gas sensors, solid oxide fuel cell anode and in the manufacture of electrochromic (Bahari, et al, 2008). NiO has a large exciton binding energy and a wide band gap ranging from 3.6 to 4.0 eV (Mohammadyani, et al, 2012), NiO has density 6.67 g/cm^3, with melting point 1955 ^{o}C (Sharma, A, et al, 2013). NiO nanoparticles show many unique optical, electrical, magnetic and chemical properties (Moravec, et al, 2011). NiO is one of the useful metal oxide and which has so many applications in different fields.

Synthesize techniques are closely related to the particle structural property such as partice size, distribution particle and morphology. There are many methods for preparation of NiO nanopowders including solid state process (Barakat, A, et al, 2013), solvothermal process, thermal process, chemical precipitation (Bahari, 2008 and Darakhshi, 2013), soft chemical synthesis route (Chakrabarty, et al, 2009) involved solid liquid seperation, washing and drying process. Another process are microwave irradiation method (Rasoul, and Mohammadyani, et al), liquid phase process, spray pirolysis process, electroplating of nickle particle (Junqing, et al, 2012). Metal organic chemical vapor deposition (MOCVD) can be use for NiO nanopowder preparation (Moravec, et al, 2011), but this method have a number disadvantages such as quite expensive, uses volatile compound and need complicated reactor. The NiO nanosheets were successfully prepared by using chemical bath deposition and hydrothermal process (Yao, Yu et al). NiO nanopowder can prepare by using polymer-matrix assisted synthesis and spray pyrolysis, but crystalite size and homogenity were small. (Dharmaraj, N, et al, 2006).

In this work, we studied NiO nanoparticle synthesis by sol-gel process and sonochemical process by using nickle nitrate hexahydrate as precursor, methanol as solvent and sodium hydroxide 5 M as additive. In this research we studied effectiveness of these method for synthesis of NiO. Sol-gel and sonochemical methods have a number of advantages over other methods such as inexpensive equipment, homogenity product and easily in processing.

2. Material and Methods

All the chemical reagents used in this experiment were of analytical grade and without further purification. Powder NiO prepared by sol- gel and sonochemical methods by using nickle nitrate hexahydrate 2 M from Merck as precursor, methanol (p.a) as solvent, sodium hidroxide as additive. 2.0 M NaOH was

added drop wise 20 mL of 1.0 M $Ni(NO_3)_2.6H_2O$ solution which was stirred by magnetic stirring apparatus, keep stirring the solution for ± 1 hour at room temperature. Light green suspension was produced. It was then dried at 100-110 ^oC for ± 1 hour in oven, the green powder was produced. The product then transferred to porcelain crucible and put in furnace at ± 450 ^oC for ± 1 hour, and finally black powder produced. Preparation of NiO by sonochemical process by using ultrasonic cleaner with power was 50 Watt and sonolysis time was ± 10 minutes for solution of NiO.

The synthesized NiO nanoparticles were characterized by X-ray Diffraction (XRD) with a diffractometer by using monochromatic CuKα with λ = 1.54060 was used for determination of crystal stucture. Energy Dispersive X-ray Fluorescence (ED-XRF) for composition analysis and morphological study was carried out by Scanning Electron Microscopy (SEM).

3. Results and Discussion

Preparation of sol for synthesis NiO by using nickle nitrate hexahydrate as precursor and methanol as solvent. NaOH 5M added to solution as precipitation agent. The reaction can be seen below:

$2\ NaOH(aq) + Ni(NO_3)_2.6H_2O\ (aq) \rightarrow 2NaNO_3\ (aq) + Ni(OH)_2.6H_2O\ (aq)$

$Ni(OH)_2.6H_2O \rightarrow Ni(OH)_2 + 6H_2O$

NiO was produced after calcination at ±450 ^oC in furnace, the reaction below:

$Ni(OH)_2 \rightarrow NiO + H_2O$

Fig. 1 a and b show the $Ni(OH)_2.6H_2O$ gels that produced by sol-gel method and sonochemical method with NaOH as precipitation agent at 100-110 ^oC for ±1 hour in oven. The color of $Ni(OH)_2.6H_2O$ gel was light green. The morphology of $Ni(OH)_2.6H_2O$ was homogen (visual) with sol-gel process. After heating process at 100-110^oC, the solvent wil evaporate. The morphology of $Ni(OH)_2.6H_2O$ was more homogen (visual) than sol-gel method (b)

Fig. 2 a and b show the NiO powders after calcination process in furnace at ± 450 ^oC for ± 1 hour. The black powders produced. The powders in visual were homogen, particle size was very small and light black. The NiO powder prepared by using sonochemical process more homogen than the sol-gel process. The product that produced by sonochemical were black, uniform, and the particle size was small.

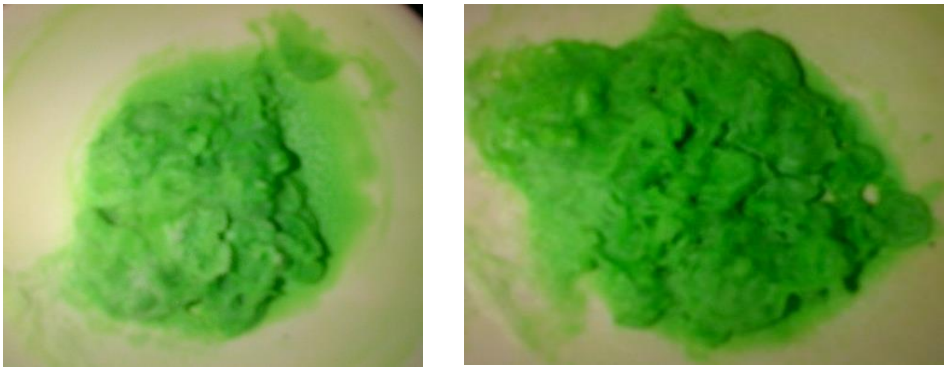

Fig. 1 a) Gel $Ni(OH)_2.6H_2O$ with sol-gel method (left) and b) Gel $Ni(OH)_2.6H_2O$) with sonochemical method (right)

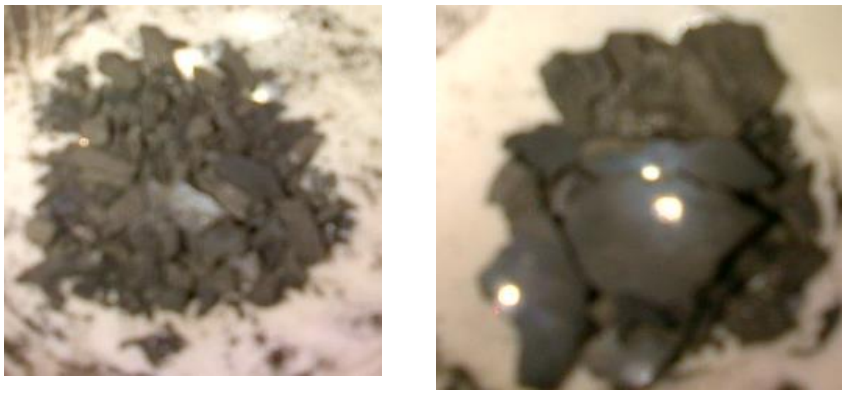

Fig.2 a) NiO powder with sol-gel process (left) and b) NiO powder with sonochemical method (right)

Fig. 3 shows the ED-XRF pattern of NiO powder by sol-gel process. From this curve can be seen that composition of NiO is 96.904 %, Al_2O_3 is 0.639 %, P_2O_5 is 0.873%, CaO is 0.141 %. From this curve showed that composition of NiO is highest.

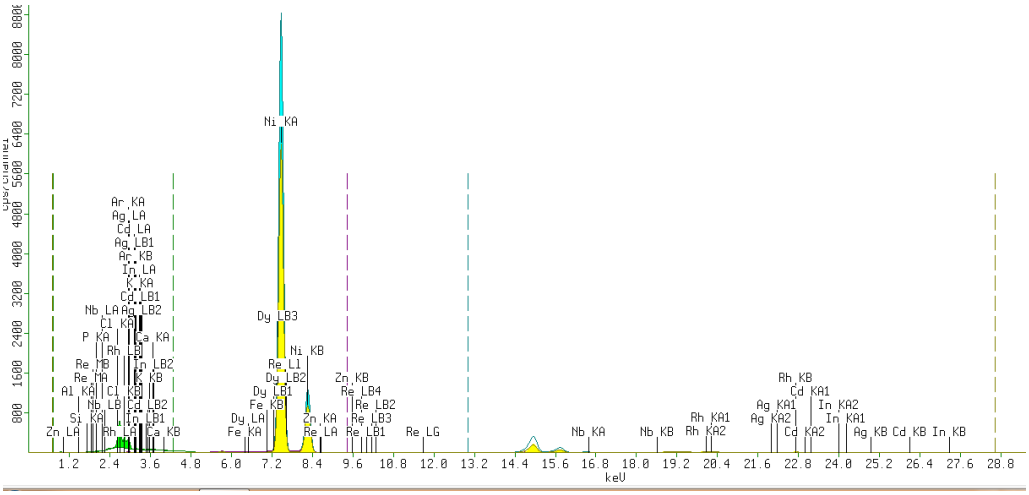

Fig. 3 ED-XRF pattern of NiO at 450 °C with sol-gel process

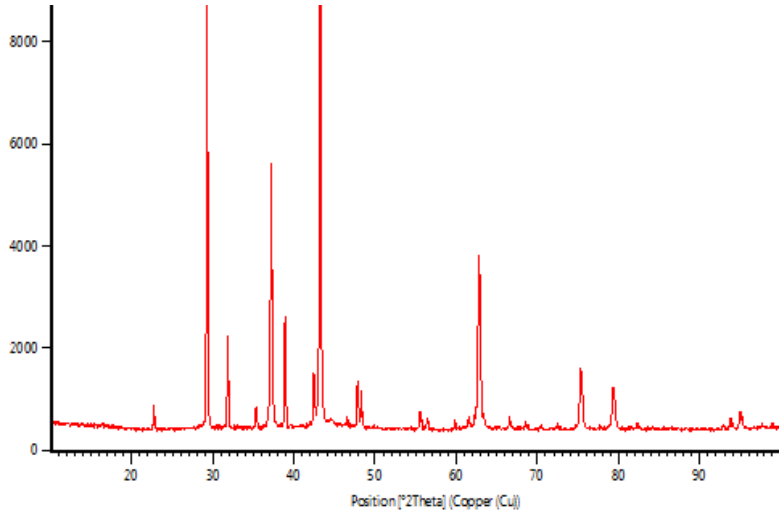

Fig. 4 X-ray diffraction pattern for NiO at 450 °C with sol-gel process

Fig. 4 shows the X-ray diffraction pattern of the black powder formed by using sol-gel method. XRD pattern of NiO powder at temperature of 450°C, the highest intensity with typical peak at 2θ = 29.31; 31.79; 37.18; 38.87; 42.46; 43.20 and 47.82. X-ray diffraction pattern of this powder is NiO in cubic form. Impurity product produced in this synthesis caused by NiO powder not washed with water. The final product appeared in black color.

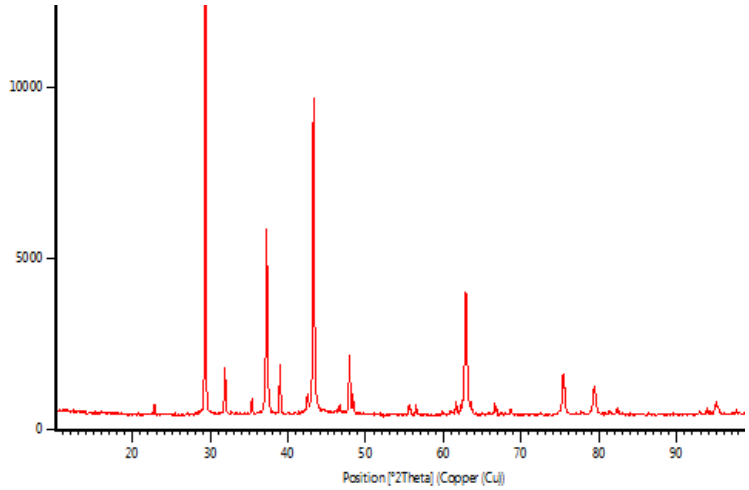

Fig. 5 X-ray diffraction pattern for NiO at 450 °C with sonochemical method

Fig. 5 shows the X-ray diffraction pattern of the black powder formed after sonochemical process. XRD pattern of NiO powder at temperature of 450°C, the highest intensity with typical peak at 2θ = 29.34; 31.84; 37.22; 43.25; 47.86. XRD analysis showed that this substance is a typical cubic NiO (JCPDS No. 01-073-1523) and impurity of $NaNO_3$ (JCPDS No. 01-079-2056). Impurity product produced in this synthesis caused by NiO powder not washed with water. The final product appeared in black color. Fig. 5 indicates high crystallinity of NiO nanopowder.According to Scherer equation, crystallite size generally decreases with broadening the longest XRD peaks. The mean crystallite size was calculated by the application of the Scherer equation:

$$D = \frac{K.\lambda}{\beta \cos\theta}$$

where:
D is the mean crystallite size of the powder,
λ is 0.15406 nm is the wavelength of CuKα,
β is the ***Full Widht at Half Maximum*** (FWHM) intensity of 2θ x (π/180),
θ is Bragg's diffraction angle, and
K is a constant usually equal to 0.89

The mean crystallite size (D) for NiO powder prepared by using sol-gel method:

$$D = \frac{0.15406\,x\,0.89}{(0.1791 x 3.14/180) x\ \cos(43.20/2)} = 72.16nm$$

The mean crystallite size (D) for NiO powder prepared by using sonochemical method:

$$D = \frac{0.15406\,x\,0.89}{(0.2047\,x\,3.14/180) x\ \cos(43.25/2)} = 41.30nm$$

The crystallite size (D) for NiO powder was 72.16 nm with sol-gel process and NiO powder size for sonochemical process was 41.30 nm. From this research, theproduct that produced by sonochemical method is smaller than sol-gel process. This small particle is very useful for adsorbent and catalyst.
Figure 5. shows the micrograph of NiO nanopowder prepared by sol-gel method that characterized by using Scanning Electron Microscopy. It is clear that in all cases, NiO nanopowder was succesfull prepared by sonochemical methods. The range particle size vary between 90-120 nm are observable in the figure. From the SEM analysis, the microstructure of NiO nanopowder was produced in spherical form. Fig.5 a-d shows the highly agregated spherical nanoparticles.

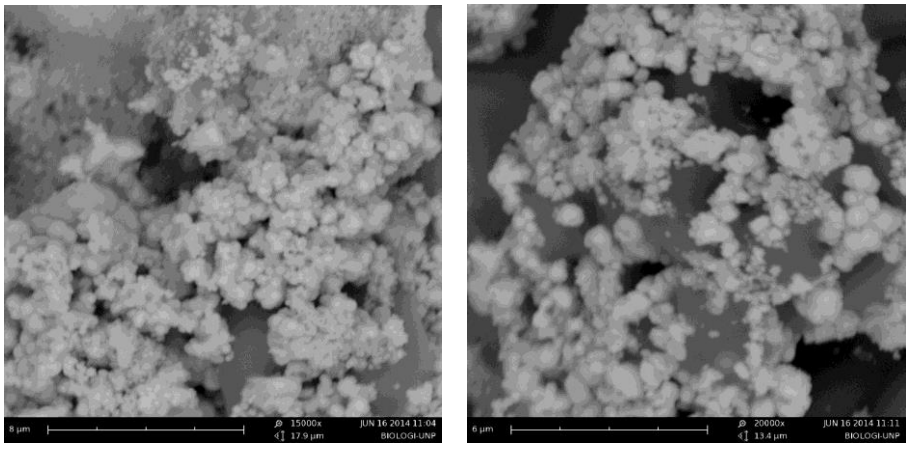

Fig. 5 SEM Micrograph of NiO powder by sol-gel method (a) 15000x, (b) 20000 x

Figure 6. shows the micrograph of NiO nanopowder synthesized that characterized by using Scanning Electron Microscopy. It is clear that in all cases, NiO nanopowder was succesfull prepared by sonochemical method. The range particle size vary between 90-120 nm are observable in the figure. From the SEM analysis, the microstructure of NiO nanopowder was produced in spherical form. Fig.5 a-d shows the highly agregated spherical nanoparticles.

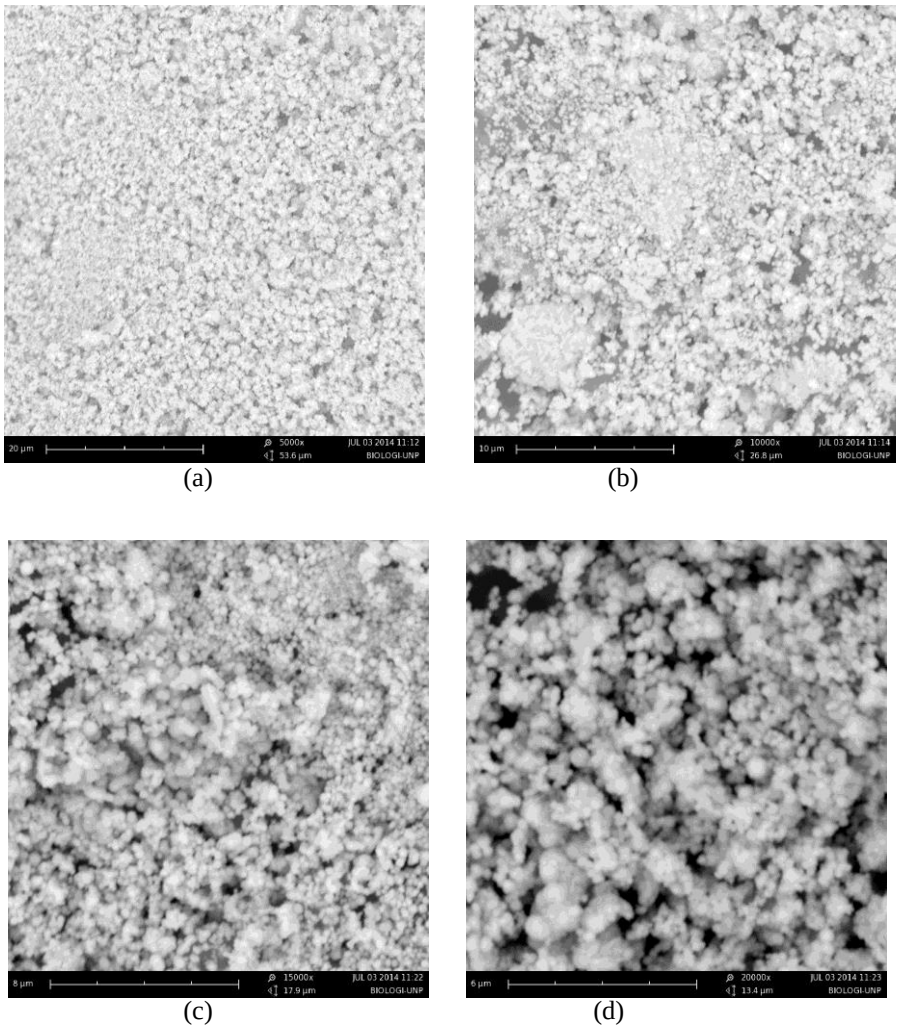

Fig. 6 SEM Micrograph of NiO powder by sonochemical method (a) 5000x, (b) 10000 x, (c) 15000x, (d) 20000x

4. Conclusion

NiO nanopowders were succesfully synthesized by sol-gel method and sonochemical method by using nickle nitrate hexahydrate as precursor, methanol as solvent and sodium hydroxide 5M as precipitation agent (additive). The NiO nanopowders were produced by calcination process in furnace at ± 450 ^{o}C for ± 1 hour, with black powder. The crystallite size of NiO nanopowder that prepared by sol-gel process was 72.16 nm by calculating with Scherer Equation. NiO powder that synthesized by sonochemical method was 41.30 nm by calculating with Scherer equation. The structure of NiO nanopowders were in cubic forms. Micrographs of NiO nanopowders were in spherical form with diameter size in range 0.1-1.0 μm with sol-gel process and diameter size in range 90-120 nm with sonochemical method. Sonochemical method more effective for NiO nanopowder preparation.

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Investigation of Surfactant Anionic Effect on Morphology and Performance of Cellulose acetate-Polystyrene Nanofiltration Membrane for Desalination

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Abstract

The effects of surfactant anionic sodium dodecyl sulphate (SDS) on the performance of cellulose acetate (CA) – polystyrene (PS) membranes nanofiltration were evaluated. The membrane prepared via immersion precipitation system. The morphology and performance of prepared membranes were studied by scanning electron microscope (SEM) and separation experiments using pure water and salt as feed. The obtained results indicate that the addition of surfactant in casting solution increases the porosity of membranes layer, enhance pure water flux and water permeation.

Keywords : cellulose acetate, membrane, polystyrene, sodium dodecyl sulphate.

1. Introduction

During the last decade, nanofiltration (NF) membranes have been heavily studied because they provide specific advantages over conventional reverse osmosis membranes for water filtration. NF operations normally produce a higher water flux and can often operate at lower pressures, which reduce energy consumption. NF membranes can be categorized into two major classes according to their material properties. Most commercial NF membranes are fabricated from organic matter such as polyetersulfone, cellulose acetate, polystyrene etc. Normally, pore sizes in NF membranes are too large to expect any significant rejection of small charge solutes, such as the salts that are present in brackish water, based on size exclusion [1].

Membrane preparation is very important way to obtain the morphology NF membrane. Technique of preparation casting solution also has much effect of forming pore sizes of NF membrane. The phase inversion via immersion is well known process for preparing a variety of asymmetric membranes. In this technique, a homogenous polymer solution containing polymer and adequate solvent with or without an additive in cast on glass plate and immersed in a coagulation bath (insome cases after a short period of solvent evaporation). The diffusive exchange of solvent and non solvent introduces liquid-liquid phase separation i.e. the formation of a polymer rich and a polymer lean phase in casting solution lowers the Gibbs free energy of mixing. The successive solidification of the phase separated solution leads to a porous, asymmetric structure. The morphology and performance of membranes depend strongly on the thermodynamics as well as kinetics of the phase inversion process [2].

Surfactants which are known as surface-active matters reduce surface tension in water and other liquid [3]. The surface activity of surfactants derives from their amphiphilic structure that posses both hydrophilic and hydrophobic parts in one molecule. Surfactants are classified into four groups depending on the charge of the hydrophilic part: nonionic (0), anionic(-), cationic (+) and zwitterionic (±). Synthetic surfactants are economically important in organic chemicals. They have very common applications in science and industry from primary processes such as the recovery and purification of raw materials to more complex processes such as improving the quality of finished products such as paints, cleanings, cosmetics, pharmaceuticals, and foods.

Cellulose acetate (CA), being an environment friendly product of sustainable resources, is an interesting polymer with respect to its low price, moderate chlorine resistance, good biocompatibility and high hydrophylicity Due to its highly hydrophilic properties, it is known as a low fouling membrane for aqueous filtration [4]. Usually, CA membrane is prepared via the phase inversion technique. Average pore size and effective porosity of CA membranes can be controlled by the polymer concentration in the casting solution. In order to improve separation properties of CA membrane, many efforts have been made. Some researchers modified membranes through changing the solvent in the membrane casting solution. Others tried to improve performance by employing different kind of additives or pore-forming agents. For instance, Arthanareeswaran et al, 2004, prepared CA ultrafiltration membranes with polyethylene glycol 600 as an additive. Sivakumar et al. succeeded in increasing water flux of CA-polysulfone blend ultrafiltration membranes by using polyvinylpyrrolidone as a pore-forming agent. Cailing et al 2007, studied to enhanced permeation performance of CA ultrafiltration membrane by incorporation of surfactant nonionic as a pore-forming agent.

The present study however attempts to enhance performance of CA membrane by using polystyrene (PS) as additive agent and sodium dodecyl sulphate (SDS) as a pore-forming agent. FTIR spectroscopy was

employed to measure the blending process CA-PS, and also the residual SDS content in the modified CA-PS membranes. Tensile test was introduced to evaluate the mechanical strength of the membranes. Scanning electron microscopy (SEM) was used to characterize the structural morphology of the membranes. The NaCl solution was used as a model salt to probe the rejection behavior of the membranes.

2. Experimental

A. Materials

CA (Mw 30.000, 39.9w% acetyl content) was procured from Brata Chemical Co. (Bandung, Indonesia), Polystyrene was from Chemical Industry Indonesia and SDS ($C_{12}H_{25}OSO_3Na$) with molecular weight 288.38, was from Sigma. Sodium chloride and dichloromethane both were purchased from Merck and Acetone as a solution was from Sigma.

B. Preparation of membranes

Homogenous solution of CA dissolved in dichloromethane was prepared using polystyrene as invariable additive and SDS as variant additive by stirring for 16 h at room temperature. The process of preparation of membrane followed blending semi-continues process. The solution then was left for 4 h to allow complete release the bubbles. The solution was sprinkled and cast on glass plate substrate and moved to the deionized water bath for immersion precipitation. The immersion process was conducted at room temperature. After primarily phase separation and formation of membrane, in order to guarantee complete phase separation, the membrane was stored in water for 24 h. This allows the water soluble components in the membrane to be leached out. As the final stage, the membrane was dried by placing between two sheets of filter paper for 24 h at room temperature. The composition of casting solution is shown in Table 1.

Table 1. Composition of CA in casting solution

CA (wt%)	PS (wt%)	DCM /Aceton	SDS (wt%)
9	1	90	-
9	1	89.5	0.5
9	1	89	1
9	1	88.5	1.5
9	1	88	2

C. Characterization of CA-PS membranes

The cross section morphology of membranes was imaged by scanning electron microscopy (SEM) using Phyllips scanning microscope and topography of membrane was imaged by atomic force microscope (AFM). The samples of membrane were frozen in liquid nitrogen and fractured. After sputtering with gold, they were viewed with the microscope at 20 kV.

The stress-strain relationships were measured with a material testing machine FTIR Spectrometer was used to investigate the blending process of CA-PS and the residual SDS in the membrane.

D. Flux and retention

The performance of the membrane was characterized using cross-flow system. This laboratory scale system includes reservoir, a pump, valves, pressure regulation and UF/NF cell. The feed was re-circulated to the reservoir and permeate was collected and weighted. The cross-flow cell house flat sheet membrane coupons with an effective area of 34 cm^2. The NaCl solution was employed as a feed for membrane performance and fouling evaluation. Each membrane was compressed with pure water at 20 psi for 1h and then the flux water was calculated by the following equation:

$$J = \frac{V}{A\Delta t}$$

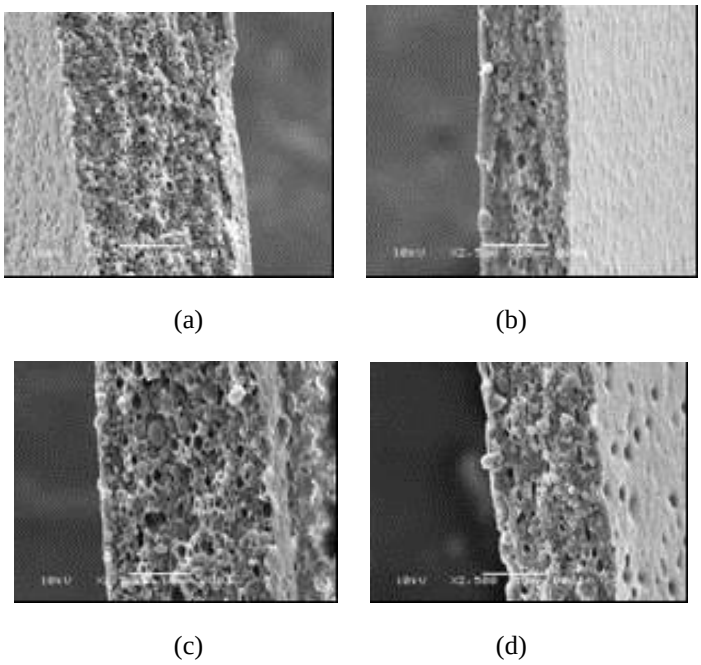
3. Results and Discussion

A. Characterization of CA-PS membranes

The CA-PS membranes have distinct advantages such as superior hydrophilicity, high antifouling property, and low price. However, their permeation performance and salt rejection property are often

unsatisfactory. PS is a polymer additive was function in mechanical property of membrane to increase strengthener's membrane. In order to prepare CA-PS membranes with suitable NF performance, SDS was added into CA-PS casting solution by semicontinues blending process. The addition of SDS in the casting solution can influence the membrane morphology and structure.

SEM photographs of cross section of CA-PS0.5, CA-PS1, CA-PS1.5, and CA-PS2 are depicted in Fig. 1(a)-(d). It is apparent that all the membranes exhibit a characteristic morphology of asymmetric membrane which consists of a dense top layer and porous sub-layer. In particular, the thickness of top layer decreased and the pore volume in the porous sub-layer increased with an increasing SDS content. This corresponds with the findings of Cailing et al. 2007.



(a) (b)

(c) (d)

Fig.1(a)-(d) Cross-sectional SEM morphology of the membrane matrix for CA-PS0.5, CA-PS1, CA-PS1.5, CA-PS2 membranes respectively.

The figure also showed the amplified SEM photographs of the membrane matrix for CA-PS0.5, CA-PS1, CA-PS1.5, CA-PS2 membranes respectively. As clearly seen, the spongy structure gradually became compact from CA-PS0.5 to CA-PS2 membrane. This constituted the possible evidence that the water content of CA-PS membranes increased with an increase in SDS.

B. Permeation properties of CA-PS membranes

Fig. 2 shows the effect of SDS concentration on pure water flux and rejection value for the membranes. The pure water flux increases gradually with increasing SDS concentration in the casting solution. It is evident that SDS could leach out from the membrane matrix. This finding is in contrast with results of Cailing *et.al*.. They reported that surfactant (PEG2000) used in the casting solution of membrane CA could not leach out from the membrane matrix due to too strong interaction between CA and PEG.

For asymmetric membrane, the pore size of the skin layer usually influence molecular sieving characteristic of nanofiltration. The salt rejection ratio of CA-PS membranes are also shown in Fig.2. All the modified CA-PS membranes exhibited high salt rejection ratio value. CA-PS1 had highest rejection ratio value. It was established that the addition of SDS in the casting solution favored the formation of smallest pores on the skin layer during coagulation process of membrane. This is also evident that utilizing additive agent with smallest molecular weight in membrane casting solution will obtain the smallest pore of membrane.

C. Study of FTIR

In order to obtain the quantitative information for CA-PS matrix membrane, in the current study FTIR was employed to measure of the crosslinking between CA and PS. Spectra of PS has specific spectra with strong sorption in 3024.2 cm-1 and indicated C-H benzene group. The wave number 1450,4 of C=C aromatic was supported the existing benzene group. Spectra of CA showed a strong sorption in wave number of 1751.2 cm^{-1} which was exhibited due to C=O ester group. This suggests that CA has acetyl group. But, there was another –OH group in 3475 cm^{-1}, this obviously indicates that acetylating reaction of CA compound was not complete. CA had acetyl number only 39.9% or equal with substitution degree of 2.8. Due to acetyl

number of CA compound, CA only dissolves in dichloromethane. Another spectrum was observed at 1049,2 cm-1 (C-O group). Availability of C=O group in CA is supported by availability of C-O group. Specific peaks of sorption at CA and PS both exhibited the composite membrane CA-PS. Spectra FTIR of showed of C=O ester group in wave number 1751.2 cm-1 and C-O group in wave number 1049.2 cm-1. This indicated the specific spectra for CA. Similar was the case of PS wherein specific spectra is exhibited in the spectra of FTIR. Sorption peak in wave number 3024.2 cm-1 for C-H benzene group and 1446.5 cm-1 for C=C aromatic group. Composite membrane CA-PS was not obtaining new compound and not reaction happened between CA and PS. Matrix membrane were built by crosslinking between CA and PS.

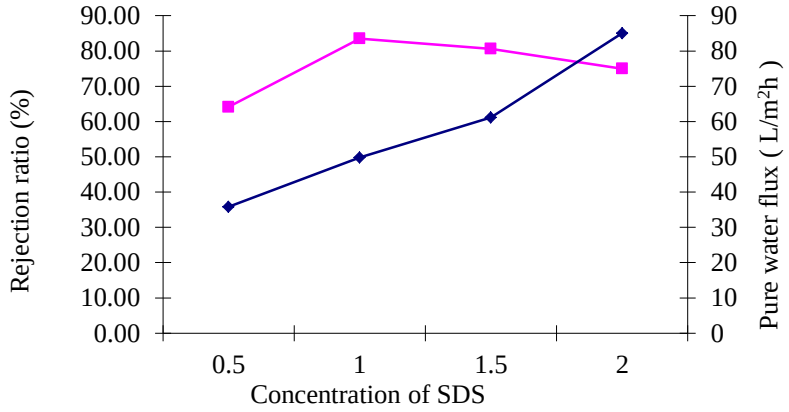


Fig. 2. Pure water flux and NaCl rejection ratio of CA-PS membranes as a function of SDS content.

4. Conclusion

From this study, it is concluded that the addition of small amount of surfactant (SDS) in the casting solution increases the formation of pore in the sublayer of membrane. This enhances the pure water flux and salt rejection ratio value. Matrix membrane were built by uncrosslinking between CA and PS.

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Isolation and Characterization of Triterpenoid Compoundfrom Indonesian Sarang-Semut Plant Tubers (Myrmecodia pendans Merr. & L.M. Perry)

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Abstract

Sarang-semut plant (*Myrmecodia pendans* Merr. & L.M. Perry) is one of the Indonesia's endemic plants. The purpose of this study was to isolate and characterize new compound from *M. pendans* tubers. This compound isolation process began with extraction of *M. pendans* tubers using soxhlet apparatus. The extract obtained then evaporated by rotary evaporator at the temperature of 40°C, then it was separated and purified by using variety of chromatography techniques. Pure compound obtained was characterized using various spectroscopic methods. We obtained compound **1** with a mass of 23 mg that supposed to be the compound from the group of triterpenoids.

Keywords:Myrmecodia pendans Merr. & L.M. Perry, sarang semut plant, and triterpenoid.

1. Introduction

Myrmecodia pendans Merr. & L.M. Perry. or sarang semut plant, a medicinal plant of the Rubiaceae family [1, 2], is known as lokon in Papua [3] as well as by other local names. It is commonly found from Burma and Indochina throughout the Philippines, Malaysia, Indonesia and Papua New Guinea, to northern Queensland, the New Hebrides and Fiji [3]. Its tuber possesses antibacterial and immunomodulatory effects, and is used for the relief of ulcer, haemorrhoid, nosebleed, backache, skin rashes, allergy, uric acid disorder, stroke, coronary heart problem, tuberculosis, tumor, cancer, hepatitis, rheumatism and diarrhea [4, 5, 6, 7, 8].

M. pendans tubers inhabited by *Ochetellus sp* ants. Its tubers generally round shape when young, later becoming oval shortened or elongated after the old. It has a network system with some typical holes. The temperature inside the tuber is quite stable, so the ant colonies nested inside the tuber [7].

In a long time, there were natural interactions between the compounds released by the ants with the compound from the sarang semut plant tubers that produce active compounds which are capable of treating various diseases. The antitumor activity of the *M. pendans* was previously reported [9]. Additionally, its immunomodulator activity was described [10]. However, just a few studies which examined pure compounds from *M. pendans*. Herein, we report the isolation and structure elucidation of the pure compound isolated from *M. pendans* tubers.

2. Materials and Methods

2.1 General Experimental Procedure

The UV spectra was obtained on Varian 100 spectrophotometers. NMR spectra was recorded with a JEOL JNM A-500 spectrophotometer using TMS as an internal standard. Open column liquid chromatography using Merk silica gel 60 GF_{254} (70-230 mesh), and TLC analysis on precoated Kieselgel 60 GF_{254} 0,25 mm.

2.2 Plant Material

Samples of the tubers of *M. pendans* were obtained from Ayawasi village, South Sorong Regency, West Papua, Indonesia. The plant was identified by a staff at the Laboratory of Plant Taxonomy, Department of Biology, Universitas Padjadjaran.

2.3 Extraction and Isolation

The dried tubers (600 g) of *M. pendans* were extracted in EtOAc using soxhlet apparatus. Evaporation of the EtOAc gave an aqueous concentrate. The extract (15 g) was chromatographed on silica gel G 60 using a solvent system of *n*-hexane-EtOAc (3:7) to yield a fraction (400 mg). The fraction was eluted with *n*-hexane and acetone (3:2) to yield another fraction (100 mg). It were further chromatographed on silica gel G 60 to yield an active compound **1** (23 mg).

3. Result and Discussion

The isolate **1** was obtained as white oil with a mass of 23 mg and did not fluoresce under UV light either at 254nm or 365nm wavelengths. This behavior shows that compound **1** contains no electronic conjugation ($n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transition). In other words, compound **1** did not have conjugated double bonds.

Based on the UV spectrophotometer measurement using methanol as the solvent, there is a maximum peak absorption at 203 nm. These data indicate that the compound **1** are thought to have a saturated bond which has free electrons (C-O) which can lead to electronic transitions $n \rightarrow \sigma^*$.

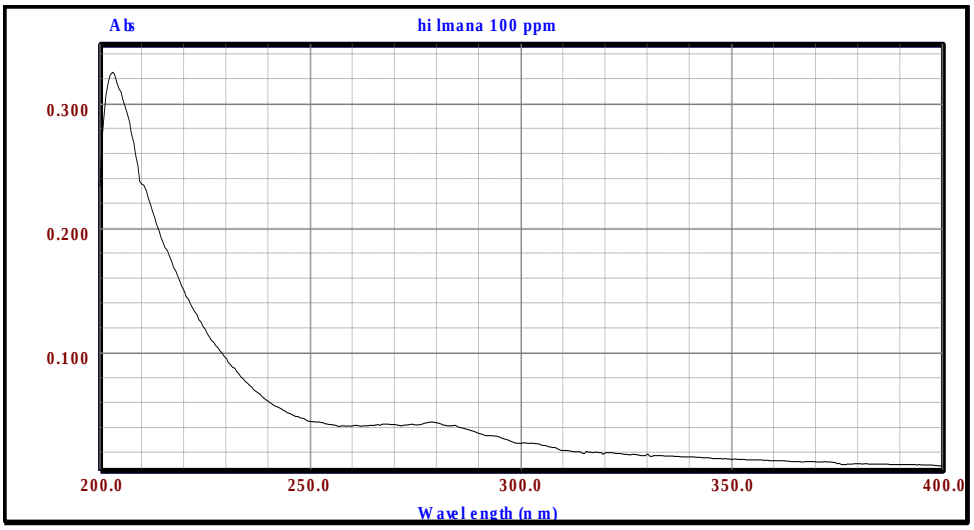

Fig.1 Ultraviolet spectrum of compound 1

Based on the TLC analysis, isolate **1** produces a spot that did not fluoresce in UV lights and have purple color. From this information, we infer that isolate **1** suppose to be the compound from the group of triterpenoids and from UV spectrum we knew that compound **1** has oxygenated carbons. It means that compound **1** is a triterpenoid with a lot of hydroxy substituents. Due to unfinished NMR spectra interpretation, we only able to provide temporary allegation for the structure of compound **1** as above.

Acknowledgements

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The Study on The Effect of Phytochemical Properties in Aloe vera Extract To Oral Candida in Cancer Survivor

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Abstract

Ethanol extract of aloe vera has known as antifungal for oral Candida Spp., because of its phytochemical properties. The purpose of this study is to investigate the effect of phytochemical properties in Aloe vera extract to oral Candida in cancer survivors who was under chemotherapy. The methods in this study are Aloe vera were extracted using maceration technique and phytochemical screening has done. Aloe vera extract has given as single use oral rinse. Candida spp. was collected before and after rinsing with aloe vera extract, using Oral Rinse Concentrate technique and inoculated at Sabouraud's Dextrose Agar (SDA). The number of candida's colony forming units were counted. The result of this research showed that Aloe vera extract has phytochemical properties such as alkaloid, flavonoid, steroid, saponin, polyphenol, quinone and triterpenoid and reduce the number of oral Candida's colony were 37.45 % and 26.43% in average. The conclusion of this study is the Aloe vera extract can be used as antifungal agents.

Keywords: Phytochemical Properties, Aloe vera Extract, Oral Candida, Antifungal, Cancer Survivor.

Nomenclature

ATCC	American Type Culture Cells
AVL	Aloe vera leaf , the extract only made from the green skin of Aloe vera plants/leaf.
AVG	Aloe vera gel, the extract only made from inner gel of Aloe vera plants/leaf.
AVW	Aloe vera whole, the extract made from the green skin and the inner gel of Aloe vera plants/leaf.
CFU/ml	Colony Forming Units/ml saliva.
PBS	Phosphate Buffer Saline.
v/v	Ratio concentration (volume/volume).

1. Introduction

Cancer patients can suffer oral toxic effects secondary to antineoplastic therapy in the form of chemotherapy, conditioned by a range of factors, including the high cell turnover rate of the oral mucosa, the diversity and complexity of the oral micro flora, and soft tissue trauma during normal oral function. Chemotherapy as one of the cancer treatment also induce the increasing number of oral candida's colonization.[1] Oral candidiasis is one of the oral complication in the treatment of cancer patients and being preceded with the colonization of Candida species in the mouth. The symptoms of oral candidiasis have negative impact on the quality of life and can impair nutritional intake of the cancer survivor.[2]

Lalla, et al., has reported that the prevalence of oral candidiasis was 7.5% pre-treatment, 39.1% during treatment, and 32.6% after the end of cancer therapy. The prevalence of oral candidiasis was 38% and the prevalence of oral colonization with fungal organisms was 72.8%, during chemotherapy.[3]

Treatment for oral candidiasis is antifungal agents, such as Polyene antibiotics and azoles, but recently the incidences of resistance has increased. An epidemiology study conducted by Schelenz S., et al., revealed that the overall resistance to azoles was 28.2% from 266 respondents. Resistance to specific drugs was also seen for fluconazole (4.5%), itraconazole (11.7%), ketoconazole (11.3%), voriconazole (0.75%) and caspofungin (41.1%), but none to amphotericin B or nystatin.[2] Thus, the development of an alternative or a new antifungal agent is important.

Aloe vera has known as antifungal because of its phytochemical properties. A comparative study of the antifungal effect of Aloe vera that has been published by Agarry O.O., et al., revealed that zone of inhibitory to Candida albicans of Aloe vera leaf extract (AVL) was 3.00 mm and 0.00 mm for Aloe vera gel (AVG).[4] Another study on AVL extract has been found the minimal fungicidal concentration for Candida albicans ATCC 10231 strains was 75% and it is important for clinical treatment.[5] There is a few study has been done for the effect of Aloe vera whole extract (AVW) to Candida species.

The anthraquinones component in Aloe vera such as aloin and aloe emodin has been suggested as an antifungal. A high content of both 1,8–dihydroxyanthraquinone derivatives (aloe emodin) and their

glycosides (aloins) has been demonstrated in Aloe vera, and presents antibacterial activity by inhibition of nucleic acid synthesis in microbes, inhibit cell growth and germ tube formation by Candida albicans.[6]

The aim of this study was to investigate the effect of phytochemical properties in the Aloe vera extract and compare both the effect of the Aloe vera leaf (AVL) and Aloe vera whole (AVW), as a single use oral rinse in reducing the Colony Forming Units of Candida species. Dental and medical condition of the patients being analyzed due to the effectiveness of those extract in reducing the Colony Forming Units of Candida species.

2. Materials and Methods

2.1 Collection of Plant materials:

Aloe vera plants picked up from an organic garden near Padjadjaran University, Jatinangor - Sumedang, Indonesia. This plants has been identified as Aloe barbadensis Miller or Aloe vera. The selected Aloe vera were 50 – 60 cm in length, 10 – 12 cm in width and the weight was 420 grams in average. There is two parts in Aloe vera leaves, the outer skin which color is green and the inner gel which color is clear, as described by Figure 1. The outer skin of Aloe vera leaves then being extracted as Aloe vera leaf extract (AVL) and the whole Aloe vera including the outer and inner part being extracted as Aloe vera whole extract (AVW).

2.2 Preparation of Aloe vera Extract:

Both of the fresh Aloe vera, AVL and AVW then macerated in 96% ethanol. One until two days after, the macerated Aloe vera was filtered through Wattman no 1 filter paper, and then evaporated using Rotary Evaporator (Rotavapor R-215, Buchi) at 30-40 degree Celsius. The extract was preserved aseptically in a brown bottle at 5 degree Celsius until used as described by the refferences.[5,7,8.] Screening of phytochemical components also done for both of the extract. In this study we used Aloe vera extract dissolved with aquadest until 75% (v/v) in concentration reached.

a.  b. 

Fig. 1. (a) Aloe vera outer skin (AVL); (b) Aloe vera whole (AVW).

2.3 Ethical clearance, Saliva Collection and Preparation:

The procedures in this study was approved by the Medical Ethics Committee from Hasan Sadikin Hospital, Bandung, Indonesia. Inclusion criteria for the subject of this study were head and neck cancer patients who was undergoing 5-FU and Cisplatin/Carboplatin chemotherapy. All of the patients were required to sign an informed consent form prior to entry into the study. Four patients rinsed with 10 ml AVL and 4 patients rinsed with 10 ml AVW, the rinsing time was between 1-3 minutes.

The concentrated oral rinse methods was used to isolate Candida species from 8 patients that fulfilled the criteria, as described by the refferences.[5,9,10] The patients have to swish with 10 ml of Phosphate Buffer Saline (PBS, pH = 7,2) for 1 minute, and then the saliva collected in a sterile medium transport. Saliva from the patients collected twice, before and after rinsing with the extract.

Candida species were cultured on Sabouraud's dextrose agar plates and being incubated for 24-48 hours in 37 degree Celsius. The total amount of Candida's colony forming units being counted as CFU/ml concentrated saliva.

3. Results and Discussion

The screening of phytochemical properties of AVL and AVW extracts were done. The results were shown in Table 1. The phytochemical properties of AVL were alkaloids, flavonoids, steroids, saponins, polyphenols, triterpenoids and quinones, but tanins were absent. In the other side, the phytochemical properties of AVW were similar to AVL with none of alkaloids and tanins.

The 75% (v/v) of AVL extract and 75% (v/v) AVW extract being tested as an oral rinse single use to the patients. The colonization of Candida species in this study before rinsing with AVL extract was 31.875

CFU/ml in average, then after rinsing decreased until 21.775 CFU/ml in average. The reduction of oral candida species colonization after rinsing with 75% AVL extract was 37,45%, as described in Table 2.

Table 1. Qualitatively Analysis of Phytochemical properties of Aloe vera Extract

Phytochemical properties	Aloe vera Leaf (AVL) extract	Aloe vera Whole (AVW) extract
Alkaloids	+	-
Flavonoids	+	+
Steroids	+	+
Tanins	-	-
Saponins	+	+
Polyphenols	+	+
Triterpenoids	+	+
Quinones	+	+

In the other side, the colonization of Candida species before rinsing with AVW extract was 26.975 CFU/ml in average, then after rinsing decreased until 19.525 CFU/ml in average. The reduction of oral candida species colonization after rinsing with 75% AVW extract was 26,43%, as described in Table 3.

Table 2. Colony Forming Units (CFU/ml) of Candida Spp.
Before and After Rinsing with 75% Aloe vera Leaf Extract (AVL)

Respondents (n = 8)	Extract, rinsing time	CFU/ml Before Rinsing (x100)	CFU/ml After Rinsing (x100)	Reduction of CFU/ml	
				CFU/ml (x100)	(%)
1	AVL, 1 minute	151	47	104	68.87
2	AVL, 2 minutes	201	127	74	36.80
3	AVL, 2 minutes	215	177	38	17.60
4	AVL, 3 minutes	708	520	188	26.55
Mean		318.75	217.75	101	37.45

Table 3. Colony Forming Units (CFU/ml) of Candida Spp.
Before and After Rinsing with 75% Aloe vera Whole Extract (AVW)

Respondents (n = 8)	Extract, rinsing time	CFU/ml Before Rinsing (x100)	CFU/ml After Rinsing (x100)	Reduction of CFU/ml	
				CFU/ml (x100)	(%)
5	AVW, 1 minute	162	128	34	20.98
6	AVW, 2 minutes	316	142	174	55.06
7	AVW, 2 minutes	179	153	26	14.52
8	AVW, 3 minutes	422	358	64	15.16
Mean		269.75	195.25	74.5	26.43

Saliva sample examination from the patients who had received 75% AVL extract as single oral rinse showed higher reduction in CFU/ml Candida species than 75% AVW extract recipients. These condition are probably due to the alkaloids as one of the phytochemical properties in AVL extract that could not find in AVW extract. The efficacy of the alkaloids as an antimicrobial is significantly high even at low concentration, which indicates a possibility of its use as antifungal.[11]

Study on phytochemical properties of Aloe vera before, showed that anthraquinones has a potential role as antifungal.[6] Another study revealed that the high molecular weight components (MW > 100 kDa) is the most effective fraction of Aloe vera that can increase in macrophage activity against Candida albicans. The acemannan (beta 1,4-acetylated mannan) can be isolated from Aloe vera with 274 – 375 molecular weight.[12] Whilst the mechanism of anti-bacterial activity is still need more investigations, it has been suggested that aloe emodin and aloins induced bacterial cell membranes disruption and the anthraquinone had strong anti-bacterial activity.[11]

It has known that the phenol component in Aloe vera have anti-bacterial agents by disrupting bacterial cell membranes, as well as by denaturing bacterial proteins. Cinnamic acid in Aloe vera is known to inhibit bacterial glucose uptake and ATP production, therefore, inhibiting bacterial growth and the coumaric acid has been shown to inhibit bacterial enzymatic activity. Aloe vera components may also function by selectively modulating the cells of the immune system. Furthermore, acemannan also inhibits bacteria adhering to epithelial cells and establishing an infection.[11]

It is likely that the anti-bacterial activity of Aloe vera extracts in vivo is due to the synergistic effects of multiple bioactive components, functioning through several mechanisms. Then more detailed purification, identification and mechanistic studies of Aloe vera as an antifungal agents are required.[11]

Medical and oral condition of these patients has been analyzed due to the effectiveness of those extract in reducing the Colony Forming Units of Candida species. Almost all of the patients (3 from 4) who had

received AVW extract was during intravenous chemotherapy and they showed less reduction in CFU/ml than the patients who had received AVL extract which was in the between the cycle of chemotherapy. The antineoplastic agents were on the peak concentration in the body while the patients during intravenous chemotherapy, and decreased oral cellular defense mechanism too. This study revealed that the Aloe vera extract as antifungal less effective in this situation (Figure 2).

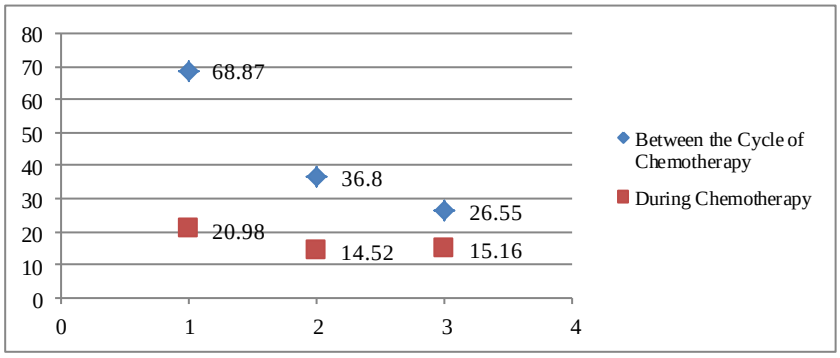

Fig. 2. (blue dots) Patients Between the Cycle of Chemotherapy and received AVL;
(red dots) Patients During Chemotherapy and received AVW.

4. Conclusion

We investigated the effect of the phytochemical properties of Aloe vera extract (AVL and AVW) to oral candida species in cancer survivor in order to find a new alternative antifungal agent. It is found that 75% Aloe vera leaf extract (AVL) as a single use oral rinse could reduce more CFU/ml oral Candida species than 75% Aloe vera whole extract (AVW). We also found that the Aloe vera extract become less effective probably due to the receiving intravenous chemotherapy.

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Antioxidant Activity And Bioactive Component Of Ethanolic Extract Of Basil (*Ocimumbasilicum* L.) Seed After Fractionation

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Abstract
Antioxidant is an important component that plays an important rule to keep health. Some researches reported that the ethanolic extract of basilicum leaves and seed contain amino acid, alkaloids, saponins, flavonoid, tannin and essential oil that have antioxidant activity potential (Raja *et al*., 2012).This study aims to determine ethanolic extract of basil seed by maceration method, fractionation by TLC and column chromatography, antioxidants activity using DPPH method, and antioxidant componenet of the most active fraction using GCMS. Ethanolic extract and the results of ethanolic extract fractionation of basil seeds using n-hexane, ethyl acetate, n-buthanol and methanol-water show that n-butanol extract has the highest antioxidant activity with IC_{50} value of 41.90 ppm. The result of column chromatography n-butanol extract using n-hexane:ethyl acetate (1:9) as mobile phase yielded 5 fractions with fraction 4 (F4 isolate) has dominant spot of active antioxidants after being sprayed with DPPH reagent, it had IC_{50} values of 39.70 ppm and total phenolic content of 0.003 mg/g. Isolate F4 suspected contains two active compounds as antioxidant which is terpenoid and phenolic compound group, namely squalene and 1,4-di-tert-buthyl-phenol identified by GCMS.

Keywords : Basil Seeds (*Ocimumbasilicum*); Ethanol Extract; Antioxidant; DPPH; Phenolic

1. Introduction

Antioxidant is an important component that plays an important rule to keep health. Antioxidant can improve state of health and reduce risk of disease such as cardiovascular disesaes. Ocimum basilicum is a plant that has traditionally been used to cure some diseases such as headache, cough, diarrhea, intestinal worms and kidney disease (Simon etal., 1999). Some researches reported that the ethanolic extract of basilicum leaves and seed contain amino acid, alkaloids, saponins, flavonoid, tannin and essential oilthat have antioxidant activity (Raja et al., 2012).

The methanolic extract of basilicum seed has total phenolic content is 3.63 ± 0.21 mg/g, antioxidant activity is 58.39 µek/g ± 3.81 (FRAP method), and IC50 value of 59.27 ppm (DPPH (2,2-difenil-1-pikrilhidrazil) method) (Nurcahyanti et al., 2011) and acetone extract of basilicum seed contain 22.9-65.5 mg/g of total phenolic (Javanmardi et al., 2003).

Therefore the aims this study to determine antioxidant compound of fractionation of ethanolic extract of the basilicum seed using GCMS. So that it can be used as a source of natural antioxidant.


Figure 1. (a) Basilicum Plant, (b) Basilicum Seed (Dokumen Pribadi, 2013)

2. Methods

Preparation of Plant Extract

Extraction was performed using maceration method. 1 Kg ocimum seed was extracted in ethanol 97% for 3 days. Extract was passed throught No. 1 Whatman papper. The filtrate obtained was concentrated under

vaccum in a rotary evaporator at 50 0C and stored at room temperature to further analyses.

Evaluation of Antioxidant Activity

1. KLT Autography

Fraction of column chromatography was evaluated its antioxidant activity using KLT autography method with the eluent is n-heksane:EtOAc (1:9). Spot that looked observed in UV light (λ = 254 nm and λ = 366 nm). Then calcutated its RF value (Asih, 2007). Then sprayed with DPPH 0,05%, and observed the formation of yellow, or orange with purple background which shows the inhibitory activity of DPPH free radical (Sukandar et al., 2010).

2. DPPH Free Radical Scavenging

The diluted solution of the extract was prepared in methanol with various concentrations. Each sample was pippeted 2 mL, then added 2 mL of DPPH 0,002%. Then the mixture was incubated for 30 minutes and measured at λ 517 nm. The optical density was recorded, percent of inhibition and IC50 were calculated.

Total Phenolic Content

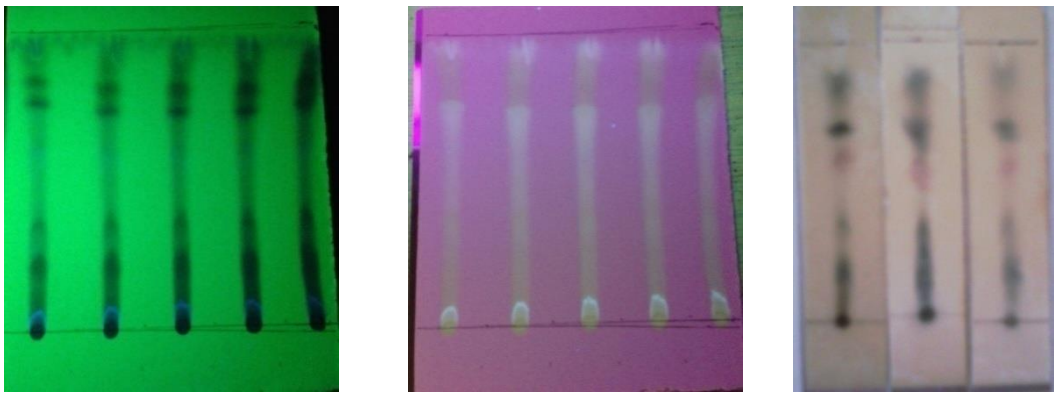
2.5 mg of sample extract was added to 2.5 mLof distilled water, 0.5 mL Folin-Ciocelteu reagent (1:1) and incubated for 3 minutes. Then add 2 mL of NaCO3 20%, heated in water bathfor 1 minute. After cooling in ice bath, measured at λ 750 nm. Total phenolic contents were expressed in Gallic acid.

Chemical Component Analyses

Chemical components were determined using GCMS. Isolatwas dissolved in methanol and injected into GCMS instrument, recorded its chromatogram and then compared with Wiley7 Library GCMS Merck Shimadzu QP2010. GCMS conditions were used as follows : column type RTx1-MS Restech Polymethylxiloxan, diameter of column 6.25 mm, temperature column50 oC, pressure of 53.6 kPa, column flow 1.00 ml/minute, linear velocity 36.3 cm/second, mobile phase of helium.

3. Results And Discussion

Ethanolic extracts of basilicum seed contain saponin, tannin, flavonoid, alkaloid, terpenoid dan quinon. N-buthanolic extract of basilicum seed has the higher antioxidant activity with the IC_{50} value of 41.90 ppm than ethanolic extract with theIC_{50}value of 67.08 ppm. Abbas *et al*., (2010) explained that fractionation purification techniques performed to obtain higher antioxidant activity fraction.



(a) (b) (c)

Figure 2.TLC analysis of n-buthanolic extract

TLC profile of buthanolic extracts using mobile phase of n-heksane:ethyl acetate (1:9) were sprayed with $FeCl_3$ 1% gives 5 fractions that are four green spots with the Rf value are 0.18, 0.34, 0.75 and 0.85 and a red spot with the Rf value of 0.67 (figure 2). Dominant spots with the same Rf value indicate active as antioxidant fraction after sprayed with DPPH is F4 fractions that has Rf value of 0.67 that indicated by color changing from purple to yellow (Sukandar *et al*., 2012).

Fraction of F4 has antioxidant activity with the IC_{50} value of 39.70 ppm, total phenolic of 0.003 mg/g. The IC_{50} value of F4 fraction less than n-buthanolic extracts (IC_{50} 41.90 ppm) and ethanolic extracts (IC_{50}

67,08 ppm) which indicated that the antioxidant activity of F4 fraction increased after fractionation. Abbas *et al*., (2010) reported that pure compound have higher antioxidant activity that the crude extracts.However, the total phenolicof F4 fraction (0.003 mgGAE/g) less than buthanolic extracts (0.24 mgGAE/g). This indicated that the high antioxidant activity of F4 fraction is affected by another compound such as terpenoid (Bando *et al*., 2004).

The results of identification using GCMS produced several chromatographic peaks which indicates that the F4 fraction not pure (figure 3).

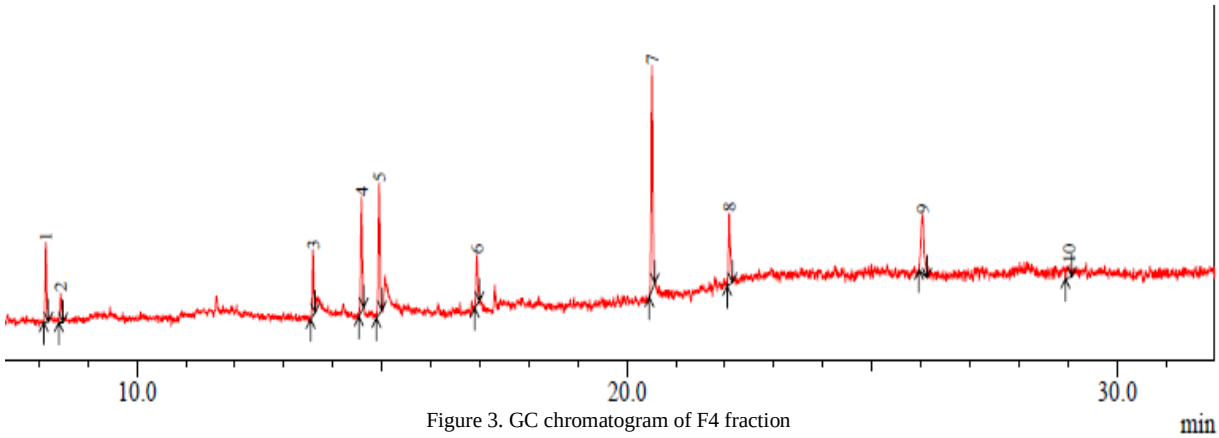


Figure 3. GC chromatogram of F4 fraction

The results of the data analysis on the Wiley Library Brand Shimadzu QP2010 GC MS are nine compounds in F4 fraction (figure 3). Among these compounds suspected that the antioxidant compound of F4 fraction is squalene which has similarity of 80% with retention time of 26.04 minutes andpeak area of 12.34 %. Squalene has relative molecular mass (m/z) 410 and molecular formula $C_{30}H_{50}$. Confort*iet al*., (2005) reported that squalene is a good antioxidant compound with the IC_{50} value of 23 ppm using thiobarbituric acid (TBA).

Squalene is antioxidant compound as *scavenger oxygen*. Squalene can prevent lipid peroxidation in human skin (Saint-Leger *et al*., 1986). Antioxidant activity of squalene was influenced by its structure similar to β-karoten is also known as oxygen extinguisher that work together with vitamin E (Bando *et al*., 2004).

The results of the mass spectrum of F4 fraction with a relative molecular mass (m/z) 410 indicate the presence of multiple peaks result of the fragmentation of the squalene spectrogram (Figure 4).

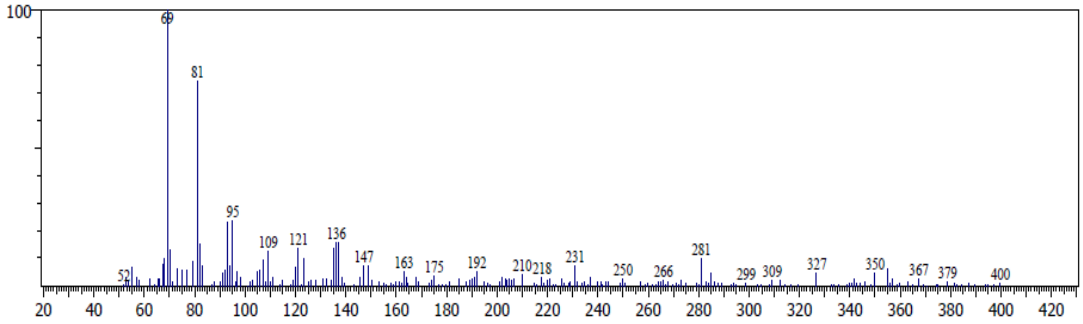


Figure 4. Spectrograms of Squalen

The estimate of the fragmentation pattern of compounds that are thought to have similarities with squalene fragmentation pattern can be seen in Figure 6. Squalene at m/z 410 (M +) ions lose propyl (M + - C_3H_7) to form ion peak m/z 367 fragment ion at m/z 327 ion corresponding to the loss of C_6H_{11} with the 83 (Oyugiet al., 2011). Furthermore, the possibility of disconnection consecutive -$C_{11}H_{20}$ M + and M + -C_7H_{10} of peak ion m/z 327 to be formed m/z 175 and m/z 81 fragment ion m/z 367 suggested the loss of 298 to form ion peak m/z 69 as the base ion.

Other compounds that supports antioxidant activity in F4 fraction was 2.4-di-tert-butyl-phenol. This compound may contribute to the total phenolic content of F4 isolates. The compound 2,4-di-tert-butyl-phenol is phenol compounds generally are antioxidant compounds (Pratt, 1992). In addition, Nurestri et al. (2010) reported that the ethyl acetate fraction isolated Pesreskia bleo compounds containing 2,4-di-tert-butyl-phenol antioxidant activity associated with a structure similar to BHT (Butyl Hydroxy Toluene), which is a synthetic antioxidant. Nurestri et al. (2009) also mentions that the compound 2,4-di-tert-butyl-phenol ethyl acetate fraction isolated leaves of Pereskia grandifolia have activity against 6 types of cancer cells, ie cells epidermoid cancer of the nasopharynx, cervix, colon, breast, lung and human fibroblasts.

GCMS analysis results, 2,4-di-tert-butyl-phenol having 90% similarity with a retention time of 8.15 minutes and 7.76% peak area. These compounds relative molecular mass spectrometer (m/z) 206 with molecular formula $C_{14}H_{22}O$ with its spectrogram and fragmentation pattern can be seen in figure 6 and 7.

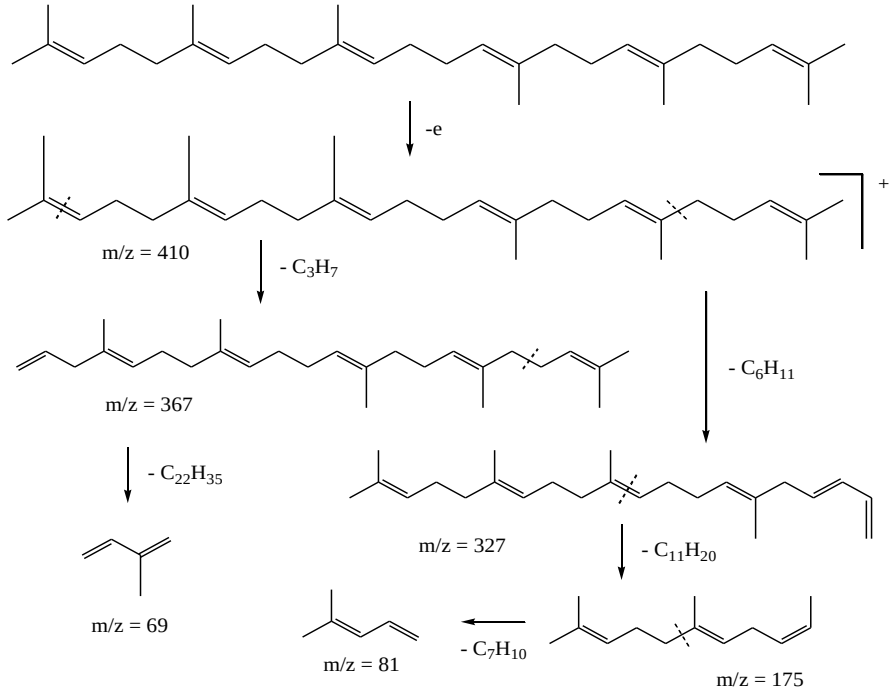


Figure 5. Fragmentation Patterns of Squalene

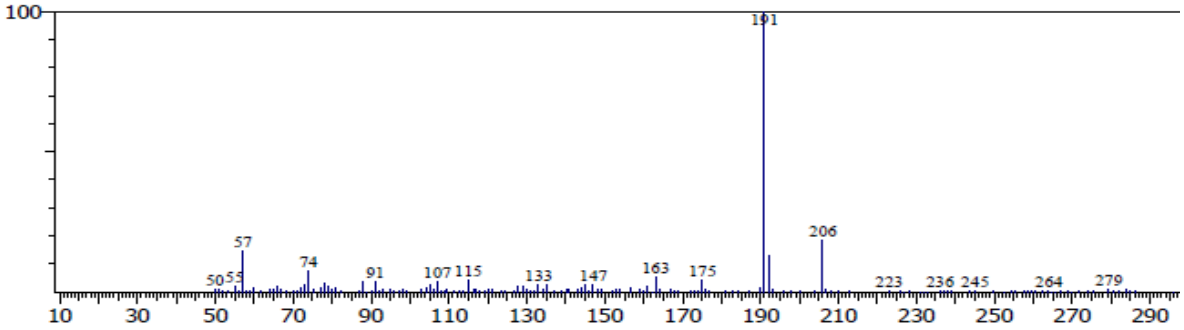


Figure 6. Spectrogram the compound 2,4-di-tert-butyl-phenol

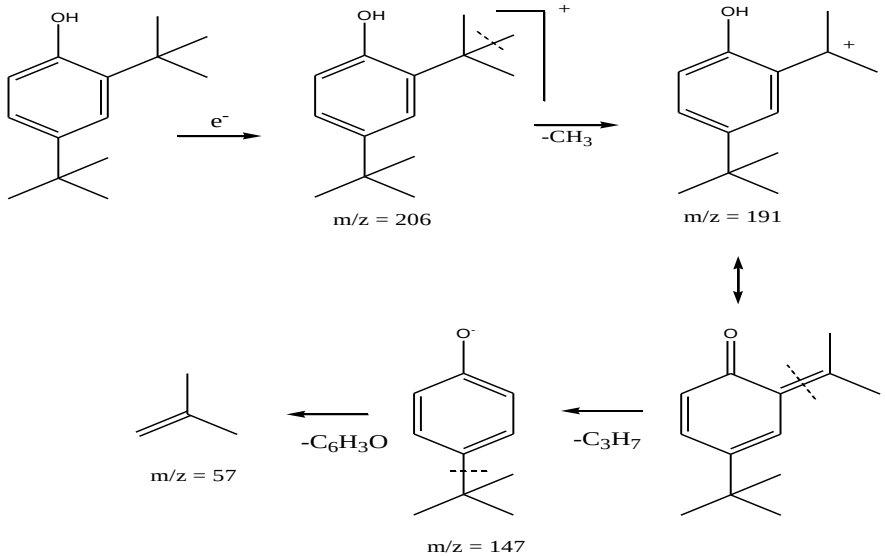


Figure 7. Fragmentation Patterns of 2,4-di-tert-butyl-phenol

The pattern of ionic fragment of compounds 2,4-di-tert-butyl-phenol showed a base ion at m/z 191 and m/z 57. The peak of the ion m/z 206 (M^+) the release of a methyl group (M^+ -CH3) and $C_{13}H_{19}O^+$ that will produce resonance in the aromatic compounds to the stable state (Korcek et al., 1969). Furthermore, will experience a disconnection M^+ and M^+ -$C_6H_3O^-$ -C_3H_7 to produce the base ion m/z 57.

4. Conclutions

The antioxidant activity of ethanolic extract of the results of the fractionation column basilicum seed haveIC_{50}values (39.70 ppm) higher than n-butanolic extract (IC_{50} 41.90 ppm) and ethanolic extract (IC_{50} 67.08 ppm). Isolat of F4 contain antioxidant compounds classes terpenoid and phenolicnamely squalena and 2.4-di-tert-butil-fenol.

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Evaluation of fucoidan bioactivity asanti-gastriculcers in mice

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Abstract

Fucoidan is a polysaccharide compounds containing sulfate group. Fucoidan is found in brown seaweed. In this study, we assess fucoidan activity extracted from brown seaweed Sargassum crassifolium origin from Binuangeun, Banten. Fucoidan extract was tested in mice in vivo. Observations were carried out during 16 days the control (without fucoidan) and fucoidan treatment.

Fucoidan were given in various concentration of 100, 200, 300, 400 ppm. On the 14th day, aspirin was given to mice with pre-treated fucoidan 400 ppm as gastric ulcer induction. The fucoidan extracts compositions showed: water content 3.11%, uronic acid 556 ppm, 0.12 ppm sulfate and 1648 ppm total carbohydrate. Results from histopathology assay in mice tissue stomach showed that 100 ppm of fucoidan can inhibit gastric ulcers caused by 400 ppm aspirin irritation. Fucoidan was associated with an increase in the mucus layer in the gastric mucosa.

Keywords: fucoidan, induced-gastric ulcer, irritation, aspirin, bioactivity

1. Introduction

Fucoidan is a sulfated polysaccharide found mainly in various species of brown seaweeds. Sulfated polysaccharides extracted from seaweeds have been proven to have valuable pharmaceutical and biomedical potential activities (Chevolot et al., 1999), such as antioxidant, anticoagulant, antithrombic, and antiviral properties (Mourão, 2004; Silva, 2005). It was also reported that polysaccharides from the brown alga *P. pavonia* (*pavonica*) exhibit anticoagulant activity.

Research on indications of fucoidan can prevent hyperplasia in rats. Aspirin known as acetylsalicylic acid, and drugs called salicylates. Aspirin is often used to reduce pain. Aspirin is known as a class of non-steroidal drugs. Aspirin was the first-discovered member of the class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs), not all of which are salicylates, although they all have similar effects and most have some mechanism of action which involves non-selective inhibition of the enzyme cyclooxygenase (Jong, 2009).

Studies have indicated that fucoidan can induce apoptosis in human lymphoma cell lines and inhibit hyperplasia in rabbits. Few studies have reported the effect of fucoidan on proand anti-inflammatory cytokines. In animal models, ingestion of fucoidan has inhibitory effects on tumors, which appears to be associated with a rise in interferon-gamma (IFN- ϒ) and interleukin-12 (IL-12) and stimulation of innate immunity (Maruyama, 2003).The fucoidan is a safe substance with potential for gastric protection (Shibata et al, 2000). Therefore, the objective of this study is to investigate the effectiveness of fucoidan on the used aspirin as a cause of gastric ulcers in mice.

2. Materials and methods

2.1 Chemicals

Brown seaweed *Sargassum duplicatum* was obtained from Banten. EtOH, acetone, NaOH, and inorganic acids and salts ($CaCl_2$, NaCl) were commercial products. CMC (carboxyl methyl celulosa) was commercial products.

2.3. Experiments

First we extracted fucoidan from brown seaweed followed (method developed by Sinurat, 2011). Before the extraction of fucoidan has been tested of species brown seaweed. Brown seaweed was rinsed with water and dried in open air then milled and freeze -dried. The solid powder of brown seaweed was dissolved and incubated in mixture solvent of MeOH-$CHCl_3$- H_2O with a ratio of 4: 2: 1 for 12 hours then washed with acetone and dried (F1). Moreover, this sample then was soaked with HCl 0,1N (1:10) (w / v) then mixing for 6 hours at room temperature. Planktonic filtered using 500 mesh, the filtrate collected. The filtrate was neutralized using NaOH 0.5 M and 4M $CaCl_2$ solution (1:10) while stirring mechanically for 60 minutes and heating at temperature 85 ^oC then filtered, the filtrate collected (F2). The filtrate was centrifuged and diluted

in water containing 0.5 M $CaCl_2$ and 5% CPC precipitation as precipitated filtrate (F3). In addition of 3 M $CaCl_2$ and ethanol with ratio (1: 2) also water then centrifuged to the filtrate will obtain the better result of fucoidan quality. The final filtrate was rinsed with0.5 M NaCl and aquabides was obtained as extract fucoidan (F4). The quality of fucoidan determination were conducted as following methods : total carbohydrate (Dubois *et al*. (1956) method), turbidimeter assay to quantify the sulfate content using $BaCl_2$-gelatin method and quantification, uronic acid (Scot method).

The second step fucoidan was be tested in mice *in vivo* as an anti-gastric ulcer followed Jong et al. method (2010). The methods were performed as follows: mice were used as animal model were divided into 7 groups. Group 1: control / only given CMC 0.5%. Group 2: control only CMC 0.5% was given every day during the previous 14 days, then mice were conditioned fasting for 48 hours after the mice were given aspirin (400 mg / kg body weight). Group 3: mice were given fucoidan at dose of 100 mg / kg of body weight every day, for 14 days and then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Group 4: rats were given fucoidan at dose of 200 mg / kg of body weight per day during the early 14 days, then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Group 5: mice were given fucoidan at dose of 300 mg / kg of body weight per day during the previous 14 days, then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Group 6: rats were given fucoidan at a dose of 400 mg / kg of body weight per day during the previous 14 days, then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Group 7: rats were given commercial fucoidan at a dose of 300 mg / kg of body weight per day during the previous 14 days, then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Used as a coating solution of CMC0.5%.

The observations carried out : 1. Blood samples were taken for analysis of pH by means of a centrifuge at 3000 rpm for 10 minutes. 2. Observations of gastric tissue changes under SEM/TEM, after first fixation in 10% formalin.

3. Result and Discussion

Table 1.The quality of of fucoidan composition from *Sargassum duplicatum*

No	Parameters	Concentration (ppm)
1.	Total carbohydrate (as fucosa)	1648
2.	Total sulfated	0.12
3.	Uronic acid	556
4.	Moisture content (%)	3.11

Fucoidan assay in vivo was done in BALIVET laboratory. Bioassay was conducted by using mice in various fucoidan feeding concentration of 100 mg / kg body weight to 400 mg / kg body weight in mice. The tests were grouped into 7 groups was compared with commercial fucoidan used as anti-gastric ulcer supplements currently available on the market.

Based on the in vivo test results showed that the use of fucoidan with a concentration of 100 ppm was able to prevent the occurrence of gastric ulcers. Gastric ulcer occurred with aspirin, is considered to injured gastric (not all showed). Aspirin is a drug containing strong acid that can injured gastric. The results of the pH measurements after the of mice surgery, for all treatments were given aspirin pH is 4.3. Physically seen the results of histopathology test, as shown in Figure 1.

Based on figure results of hispatology performance that given aspirin without fucoidan (Figure B) caused blood clots this show that ulcer gastric in stomach tissue. Different performance on (Figure A without aspirin and C given aspirin and fucoidan) not see many blood clots. This show that fucoidan can be prevent ulcer gastric with make be layer in mucosa because fucoidan make so it does not injure the lining of the stomach tissue. The mucosa is always covered by a layer of thick mucus that is secreted by tall columnar epithelial cells. Gastric mucus is a glycoprotein that serves two purposes: the lubrication of food masses in order to facilitate movement within the stomach and the formation of a protective layer over the lining epithelium of the stomach cavity. This protective layer is a defence mechanism the stomach has against being digested by its own protein-lysine enzymes, and it is facilitated by the secretion of bicarbonate into the surface layer from the underlying mucosa. The acidity, or hydrogen ion concentration, of the mucous layer measures netral at the area immediately adjacent to the epithelium and becomes more acidic at the luminal level. When the gastric mucus is removed from the surface epithelium, small pits, called foveolae gastricae, may be observed with a magnifying glass (web-1).

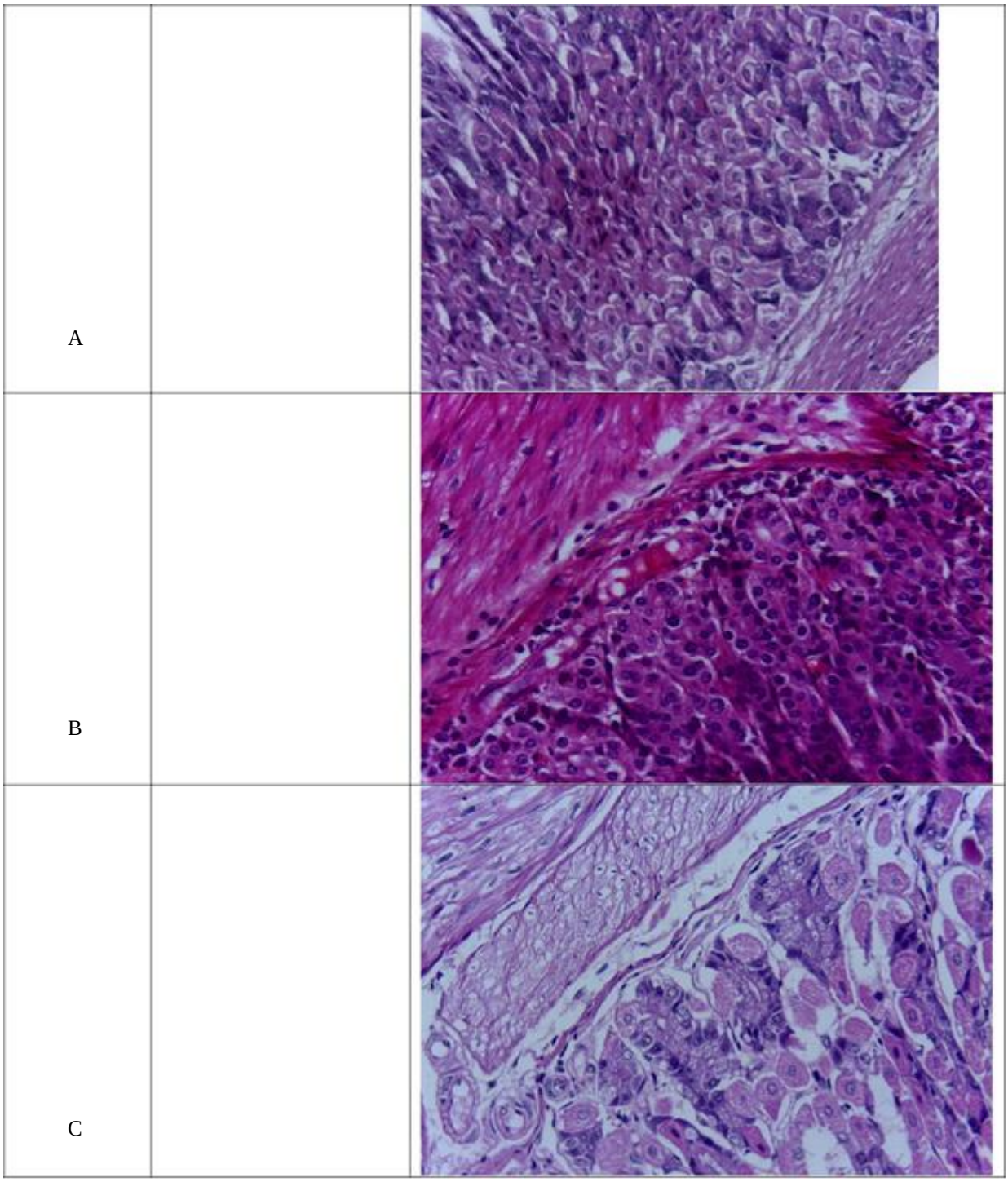

Figure 1. Test hispathology in stomach tissue of normal an ulcer experimental mice.

The toxic dose of aspirin did not fully dissolve in normal saline or water. Hence it was preferred as using a vehicle and the vehicle was also given for the control animals. Carboxy methyl cellulose is generally being used as the vehicle because of its high viscosity, non-toxicity, biodegradability and safe (Malairajan et al, 2008).

These findings support the hypothesis that fucoidan effectively attenuates the inflammatory cytokines release mediated gastric mucosal damage on give aspirin. As ulcer healing is a complex process involving various factors, this study is at too primitive stage to conclude its exact action. Mucosal damage can be easily produced by the generation of reactive metabolite. An improvement in mucus production guides the healing process by protecting the ulcer crater against the endogenous aggressors, like stomach secretions and oxidants as well as against exogenous damaging agents, such as NSAIDs. The ulcer prevention or healing by fucoidan was associated with an increase in the mucus layer in the gastric mucosa (Chang et al, 2005).

Groups	Gastric organ	Remarks
A	Normal	Mice surgery in stomach tissue first day (before treatment)
B	Mild ulcerative gastritis	Mices were given 0.5% carboxyl methyl cellulose (100,200,300mg /kg body weight, p.o.) for 14 days and fasted for 48 h and post-orally gavaged with aspirin (400 mg/kg body weight
C	Fucoidan-pretreated ulcerated	Mices were given fucoidan (100,200,300mg /kg body weight, p.o.) for 14 days and fasted for 48 h and post-orally gavaged with aspirin (400 mg/kg body weight, suspended in 0.5% carboxyl methyl cellulose)

4. Conclusion

The yield of was 5.56% crude fucoidan from seaweed powder dried. Based on organ histopathology test of mice obtained using 100 ppm fucoidan can inhibit gastric ulcer irritation after given aspirin 400 mg/kg body weight. Fucoidan was associated with an increase in the mucus layer in the gastric mucosa.

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Fracture Pattern of Controlled Groundwater Flow in the Volcanic System Case Study in Ciherang, West Java, Indonesia

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Abstract

In general, the volcanic region has highly potential of a water resource. However, the volcanic area also has a complex variation in the deposition process and fracture is also growing in the area. Volcanic rocks have a variation of porosity, with the spread of the highly different within a short distance. The amount of rock that has impermeable properties and the other has porous properties. Those rocks should be identified as vertical and lateral spreading. In fact, the groundwater in the area is not only flowing in the volcanic rock pores only, but also in the flow caused fractures that developed by the volcanic and tectonic processes. Groundwater in these fractures has to consider for water resource calculation. Therefore, fracture media has become one of the important parameters in the calculation of water resources. Through the lineament analysis and fracture density method with Digital Elevation Model (DEM) Raster can be seen growing fracture characteristics and classified into a fracture pattern that has a function in controlling the flow of groundwater in this volcanic region. Ciherang area which is located between Mount Salak and Mount Gede-Pangrango and bypassed by tectonic faults, has a complex fracture pattern. Based on the results of research in the area Ciherang have at least four different fracture patterns with each character. The fracture pattern has happened as a result of the imposition of volcanic rocks, there are also fractures are influenced by tectonic faults, and fractures caused by both of the processes. Population of fractures that have a high instensity and fractures that have a trend relatively north-south direction is claimed as a porous media in surrounding area. Then, these fracture patterns have been validated by Hydrochemistry and Hydroisotope to proof the existence of fracture patterns in volcanic region to control groundwater flow.

Keyword : Fracture Pattern, Groundwater Flow, Volcanic, Hydrochemistry, Hydroisotope

1. Introduction

Mount Salak is a mountain was formed in the period of quarter volcanism with a fractures that has its own characteristics. This volcanic area has a high potential of groundwater. Mount Salak has a large content of groundwater caused by high fracture intensity. This suggests that the fractures in volcanic areas have contributed a great potential groundwater resources. (Hendarmawan et al, 2010).

Fracture patterns mapping is important to gain knowledge of the fracture system prevailing in this area, both of which are influenced by tectonic fractures or pattern formed on the clotting time of volcanic rocks. As revealed by Denny et.al in his paper on methods of structure mapping that control the aquifer, the fracture as a structural element of regional or local geology strongly influence the determination and the presence of groundwater recharge zone (Denny et. al, 2006).

Research related to the fracture has been done in previous studies. Many research do in two-dimensional form of lineaments in the valleys and hills of analog or digital maps, satellite imagery and DEM, with lineament analysis used as reference to determine the cracks intensity level of an area (Sander, 2006).

2. Regional Geologic Setting

Regionally, the area Ciherang belonging to the Geological Map Sheet Bogor (Efendi et all, 1998). Based on regional geological map Sheet Bogor is known that the oldest rocks exposed in the study area consists of pumice tuff rocks which belong to the Old Volcano to the east, north and south of the study area. Composed by lithology in the form of lava and andesite lavas included in Rock Volcano Pangrango. Lithology in the west area of research in the form of lava, tuffaceous breccias and lapilli, lava flow manifold andesite basalt rocks that belong to the Mount Salak volcano. These rocks belong to the Quaternary volcanic rocks.

Tectonic activity in this area began in the Early Tertiary, followed by Plio-Pleistocene tectonic activity that reactivate the product of tectonic Early Tertiary period, forming fault-fault trending generally north-east - south-west and northwest - southeast. Cracks are formed into a weak zone for the emergence of young volcanic rocks of Quaternary age. The geology in the investigation area are generally prepared by groups of Quaternary age rocks, sediments form an inseparable young volcano composed of sandy pumice tuff,

tuffaceous breccia lava Pangrango of sediment, the sediment is thick enough. Underneath the form of an old volcanic deposits integral a structure consisting of andesitic breccia - basaltic, andesitic lavas, tuffs and agglomerates. To the south developing fine to coarse clastic sediments of Tertiary age that have terlipatkan and faulted (Edi Murtianto, 1991).

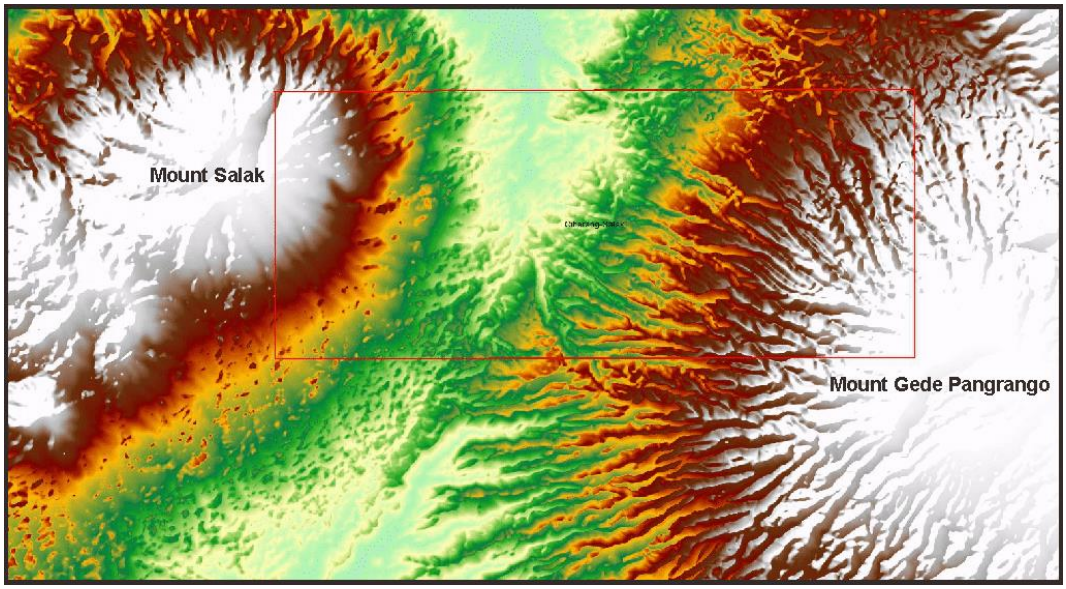

Figure 1. Research Area located between Mount Salak and Mount Gede Pangrango at Ciherang, West Java, Indonesia.

Most researchers agree that the volcanic eruption in this area relates to the activity of plate collision. Based on geophysical data, it is known that the subduction pathways in the southern island of Java, is a continuous zone, but the volcanic eruption on the surface is separated from one another. This phenomenon indicates that the activity magmatisma / volcanism does not by itself come to the surface, but there must be a gap / fracture which serves as a medium for the discharge of magma to the surface. Cracks / fractures is always a fault lines, for example on the island of Sumatra, the position of the active volcanoes are in the volcanic arc environment on the surface appearance was in the Sumatra Fault Zone

With geological facts described above, it is in Java which is also found volcanics (from the age of Paleogene to the present) associated with fault zones confirmed. Examples Katili and Sudradjat (1984) connects the presence of volcanoes around London and the surrounding areas with Cimandiri Fault zones and fault Citanduy. Some other examples are the appearance of Volcano Ciremai in Cirebon-Kuningan which is at the intersection of the fault zone and Fault Baribis Citanduy; Volcano Tangkubanprahu-Burangrang are in fault zones Cimandiri; and Mount Krakatau in the Sunda Strait was in the Sunda Strait fault zone.

3. Method

Slope is the steepness or the degree of incline of a surface. Slope cannot be computed from the lidar points directly; one must first create either a raster or TIN surface. Then, the slope for a particular location is computed as the maximum rate of change of elevation between that location and its surroundings. Slope can be expressed either in degrees or as a percentage. Aspect is the orientation of slope, measured clockwise in degrees from 0 to 360, where 0 is north-facing, 90 is east-facing, 180 is south-facing, and 270 is west-facing. Hillshading is a technique used to visualize terrain as shaded relief, illuminating it with a hypothetical light source. The illumination value for each raster cell is determined by its orientation to the light source, which is based on slope and aspect.

DEM can be obtain from various sources SRTM (90 x 90m global DEM), 30 x 30 m or Manually by interpolation, in the present study we used ASTER DEM for getting shaded relief maps, In order to identify linear topographic features from the DEM, eight shaded relief images were generated. The first step is the production of four separate shaded relief images with light sources coming from four different directions. The first shaded relief image created had a solar azimuth (sun angle) of 0°, and a solar elevation of 30°. An ambient light setting of 0.20 was used, which produces a good contrast. The ambient light setting is simply a scaling factor in the imagine topographic program.

The other three shaded relief images were created with three contrasting illumination directions 45°, 90°, and135°(Figure 3). The second step is to combine four shaded relief images to produce one shaded relief image, for this purpose, the combinations of the four shaded relief maps are computed by using GIS overlay

technique, where the four shaded relief images are overlaid to produce one image with multi - illumination directions 0°,45°,90°,and135°. Finally, this image has been used for automatic lineaments extraction over the study area (Abdullah et al., 2010).

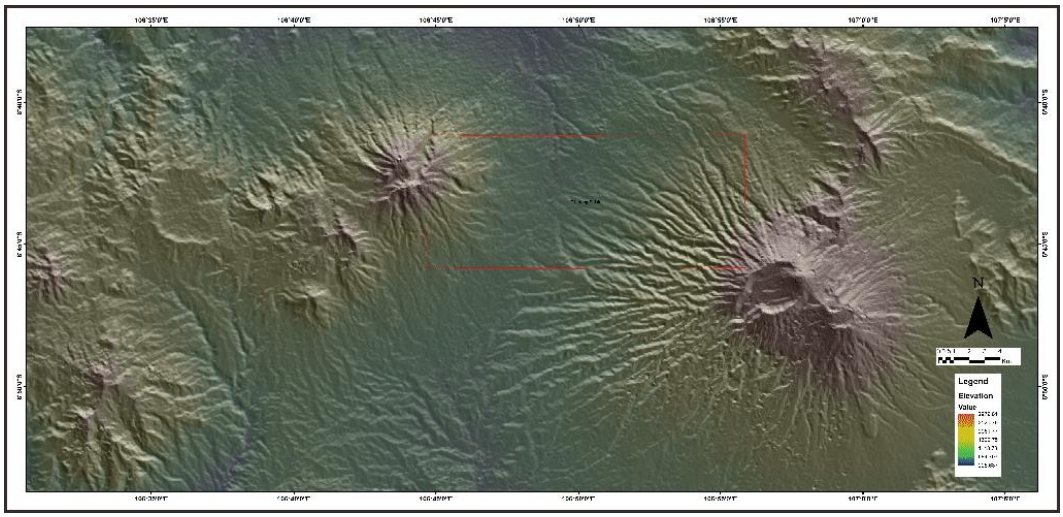

Figure 2. Digital Elevation Model (DEM) has been used for analysis of lineament with purpose of determination of fracture pattern in research area.

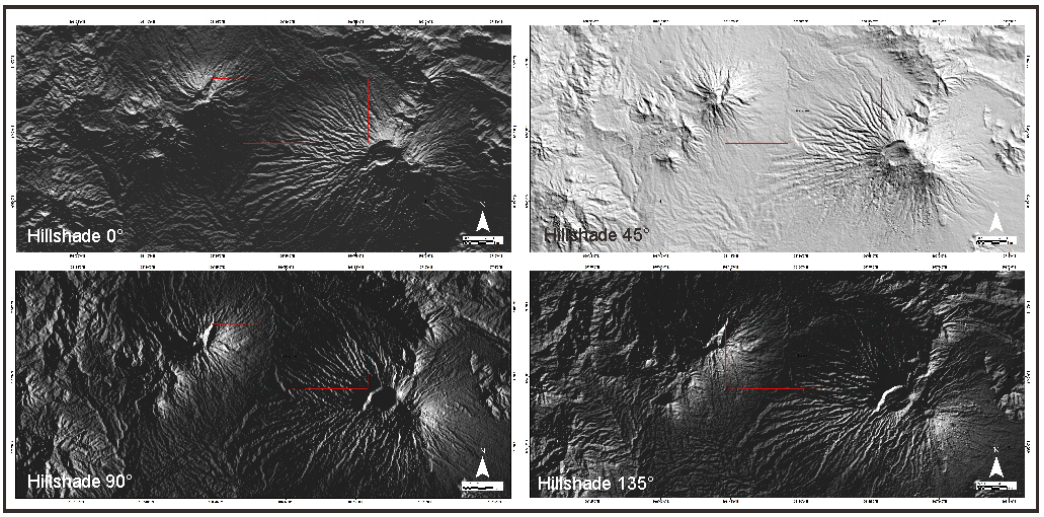

Figure 3. Hillshade of Digital Elevation Model (DEM) with azimuth of sun angel 0°, 45°, 90°, and 135°

4. Result and Discussion

The result of the Digital Elevation Model merging hillshade method showed obvious morphological to determine its lineament, but in some places is not clear enough to be analyzed. Lineament on the clear morphology can be detected by the method of automatic lineament as described previous, but for unclear morofologi have to use manual methods to analyze its lineament. Therefore, it is necessary to use image processing DEM slope aspect of the hills and valleys.

The lineaments that formed during the formation of volcanoes tend to have a radial pattern, the same as the radial pattern in river pattern. Other lineaments appears also as a result of normal faulting during volcanic processes, or at the time of post-volcanic loading. These fractures become porous media on volcanic deposits. Therefore fractures becomes an important parameter as secondary porosity in the groundwater aquifer.

Besides these two types of faults, the existence of an active regional fault of the Miocene epoch, which is referred to as the Pelabuhan Ratu Fault, passing along the study area and be an impact on the pattern of fractures. The impact does not always increase the fracture porosity in the media, but can also reduce the level of porosity in the fracture media.

Generally, the distribution pattern of fracture can be divided into two groups: the first group are fractures that are influenced by volcanic processes, as indicated by clear lineament on its morphology. The second

group are fractures that are influenced by tectonics, which is indicated by the morphology unclear in analyzing its lineament. This two groups can be shown by slope aspect analysis. (Figure 5). In more detail, there are several patterns of fracture are affected by both volcanism and tectonic processes.

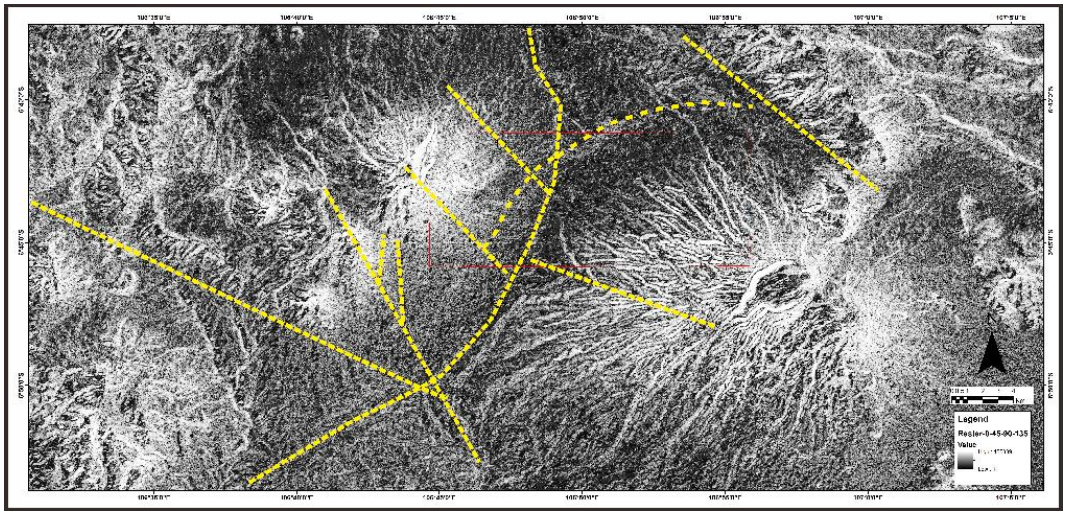

Figure 4. Overlaying of hillshade images to combine from four different sun angle resulting of pattern of lineament but many location unclear to be analyzed.

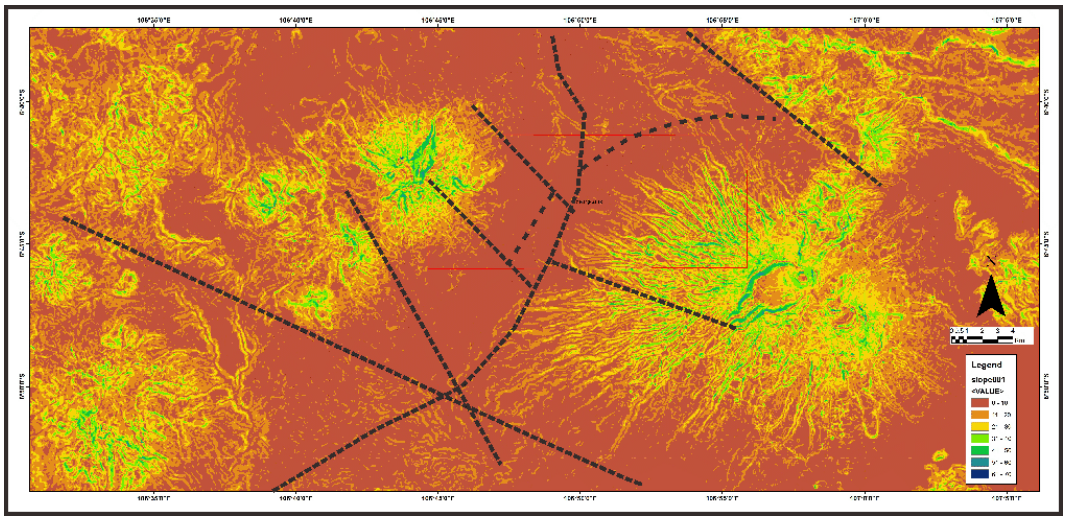

Figure 5. Slope aspect in the study area showed a different morphology to analyze lineament.

As a detil result of fracture pattern anlyzing, ther is several fracture pattern has been developed by volcanism and tectonism in this research area. There are at least four different types of fracture pattern in Mount Salak area, which are formed by volcanic and tectonic processed. First type formed by volcanic processed, second and third types formed by both of volcanic and tectonic processed, and four type is affected by tectonic processed. (Figure 6).

The four types of fracture patterns described as follows:

- Type A, is affected by volcanic process, has a relatively North-South direction, good porosity of aquifer.
- Type B, is affected by both volcanic and tectonic process, has a relatively Northwest-Southeast direction., good porosity, but in a few place are impermeable.
- Type C, is affected by both volcanic and tectonic process, has a relatively East-West direction., good porosity, but in a few place are impermeable
- Type D, is affected by tectonic process, has a relatively the same direction with trend of Pelabuhan Ratu fault along research area, and the possibility of this fracture pattern is impermeable in many locations.

In comparation from four types of fracture patterns and their characterization, type A has a possibility to be the best fracture pattern as a porous media and also become a good aquifer with potential groundwater content in Ciherang research area. The model of this fracture has been validated by Hydrochemistry and

Hydroisotope with stable isotope deuterium oxyde (H2 and O18). As we know those stable isotopes is a finger print to know the origin of groundwater flow.

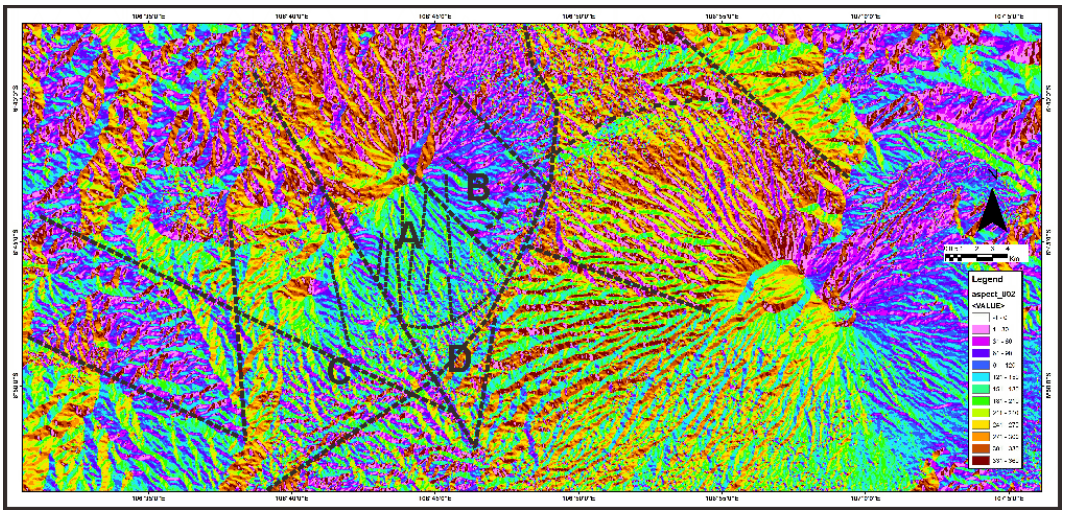

Figure 6. Four types of fracture pattern in Ciherang research area.

5. Conclusion

Volcanism and tectonic process has highly affected in research area. The influence of their processed made many different in developing of each fracture pattern characterization. The fracture media become an important parameter for groundwater, which is fracture media as an secondary porosity in volcanic aquifer system. In the early stages of research, lineament analysis can be used to determine the fracture pattern on the surface morphology of the research area.Based on this research the fracture pattern with affected by volcanic processed have a better porosity then fracture pattern developed with affected by regional fault.

As an early stage of research related to the fracture pattern controlled groundwater flow in volcanic system, which in this research focused only on the surface area, then the research will be continued on the next stage of the research of fracture patterns that occurs in the subsurface area. The purpose of next reseach is to determined the fracture pattern vertically in subsurface area. Determination of fracture that continues until subsurface become more essential to know the groundwater flow system in volcanic rocks.

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Coolant Wastewater Treatment From Rest of Milling Aluminium Production at Indonesian Aerospace Ltd. With Electrocoagulasi Method

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Abstract

Research has been done on the coolant waste water treatment residual aluminum milling process in Indonesian Aerospace Ltd. using Electrocoagulation Method. Research conducted in a laboratory scale reactor using a 10-liter and 6 pairs of aluminum electrodes. Variations of the time used for 0, 10, 20, 30, 40 and 50 minutes with a current of 5.25 Amperes, waste pH 5.5. While the reference is a parameter TDS, TSS, BOD, COD and pH. It was found that the most effective time is 40 minutes with 99.02% remover TDS, TSS 98.16%, 99.73% BOD, COD 99.72% and a final pH of 8.7. This data is used as reference for to design an industrial scale, including the estimated cost of processing. Provided that for the waste water capacity 3M3/day, then the reactor capacity required is 429 M3, 3KVA AC current, capacity filter press 3M3/day (6 plate, 630X630 mm, pressure 8 bar), Equalization basin 3M3/day, buffer basin 3M3, Effluent basin 3M3, Carbon Filters 3M3/ day. The total of electrical power 34KWH/day. If electricity costs IDR830/kwh then the operating costs in this processing is IDR16.982/M3, including the cost of electricity, sludge processing, electrode replacement and the area (5 M x 9 M).

Keywords: Electrocoagulation, Waste, Coolant.

1. Introduction

. To meet the needs of subsistence goods continuous, then every state is always innovating to create various types of industries to produce raw materials and finished goods. So that industry growth will be more fertile and diverse. The effects of these developments are always accompanied with waste products, they are solid, liquid and air waste, In a water waste is divided into Liquid and Toxic Hazardous Waste (B3) and Non-B3 waste. The waste water must be treated before discharged into water bodies / rivers, so hopefully will not be pollute the surrounding environment. On the contrary, if the waste water without prior treatment, it can spoil the quality and preservation of the environment including the safety of human life.

Waste water treatment generally divided into 3 treatment systems, I.e. biology, chemistry and physics, or a combination of the three systems but they are ineffective and un-efficient. This condition requires us to continue to innovate for new technological breakthroughs that can overcome this problem I.e. with Electrocoagulation Hazardous Technology System. This technology/method can process the coolant waste in a relatively quick time, small land and economist. More over it can decrease parameters waste that can meet the quality standards determined by the government, through the Kementerian Lingkungan Hidup Number : Kep.51 / MENLH / 10/1995, Annex B II, regarding a Standard for Industrial Waste water.

The Conditions in Indonesia as an archipelagic nation becomes a challenge for the transport to shorten the travel time among islands. The advances of science and technology are growing rapidly can be utilized to improve the efficiency and effectiveness of transportation, one of them is by the development of Aircraft Industry. One of the strategic industries that has participated in the development of high-tech is Indonesian Aerospace, Ltd. One of many of aircraft manufacturing process, there are stages by milling the raw material (Aluminum Alloy) to be aircraft components. This process requires a liquid coolant.

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The coolant is the cooling system in the process of milling, lathe, cutting and others. Beside that, it can raise the remaining pieces (chips), metal powders, surfaces and protects the blade from overheating and cracking. The definition of waste Coolant in this study/research is a waste of activity after using liquid coolant milling. The types of pollutants are; dissolved metals, trams oil, organic compounds (methilpentane-diol, phenoxy- isopropanol), and bacteria.

2. Research Methodology

The basic principle of the system Electrocoagulation (EC) is an electrochemical process, which occurs in the oxidation reduction process wastewater samples with the help of electric current. This process is used as a

method of waste water treatment, in practice almost completely does not require chemicals. The effectiveness of EC can decrease COD, BOD and turbidity from organic and inorganic waste water, The capability of this EC depend on the processing time and supplied current.

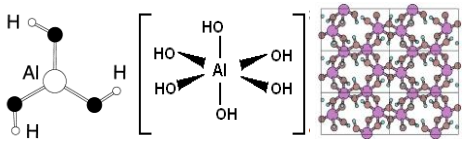
In Electrode Reactions, In this study/research using aluminum electrode. The process of oxidation reduction occurred in the reactor which has a pair of Anode and Cathode electrodes. The water content in the sample will have electrolysis, I.e. the decomposition of water molecules into ions. The reaction is

$$2\ H_2O \rightarrow OH^- + H^+$$

The OH^- ion is active and react with aluminum ions from the anode to form $Al(OH)_3$.

The molecular structure of $Al(OH)_3$ or Aluminum Hydroxide is amfoteric compounds, these compounds can react to acidic or alkaline conditions. The molecular structure of $Al(OH)_3$ is tetrahedron and a monomer molecule to the others $Al(OH)_3$. When two molecules of $Al(OH)_3$ binds, it will form a dimmer of $Al(OH)_3$ to form the molecular octahedron structure. Dimmer is more stable than monomer. Meanwhile, if there are a lot of $Al(OH)_3$ that binded together, it will form a stable polymer deposition.

Animation of the structure of the monomer, dimmer and polymer $AL(OH)_3$ can be seen in picture below:



Monomer **Dimer** **Polimer**

$Al(OH)_3$ has a strong absorption of the waste, so the result to be heavier and in accordance with the gravity force will settle. Due to the above reaction contained gas bubbles, then some floc and other impurities contained in waste water solution will rise to the surface forming bigger and bigger bubbles.

In Electrode Anode, an oxidation reaction will occur Aluminum metal becomes trivalent aluminum 3^+ ions, if there are samples in the water, the reaction will be:

$$Al \rightarrow Al^{3+} + 3\ e^-$$
$$Al + 3\ H_2O \rightarrow Al(OH)_3 + 3\ H^+ + 3\ e^-$$

Furthermore, with the base condition the reaction will be:

$$4\ OH^- \rightarrow 2\ H_2O + O_2 + 4\ e^-$$

At the cathode electrodes, will occur the reduction reaction against ions in pollutants from other additives or flake /chip metal contained in the sample, forming a smaller valence or simple. If the metal is we symbolized by "M", so :

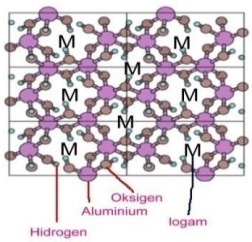
$$M^+ + e^- \rightarrow M$$

For example, if the waste water contained of Cd^{2+} ions, so the ions Cd^{2+} was reduced will be :

$$Cd^{2+} + 2\ e^- \rightarrow Cd$$

Then, the metal "M" will be bound or absorbed into the floc. As we know that Aluminum from a polymer has a charge density is large, so that the metal M will be absorbed into the cavity of the polymer floc. Forming a stable covalent bond.

Animation M are bound in a flock such as Figure 2 below:



While in the acidic conditions of the sample, H^+ ions are reduced to hydrogen gas

$$2\ H^+ \quad + \quad 2\ e^- \quad \rightarrow \quad H_2$$

In Waste Oil Coolant contained Tram oil which will inhibit the process of oxidation at the anode. Tram Oil will be attached to the surface of the anode so that the release of Metal Ions and electrons will be prevented from oxidation reactions and finally the oxidation reactions will not happen. It can be seen from the voltmeter that it will get smaller by degree to show zeros. For that, prior to construction, Tram oil must be separated first. Tram Oil which has been separated can be used as an alternative fuel.

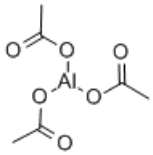
Other component in the waste coolant is Paraffin ($C_{14\text{-}17}$) which is a long-chain hydrocarbon compounds, the Paraffin in waste coolant will easily oxidized to form simpler hydrocarbon compounds, I.e. forming compounds Alcohols, carbonyl or carboxyl bonded to aluminum ion. Where R-CH3 be oxidized to R-CH_2OH then R-CH_2OH react with $Al(OH)_3$ form a complex compound of $Al(OR)_3$.

$$R\text{-}CH_2OH + Al(OH)_3 \rightarrow Al(OR)_3 + H_2O$$

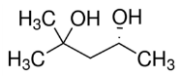
Partly R-CH_2OH will be continuesly oxidized to form aldehydes (carbonyl) and Carboxylic (-COOH);

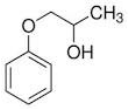
$$R\text{-}CH_2OH \rightarrow R\text{-}CHO \rightarrow R\text{-}COOH$$

Then carbonyl / carboxyl compounds will bind with Al^{3+} ions to form polymeric Carboxylic Aluminum compounds. If passes filter, will get a white precipitate/sludge.



Organic compound can be found in the waste coolant, for example; Methilpentane-diol and phenoxy isopropanol. At Organic compounds will be found some bonding with carbonyl groups / carboxyl, these compounds easily oxidized so that it can cut between the carbon chain. So it becomes the simpler/smaller compounds form.





Methilpentane-diol Phenoxy isopropanol

Methilpentane-diol, this compound has 2 OH^- group, which will bind the coordinate covalent bond with Aluminium element of $Al(OH)_3$ so that forming more complex and heavier compounds, this condition will cause precipitation.

In this Phenoxy isopropanol processing, it will occure the disconnection of oxygen-carbon bond, thus forming two intermediate molecules which simpler than before, I.e. ion isopropanol and cyclic carbon bond. Isopropanol will then bind to the floc by releasing one molecule of water (hydrolyzed). Polymer of this bond can release 3 water molecular so that the molecular structure of water forming a hexagon ring that is not flat.

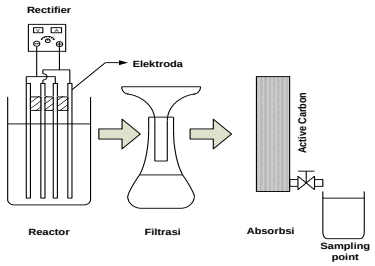
In addition of an electric current, there will be changes in the position of the OH^- group in isopropanol to form other isomers. In the EC reaction will occur electron transfer continuously from the anode, so that it will be visually seen the bubbles - bubbles of gas (O_2 and H_2) at the electrodes and produce white $Al(OH)_3$.

The amount of dissolved aluminum anode is directly proportional to the time and supplied currents, and inversely proportional to the value Valence. This relationship is expressed in Faraday's law, as follows:

$$\mathbf{w = \frac{I\ x\ t\ x\ Mr}{n\ x\ F}}$$

Description :
W = Weight of Electrode Soluble (grams)
I = currents (Ampere)
T = time (sec)
Mr = Heavy Atomic Anode
N = Valence Electron Value
F = Faraday Constans, 9600 Coulomb / mol

At the laboratory scale, schemes of work as shown in figure 3 below;



3. Result

Based on the results of the research can be found the opimal time and current, and it can also be obtained some results of a study on the concentration of TDS, TSS, BOD, COD and pH end. Above datas can be used as a tool to Design, draw and including to define the specification, installation the Electrocoagulation Process for Industrial Scale. Then it also can be obtained the costs of production per cubic metric, taking into account the cost of electricity, maintenance and disposal of sludge. The results can be seen in the Table 1. Below

Tabel 1. Analysis Results

No.	Parameters	TDS	TSS	BOD	COD	PH
1	quality standards	2000	200	50	100	6,0 - 9,0
2	Initial Waste	2650	815	15986	35204	6
3	10 minutes	2014	320	6750	19024	7
	% Removal	24	60.74	57.78	45.96	
4	20 minutes	970	210	1750	7505	7.5
	% Removal	63.4	74.23	89.05	78.68	
5	30 minutes	240	97	325	784	8.2
	% Removal	90.94	88.1	97.97	97.77	
6	40 minutes	26	15	43	98	8.7
	% Removal	99.02	98.16	99.73	99.72	
7	50 minutes	36	13	47	102	9.5
	% Removal	98.64	98.40	99.71	99.71	

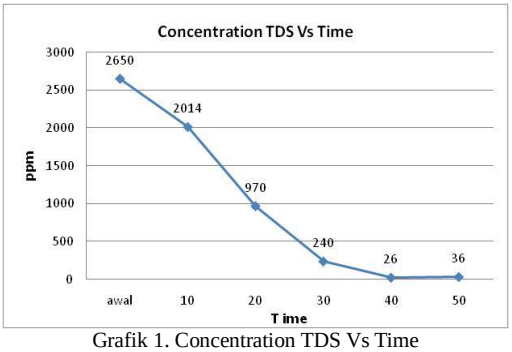
The Degree Of Acidity

In accordance with Table 1 above, with reaction process in the reactor will be an additional pH of 6. This occurs due to the addition of OH^-ion and acid reduction in the sample. Reaction will be effective until the 40 minute, then on 50 minutes the pH will increase that exceeds the quality standard (pH 9).

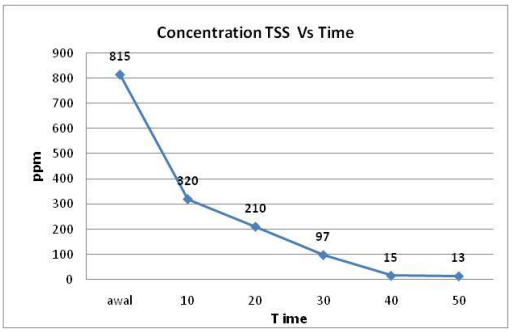
Changes in TDS and TSS in the sample decreased gradually in accordance with the length of the time process and comparable with current supply. Pollutants dissolved and suspended will experience reduction oxidation reactions that result in the form of pollutants into stable compounds precipitates. Smallest decrease in TDS occurs during the 40 minute at 26 ppm. If the addition of processing time to 50 minutes will increase the TDS to 36 ppm. This change indicates that in solution there is excess OH^- ions / floc. This happens because of the influence of the Al^{3+} ion content of the anode who participated dissolved. In addition, this indicates that the pollutants in the sample has been exhausted or very little. The graph of this change can be seen in the graph 1. Below ;

Then, the changes in COD and BOD can be seen in the graph 4 and 5 below; Obtained that are very effective for lowering BOD and COD at minute 40 with BOD to 43 ppm and COD to 98 ppm, the degree of acidity occurs to pH 8.7. And all three of these parameters have been to meet the Quality Standard. but, when the processing time is continued until minute 50 it will increase the value of BOD and COD.

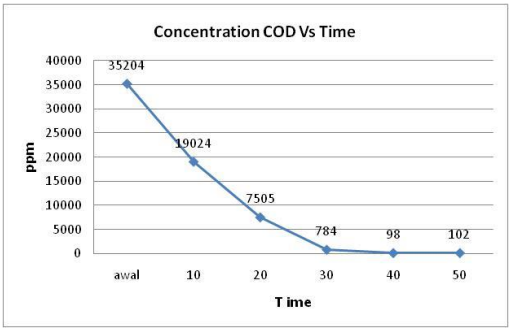
Graphics 6 are Percentage a decrease of TDS, TSS, BOD and COD. Overall condition, the best removal percentage are the 40 minute i.e. TDS at 99.02%, TSS = 98.16%, BOD = 99.73% and COD = 99.72% whereas pH 8.7.



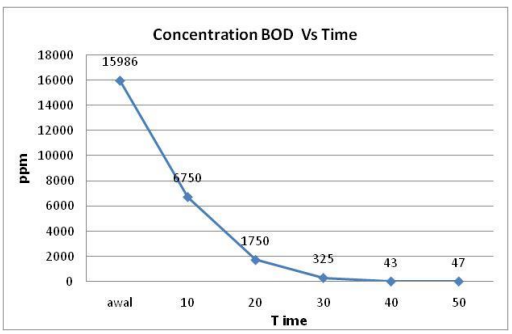
Grafik 1. Concentration TDS Vs Time



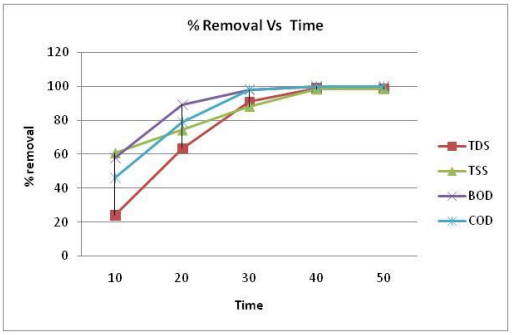
Grafik 2. Concentration TTS Vs Time



Grafik 4. Concentration COD Vs Time



Grafik 5. Concentration BOD Vs Time



Grafik 6. Precentage Removal Vs Time

For make specifications design and technical are based on the results of the datas above, which we pour in Table 2. Below ;

Tabel 2. Parameters Acuan Desain

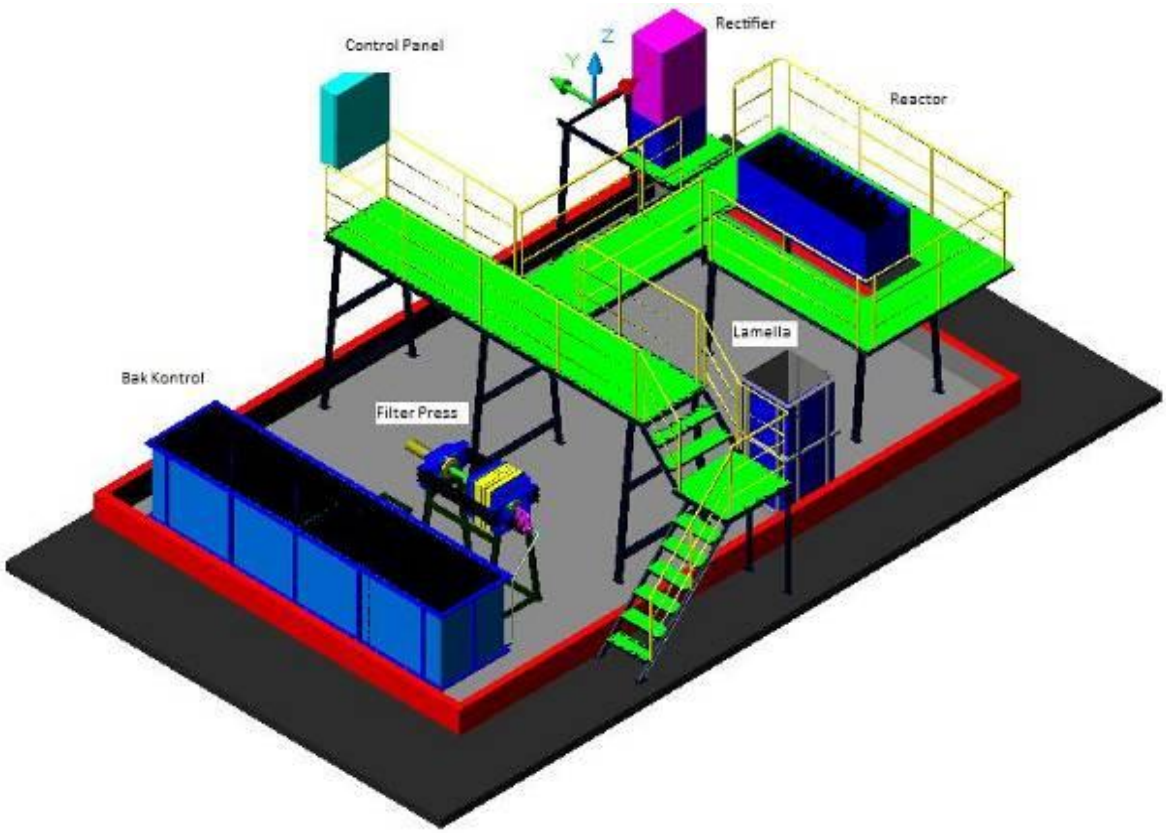
#.	Parameters	value
1	Electric current	5,25 Ampere
2	Voltase	0,78 Volt
3	Time	40 minute
4	Capacity	3 m3/day

Base on the results of the design calculations, we found out that the reactor capacity is 429 liters is needed as much as 1 piece with the operational lifetime of 40 minutes. Electrical power at 3 KVA AC. Wastewater tub (equalization), and tub effluent buffer, each for 3 M3. Specifications Filter press is used with a capacity of 3 m3 per day, Frime size 630X630 mm, plate 6 units, pressure 8 bar, pressing Plate 32 tons, 4 HP motors Hydrulic.
Land required for 1 unit of processing is 5M x 9M.

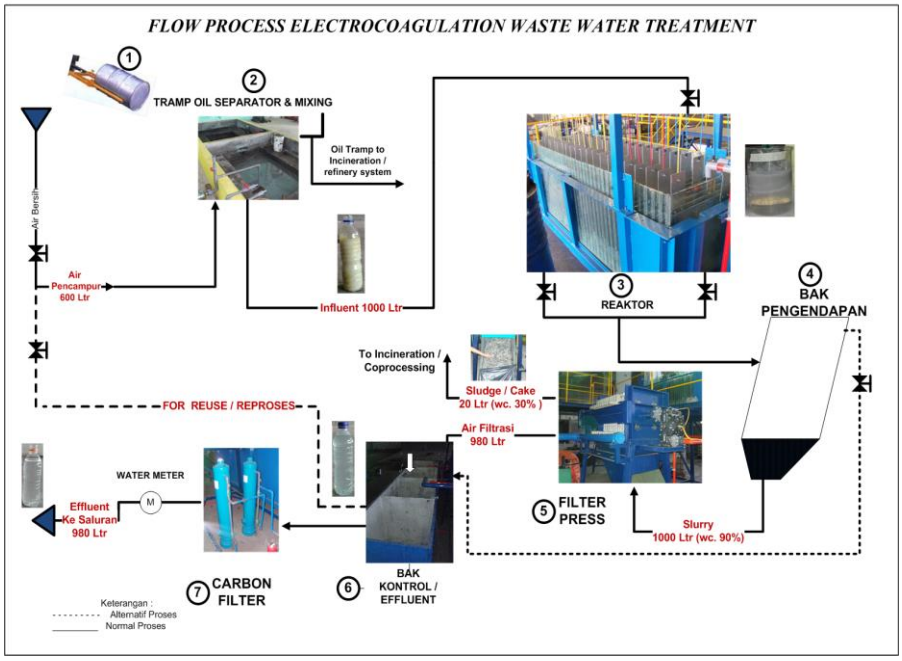
Processing costs

Processing Costs with Electrocoagulation Method here covers all costs to be issued based on, the cost of electricity, replacement electrodes and sludge disposal to 3rd party amounted IDR16.982.-/M3 waste water. With a record if electricity rates IDR830/KWH.

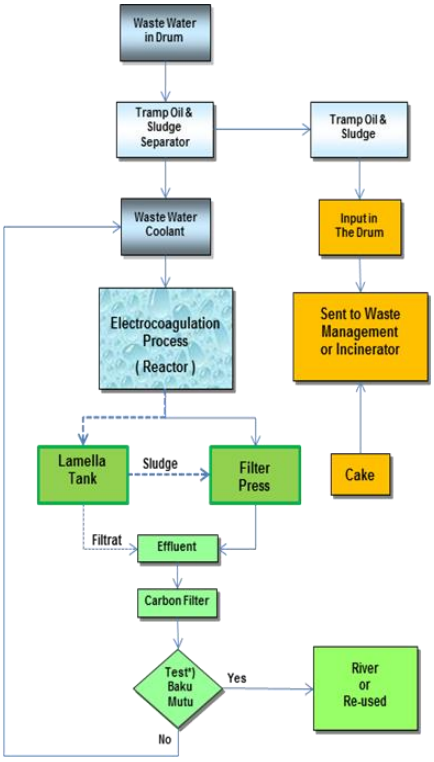
Design tools can be seen in picture 4 below ;



Picture 4. Lay Out 2D-Design Electrocoagulation



Picture 5. Flow Process Electrocoagulation



Picture 6. Flow Chart of Process Electrocoagulation

4. Conclusions

Electrocoagulation technology able to treat wastewater coolant from processes aluminum milling at Indonesia Aerospace Ltd . During the treatment process by the Electrocoagulation method does not produce adverse reactions which harm the environment, i.e. liquid, solid or gas. the processing system is very simple and very friendly environment by releasing oxygen gas into the air. On a large scale the capacity of 3 M3 / day, cost of processing operations at IDR16.982,-/M3. Area required for this tool 5M x 9M.

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The Potential of Energy Eficiency on Recycle Paper Bioprocess

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Abstract.

The increasing use of recycled paper by the paper industry to support the Green Industry in the Indonesian Pulp and Paper Industry. But technically, there are some disadvantages of recycled fiber usage such as low drainage rate in which boosted the high energy consumption on drying process. As already known, the drying process consumes the highest energy in papermaking. To overcome this problem, the research using the concentrate of endoglucanase Egl-II has done. The steps of research included production of endoglucanase Egl-II; concentration by ultrafiltration method, modification of recycled paper fibers using endoglucanase Egl-II; characterization of paper sheet; and evaluation of energy consumption in drying the sheet of paper. The results showed that endoglucanase Egl-II had increase drainage rate of recycled fiber stock, which indicate by the freeness number. The number had increased by 80 ml CSF (Canadian Standard Freeness) from 190 ml CSF to 270 ml CSF, and fiber retention increased by 0,63% from 99,31% to 99,94 %. The dissolved cellobiose test results of recycled fiber stock by the enzyme treatment at low dosage showed that no degradation of cellulose, no change into soluble sugars. Characterization of sheet of paper that has been modified with endoglucanase Egl-II based FTIR spectra showed no change in functional group, SEM test results showed on the fiber surface fibrils grow more, and the results of the XRD analysis showed area reduction of amorphous regions is about 6%. The potential for energy efficiency is calculated through thermodynamic approach is about 15 %.

Keywords: Area reduction of amorphous regions, endoglucanase Egl-II, energy efficiency, fiber modification, recycled paper.

1. Introduction

The trend of utilization of recycled fiber in Indonesian paper industries are increasing recently, along with the launching of government policy regarding the green industry. Green industry is environmentally friendly industry, which aligns development with preservation of the environment as well as giving priority to efficiency and effectiveness of the use of resources in a sustainable manner. In addition, the use of recycled paper is very promising because of its cheap price, ie the price of a local recycling cardboard around 1200 IDR/kg, compared with the price NUKP, around 8600-8700 IDR/kg. Cardboard or corrugated-board box is a type of recycled paper easiest and most widely collected [1]. Corrugated-board box is formed of corrugated-board. Corrugated-board made up of liner paper and fluting medium [2]. The raw material of liner paper is needle unbleach kraft pulp (NUKP) and old corrugated carton (OCC). Fluting medium is made from a mix waste paper and OCC.

But technically, there are some disadvantages of recycled fiber usage such as low drainage rate in which boosted the high energy consumption on drying process. As already known, the drying process consumes the highest energy in papermaking. This process consumes energy of 470 Tbtu or paper machine consumes electricity of 192 kWh/t and steam 806 kWh/t [3].

In the paper-making process, the drainage rate observed through the freeness numbers, which can be tested using two types of methods: Schopper Riegler and Canadian Standard freeness. In the Canadian Standard freeness method, the principle of the test is drainage through a fibre mat formed during the test on a perforated screen plate of a given volume of an aqueous pulp suspension into a funnel provided with a bottom and a side orifice. Determination is made of the volume of filtrate discharged from the side orifice. The volume of the discharged filtrate, in millilitres, is the Canadian Standard freeness of the pulp. The less the volume of the filtrate were measured indicate that water retention is getting stronger by the pulp. According to ISO 5264-1: 2011, the supply of stock is done by refining the pulp at 1.5% consistency without load or the load until the freeness of 300 ml CSF or 40 °SR. The freeness of recycled paper from the outlet refiner average of 180 ml CSF (Fig. 1). It means recycled paper pulp is hold water very strong, so that the drainage rate is under standard or the quality is low.

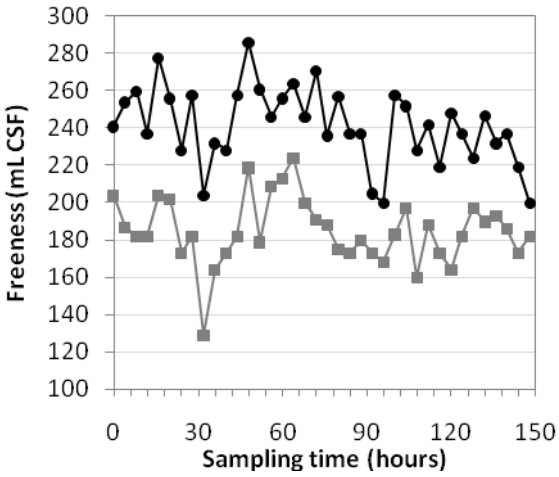


Fig. 1. Freeness of inlet and outlet of the refiner in recycled paper mill that produces liner and medium paper.

Efforts to improve the drainage rate has been performed using the endoglucanase and exoglucanase. According to [4], endoglucanase III from *T. reesei* Cel 12A is very effective in improving the drainage rate from the secondary fiber. In practical applications, endoglucanase I TrCel7B not cause a decrease in the strength of the paper on the treatment of secondary pulp. Exoglucanase applications on recycled paper production is done by a controlled treatment increases the strength of the paper [5]. The recombinant enzyme is better than native bacterial enzyme, because enzymes produced more pure and have a better ability to bind and degrade cellulose cellulose [6].

Fiber modification using endoglucanase is done by incubating the fiber in the process of stock recycled pulp preparation [7]. The incubation process is done after the refining process to avoid friction that can stimulate the degradation of the fiber. Endoglucanase is easier to hydrolyze the amorphous portion on the surface of the cellulose fibers than the crystalline cellulose fibers. The surface of the cellulose fibers condition is associated with water retention ability by the fiber. The amorphous regions of cellulose fibers are readily penetrated by water, by contrast crystalline regions are difficult to penetrated [1].

The main thing that distinguishes the papermaking from virgin pulp and recycled pulp is at the step disintegration and refining of the pulp [1]. The process that occurs during papermaking leads to changes in fiber properties on recycled pulp. Basically, the changes that occur in recycled fibers caused by the shorter of the fiber length and the lower of fiber flexibility than virgin pulp [8]. In the paper sheet formation, stock containing water need to set aside the water in order to obtain a dry sheet. According to [9], thermodynamically, the allowance of water in the drying process using the following equation:

$$Q = mC_p(T_b - T) + m\lambda \quad (1)$$

By: Q = energy for drying (J); M = mass of water that drained (g); Cp = heat capacity of water (J / g.K); T b = boiling point of water (K); T = initial temperature (K); λ = latent heat at the boiling point of water (J / g).

Enzyme treatment can increase the freeness which means water that is on the wet sheet less than the untreated enzyme. Suppose the mass of water in the sheet without treatment (m_0) and the mass of water in the sheet after the enzyme treatment (m_1), then $m_0 > m_1$. Thus the energy to eliminate water in wet sheets that have undergone enzyme treatment was lower than that of the untreated enzyme ($Q_0 > Q_1$).

Endoglucanase Egl-II has an optimum pH in the neutral pH [10], so that appropriate to be applied in the paper industry. The process of making paper at neutral or alkaline pH is more efficient than at acidic pH [11]. This research needs to be conducted to determine the potential of energy efficiency on recycled paper bioprocess using concentrated endoglucanase Egl-II which is an Indonesian product.

2.Material and Method

2.1. Materials.

Samples of recycled paper used is low quality old corrugated carton/OCC (local OCC for instant noodle) obtained randomly from the area Baleendah and Dayeuhkolot, Bandung, West Java. OCC from papermill A and papermill B, distilled water that meets the requirements for freeness testing. Endoglucanase Egl-II expressed by *Bacillus megaterium* which carries plasmid- $PMM_{1525\text{-}egII}$ ekstrasel. *B. megaterium* was obtained from the Laboratory of Biochemistry, Institute of Technology Bandung. Cellulase from *Aspergillus niger*, (Sigma, Index-No.: 647-002-00-3). Pa chemicals pro analyst consisting of tryptone, yeast extract, NaCl, tetracycline, peptone, xylose, bovine serum albumin, carboxy methylcellulose, dinitrosalisilat acid, cellobiose, Bradford reagent, and water distillate. Polysulfone membrane (PM) with a pore size of 10 kDa, 30 kDa and 50 kDa for endoglucanase concentration by ultrafiltration.

2.2 Method

Production of endoglucanase Egl-II and concentration by ultrafiltration method. Production, activity measurement [12], and determination of protein content [13] of carried out in accordance with the method in [14]. Endoglucanase Egl-II was concentrated by ultrafiltration method [15; 16].

Modification of recycled paper fibers using endoglucanase Egl-II.

Recycled fiber stock was prepared by the procedure according to [17]. Initial freeness of recycled fiber stock was 490 ml CSF (Canadian Standard freeness). The stock used in this study has a freeness number 190 ml and 3% consistency. Recycled fiber modification was done by incubating the mixture of endoglucanase Egl-II and the stock. Total activity (both endoglucanase Egl-II or commercial cellulase) which is used to modify the fiber is 0; 0.1; 0.3; 0.7; 1.0; 1.5; 2.0; and 4.0 U/g of oven dry pulp. Hydrolysis of recycled fiber stock was done by the procedure according to [17].

To observe the changes that occur in the recycled fiber stock without modification and modified by using the enzyme in various enzyme activities, the stock are measured freeness numbers (water drainage capability), with reference to the method of ISO 5267-2: 2008 Pulps - Determination of drainability - Part 2: Canadian Standard freeness method; retention of fibers by first past retention method (FPR); fines content, and reducing sugar content. Reducing sugar released was determined by dinitrosalicylic acid reagent (DNS) [12] using cellobiose as a standard, to determine the effect of enzymes on cellulose degradation as the main chemical component in the paper into glucose or cellobiose. Fines content assay is performed to determine the percentage of fine cellulose fibers that pass during the papermaking. Each measurement is carried out three times (triplo).

Making laboratory sheets of pulp for characterization of paper sheet. The steps of making laboratory sheets are making wet sheets, pressing, and drying the sheets. Making handsheet is done with reference to the SNI ISO 5269-1: 2012 Pulps - Preparation of laboratory sheets for physical testing – Part 1: Conventional sheet-former method. For each variable is made of at least 6 (six) laboratory sheets. To ensure the functional groups changes that occur in the cellulose fibers of the recycled paper that effected by endoglucanase Egl-II and commercial cellulase, were examined by Fourier transform infrared spectrophotometri (FTIR), the changes that occur on the surface of the fiber, were observed with scanning electrone microscope (SEM). The changes in the inner structure that caused by the drying were learned of the crystallinity changes as measured by X-ray Difraction (XRD).

Evaluation of energy consumption in drying the sheet of paper. Determination of the potential of energy efficiency in recycled paper bioprocess is done by measuring the moisture content of the wet sheet before and after pressing of modified pulp suspension. Activity of enzyme from either endoglucanase Egl-II or commercial cellulase commercial enzyme used to modify waste paper fibers were 0; 0,1; 0,3 dan 1,5 U/g oven dry pulp. Potential energy efficiency that calculated by thermodynamic approach is done by measuring the moisture content changes of wet sheets of recycled paper fiber stock, during drying in an oven at 105 °C, the water content measurements performed at intervals of 15 minutes until the sheets become dry. Activity of endoglucanase Egl-II used to modify waste paper fibers were 0; 0.05; 0.1 and 0.2 U / g of oven dried pulp.

3. Result and Discussion

The result of production of endoglucanase Egl-II and concentration by ultrafiltration method.

Crude endoglucanase Egl-II has a total activity of 0.212 units/ml and a specific activity of 22.282 units/mg protein. Concentration by ultrafiltration method increases the total activity of the enzyme from 0.212 to 0.678 units/ml, increasing the protein content from 0.010 mg/ml to 0.021 mg/ml and specific activity increased from 22.282 to 32.286 units/mg protein. While commercial cellulase from *Aspergillus niger* has a specific activity of ~ 0.8 units / mg.

Table 1 Enzyme activity, protein content and specific activity of the crude and concentrated of endoglucanase Egl-II in comparison with commercial cellulase from *Aspergillus niger*

Sample	Enzyme activity (Units/ml)	Protein content (mg/ml)	Specific activity (Units/mg protein)
Crude endoglucanse Egl-II	0.212	0.010	22.282
Commercial cellulase from *Aspergillus niger*	-	-	0.8
Concentrated of endoglucanase Egl-II	0.678	0.021	32.286

From the analysis of protein content in Table 1, it can be seen that after ultrafiltration the protein content increased by approximately 2 times, so there has been a concentration of protein content.

The result of recycled paper fibers modify using endoglucanase Egl-II.

The effect of treatment with Egl-II to changes in the value of freeness, retention of fibers, fines and levels of soluble cellobiose concentration of pulp fibers recycled paper can be seen in Table 2. Measurement of freeness numbers performed to determine the effect of endoglucanase Egl-II of the drainage stock pulp. Effect of enzymes on the retention of fiber expressed as % FPR. Fines content test is performed to determine the percentage of fine cellulose fibers that lost during the papermaking. Cellobiose concentration test was conducted to determine the effect of endoglucanase Egl-II to the degradation of cellulose as the main chemical components on recycled paper into soluble saccharides.

Table 2. Effect of endoglucanase Egl-II and commercial cellulase to changes in the value of freeness, FPR, fines content and cellobiose content of recycled paper fibers stock

Enzyme dosage (U/g oven dried pulp)	Parameter uji (satuan)							
	Freeness (ml CSF)		Fiber retention (%FPR)		Fines content (%)		Cellobiose content (mM)	
	Egl-II	Commercial cellulase	Egl-II	Commercial cellulase	Egl-II	Commercial cellulase	Egl-II	Commercial cellulase
Blanko	190	190	99.31	99.31	0.69	0.69	0.0	0.0
0.1	270	220	99.94	99.76	0.06	0.24	0.0	0.0
0.3	210	210	99.51	99.75	0.49	0.25	0.0	0.0
0.7	210	200	99.39	99.68	0.61	0.32	0.0	0.0
1.0	220	210	99.31	99.74	0.69	0.26	0.0	0.0
1.5	230	210	98.81	99.14	1.19	0.86	0.0	0.0
2.0	240	220	98.42	99.20	1.58	0.80	0.0	0.0
4.0	250	240	98.68	99.28	1.32	0.72	0.0	0.0

Freeness target of pulp for the papermaking is about 300 ml CSF. Initial freeness of recycled paper pulp fibers used is190 ml CSF. Optimum dosage of the applications of endoglucanase Egl-II can be seen in Table 2 is 0.1 units /g oven dried pulp of recycled paper, Egl-II has increased the freeness of 80 ml CSF, from 190 ml to 270 ml, close to the target desired freeness. The use of higher dosages of enzyme (0.3 to 4.0 U / g of oven dried pulp of recycled paper) has resulted in a lower freeness, with an increasing trend. When compared with commercial cellulase application, it has increased in the freeness of recycled paper, the use of endoglucanase Egl-II provides better results. According to [8], pulp treatment with cellulase improve drainage at low dosage. The effect of different raw materials to the modified fiber can be seen in Fig. 2.

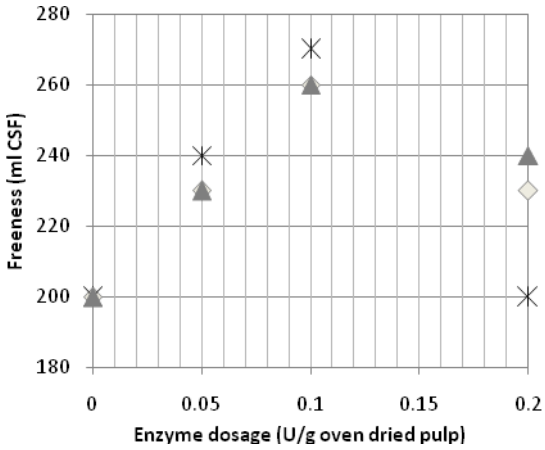


Fig. 2. Changes in the freeness of recycled fibers pulp modified by endoglucanase Egl-II on some OCC, ◊: local OCC, *: OCC from papermill A, ▲: OCC from papermill B.

Applications of endoglucanase Egl-II at low dosages (0.05 and 0.1 U / g of oven dried pulp) to three different OCCs, namely local OCC, OCC from papermill A and OCC from papermill B. The result can be seen in Fig. 2 that these has given similar pattern changes of the freeness, did not show significant differences. Optimum dosage of these are 0.1 units/g oven dried pulp of recycled paper.

Fibers modification by lower dosage of endoglucanase Egl-II (0.1; 0.3 and 0.7 U/g dry oven pulp of recycled paper) have increased fiber retention but in higher dosage decreased it (Table 2). The highest fiber retention is modified by 0.1 U/g oven dried pulp of endoglucanase Egl-II, has increased from 99.31% to 99.94% (up 0.63%). Fiber retention have increased because of the high binding ability of cellulose binding domain (CBD) of endoglucanase, thus increasing inter-fiber bonding. Due to increased retention, fines content decreased.

Cellobiose content assay of filtrate of recycled fiber pulp which has treated by all dosage of endoglucanase Egl-II and commercial cellulase have showed no soluble saccharides (Table 2). It means that treatment by these enzymes at those dosages does not cause degradation of cellulose into soluble saccharides. Complete hydrolysis of cellulose by endoglucanase result cellobiose as main product. Cellobiose are soluble in water. Controlled hydrolysis of cellulose by endoglucanase Egl-II and commercial cellulase modify the fiber without causing degradation of cellulose fiber into cellobiose.

The result of characterization of paper sheet.

(a) Effect of treatment by endoglucanase Egl-II in the functional groups of recycled paper fibers.

Determination of the functional groups by FTIR has done to study the changes by endoglucanase Egl-II and commercial cellulase treatment that occur in the cellulose fibers of the recycled paper.

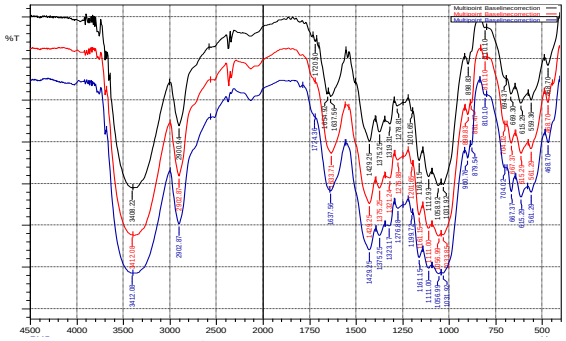
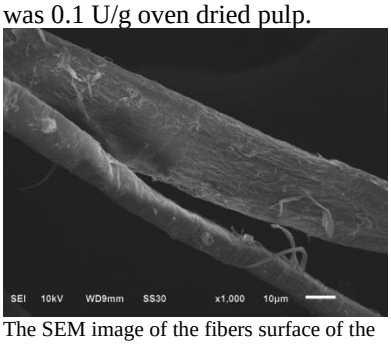


Fig. 3. The FTIR overlay spectrum of --- = local OCC (blank), --- = paper sheets modified by endoglucanase Egl-II, and --- = paper sheets modified by commercial cellulase

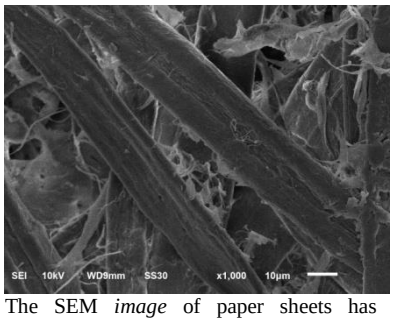
The FTIR overlay spectrum (Fig. 3) has shown that no change in functional group in paper sheets modified by endoglucanase Egl-II and commercial cellulase 0.1 U/g oven dried pulp, compare to the blank. Controlled hydrolysis of cellulose by endoglucanase Egl-II and commercial cellulase modify the fiber without causing changes in the functional groups of recycled paper fibers.

(b) Effect of treatment with endoglucanase Egl-II on recycled paper fiber surface.

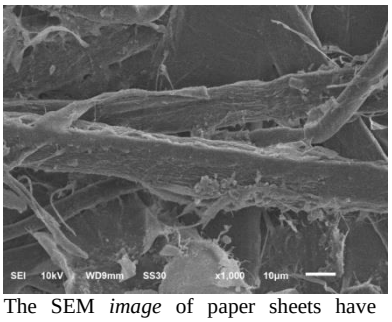
Observation by SEM methods performed to ensure the changes that occur on the surface of the fiber. Fig. 4a has shown the SEM image of the fibers surface of the blank (local OCC), paper sheets have modified by Egl-II (Fig. 4b) and paper sheets have modified by commercial cellulase (Fig. 4c). The enzyme dosage used was 0.1 U/g oven dried pulp.



The SEM image of the fibers surface of the blank (local OCC)



The SEM *image* of paper sheets has modified by Egl-II



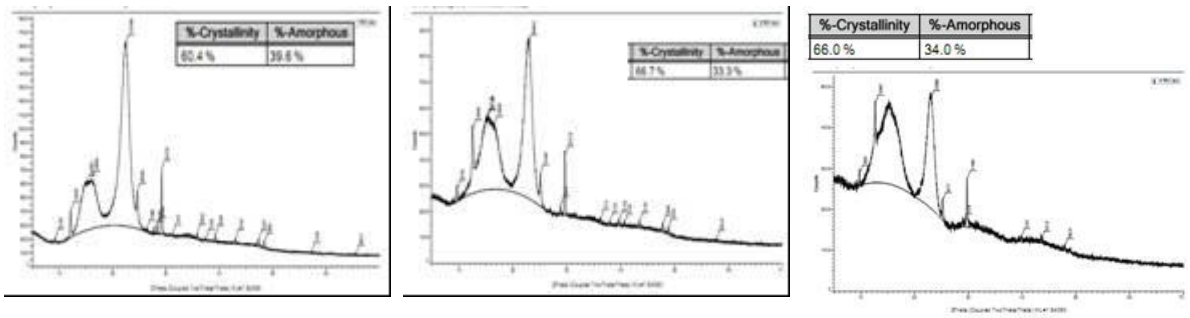
The SEM *image* of paper sheets have modified by commercial cellulase

Fig. 4. SEM image of the fibers surface of the recycled paper.

The fibers surface of the blank appear smooth, there are only a few of fibrils, while the fibers surface of paper sheets have modified by Egl-II appear more fibrils so that increasing inter-fiber bonding. Fiber modification by commercial cellulase has caused appear more fibrils on the surface of the fiber, but is accompanied by the presence of cracks. According [5], treatment with cellulase enzymes in the secondary fibers grew more fibrils.

(c) Effect of treatment with endoglucanase Egl-II in crystallinity on recycled paper fibers.

XRD measurement method performed to ensure that changes in the inner-structure occurs in recycled paper fibers due to the drying process. Inner-structure changes in a comparison of the percentage of crystallinity and amorphous structure. Fig. 5a has shown the XRD spectrum of the blank (local OCC), paper sheets have modified by Egl-II (Fig. 5b) and paper sheets have modified by commercial cellulase (Fig. 5c). The enzyme dosage used was 0.1 U/g oven dried pulp.



The XRD spectrum of the blank (local OCC)

The XRD spectrum of the paper sheets have modified by Egl-II

The XRD spectrum of the paper sheets have modified by commercial cellulase

Fig. 5. The XRD spectrums of the recycled paper sheets

The XRD analysis of the recycled paper sheets have done to study the changes in the inner-structure caused by the drying. The results have shown that fibers modifications by endoglucanase Egl-II improving crystallinity and reducing amorphous regions about 6%. Crystallinity regions increased from 60.4% (Fig. 5a) to 66.7% (Fig. 5b) and 66.0% (Fig. 5c). Amorphous regions reduced from 39.6% (Fig. 5a) to 33.3% (Fig. 5b) and 34.0% (Fig. 5c).

The results of energy consumption evaluation in the drying of wet paper sheet.

Changes in the moisture content of the wet sheet before and after pressing of the stock of recycled paper fibers which have modified by endoglucanase Egl-II and commercial cellulase of 0.1; 0.3 and 1.5 U/g oven dried pulp, compare to the blank, can be seen in Fig. 6.

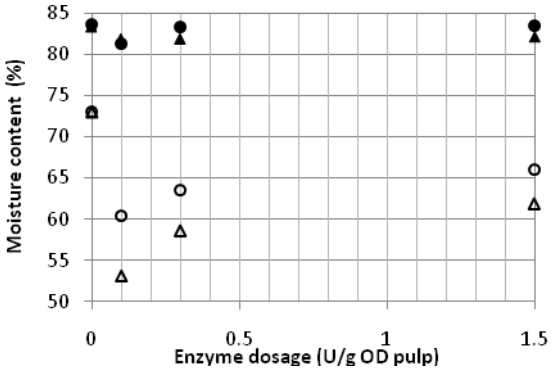


Fig. 6. Changes in the moisture content of the wet sheet before and after pressing of the stock of recycled paper fibers. ●: The results of wet sheet modified by Egl-II, ○: The results of wet sheet after pressing modified by Egl-II, Δ: The results of wet sheet modified by cellulase commercial ▲: The results of wet sheet after pressing modified by cellulase commercial.

The greatest changes in the moisture content of the wet sheet before and after pressing of the stock of recycled paper fibers which have modified by endoglucanase Egl-II and commercial cellulase is in treatment at a dosage of 0.1 U/g OD pulp, that is equal to 13 %. To calculate the energy efficiency potential through thermodynamic approach has been carried out measurements of changes in the moisture content of the wet sheets of of the stock of recycled paper fibers which have modified by endoglucanase Egl-II of 0; 0.05; 0.1 and 0.2 U/g OD pulp during drying in an oven at 105 °C. Measurement of moisture content is done every 15 minutes intervals until the sheets become dry. The result of this determination can be seen in Fig. 7.

Refer to Fig. 7, it can be seen that the greatest changes in the moisture content of the wet sheet at every 15 minutes intervals drying until the sheets become dry which modified by Egl-II 0.1 U/g OD pulp. This treatment can reduce the moisture content of wet sheets of 80.76% to 78.52% before drying (0 minute drying time) and reduce the moisture content of 43.97% to 35.38% (15 minutes drying time).

Approach to calculating potential energy efficiency on recycled paper bioprocess. From the determination of moisture content changes of the wet sheets at every 15 minutes intervals drying until the sheets become dry which modified by Egl-II in variation of enzyme dosage (Fig. 7) has been carried out through the calculation of the potential for energy efficiency thermodynamic approach [9], the results can be seen in Fig. 8.

Refer to Fig. 8, the results of the energy efficiency potential is calculated by thermodynamic approach [9] are shown that the highest energy efficiency which modified by Egl-II 0.1 U/g OD pulp. This treatment can reduce 15% of the drying time energy at 15 minutes the second to fifth (at every 15 minutes intervals drying until the sheets become dry).

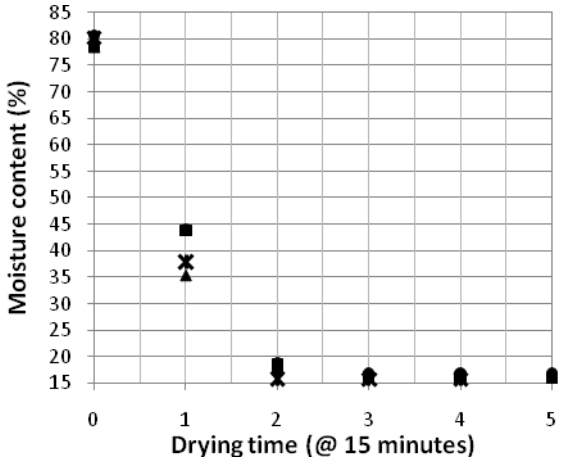


Fig. 7. Changes in the moisture content of the wet sheets which modified by Egl-II at every 15 minutes intervals drying until the sheets become dry. ●: Blanko, ■: The wet sheet has modified by Egl-II 0.05 U/g OD pulp, ▲: The wet sheet has modified by Egl-II 0.1 U/g OD pulp, *: The wet sheet has modified by Egl-II 0.2 U/g OD pulp.

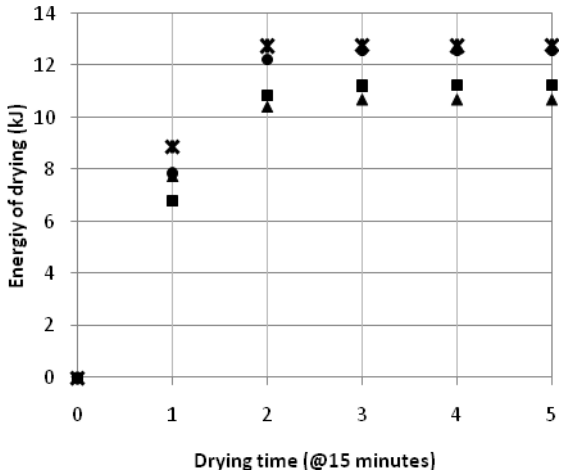


Fig. 8 the results of calculation of the energy efficiency potential through thermodynamic approach of of the wet sheets which modified by Egl-II at every 15 minutes intervals drying until the sheets become dry. ●: Blanko, ■: The wet sheet has modified by Egl-II 0.05 U/g OD pulp, ▲: The wet sheet has modified by Egl-II 0.1 U/g OD pulp, *: The wet sheet has modified by Egl-II 0.2 U/g OD pulp.

4. Conclusion

The results showed fiber modification by endoglucanase Egl-II 0.1 U/g OD pulp can be used to reduce drainage of recycled paper pulp fibers, shown by the increase in the number of 80 ml CSF freeness of 190 ml CSF initial freeness to 270 ml CSF, increasing the retention of the fiber by 0.63% from 99.31% to 99.94%. Cellobiose content assay of filtrate of recycled fiber pulp which has modified by 0.1; 0.3; 0.7; 1.0; 1.5; 2.0; and 4.0 U/g of OD pulp of endoglucanase Egl-II and commercial cellulase have showed no degradation of cellulose into soluble saccharides.

FTIR spectrum overlay of local OCC (blank), paper sheets modified by Egl-II 0.1 U/g OD pulp, compared with paper sheets modified by commercial cellulase 0.1 U/g OD pulp, showed no change in the functional groups. The SEM image of the fiber surface appears smooth blank, there are only a few sheets of fibrils, while treatment with Egl-II grow more fibrils, treatment with commercial cellulase was growing more fibrils but is accompanied by the presence of cracks on the surface of the fiber. The results of the XRD analysis showed area reduction of amorphous regions is about 6%.

Applications of endoglucanase Egl-II 0.1 U/g OD pulp can reduce the moisture content of wet sheets of 80.76% to 78.52% before drying (0 minute drying time) and reduce the moisture content of 43.97% to 35.38% (15 minutes drying time). the energy efficiency potential is calculated by thermodynamic approach [9] are shown that the highest energy efficiency which modified by Egl-II 0.1 U/g OD pulp. This treatment can reduce 15% of the drying time energy at 15 minutes the second to fifth (at every 15 minutes intervals drying until the sheets become dry).

Aknowledgement.

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The Effectivenees of Laboratory Experiment Method to Increase Activity and Student's Achievement on Teaching Salt Hydrolysis

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Abstract

The objectives of this research are to get the effectiveness of laboratory experiment method to increase students' achievement, students' activity and the significance correlation between students' achievement and students' activity. This research was done in 3-s state senior high school (SMA N) in North Sumatera-Indonesia, those are SMA N 1 Lubukpakam, SMA N 2 Medan, and SMA N 3 Medan. The samples are 2 class from students in SMA RSBI and Non RSBI which taken by purposive random sampling. The research instruments are achievement test and non test in list activity form, which have been validated. The research data analyzed by description and inferential statistic methods. The results data shows that (1) Using laboratory experiment method is effective to increase students' activity on teaching hydrolysis chemistry, (2) Using laboratory experiment method effective to to increase students' achievement on teaching hydrolysis chemistry, (3) There is significant corelation between student's achievement and student's activity on classroom that is taught by using laboratory experiment method.

Keywords : Laboratory experiment method, teaching hydrolysis chemistry, student ahievement, student activity, Senior High School (SMA) students.

1. Introduction

Learning must be done individually by students, learning is experiencing, and learning cannot be delegated to others. According to Edgar Dale, argued that learning is best learned through direct experience. In learning through direct experience of the students do not simply observe directly but he should live, directly involved in the action, and are responsible for the results.

Chemistry is an experimental science, cannot be learned only by reading, writing or listening it. Chemical Sciences not only learn to master a body of knowledge of facts, concepts, principles, but also is a process of discovery and mastery of the procedures or the scientific method. Therefore, in teaching chemistry there are two important issues that must be considered, namely the chemical as a product of the scientists in the form of facts, concepts, principles, laws, and theories of chemistry as a process of scientific work.

Subject matter of abstract and concrete chemistry requires direct observation by the students towards the object and the material being discussed. Therefore, by using practical teaching methods are very effective delivery of teaching materials for students will be confronted with real situations. Practical methods of implementation in the laboratory is expected students will have the ability to think scientifically, is able to find scientific facts, identify, think critically and be able to accept criticism from fellow students owned a difference. Students are asked to experience for her/his, seek the truth and draw conclusions from what has been taught.

Experimental method is a way of teaching which provides the opportunity for students to find some facts that they need or want to know by themselves. This method emphasizes the activities that must be experienced personally, sought and found their own data and solutions.

Learning chemistry is closely associated with experiments suitable with the characteristic of chemistry as an experimental science. There are 2 important things that must be noticed in studying the chemistry that is chemistry as a result of the finding of experts such as principles, laws, theories and the chemistry as process that is scientific work such as laboratory experiment. One effort to improve student's achievement is to use laboratory experiment method. By laboratory experiment method, activities students will be more focused attention on the learning process and not on other things as well as students have the opportunity to develop the ability to observe all things that are involved in the process and can take the expected conclusions.

Learning activity are all activities that done by a student in the context of learning to achieve the goals. Without any activity so the learning process will not be going well. Activity of students in the learning process not only to listening and writing. Increasingly more activities that done by students in learning, so the process of learning that happen will be better. According Sadiman 2008, studied activity is a principle or rules that very important in teaching and learning interactions. So to make it easier for students to learn the salt hydrolysis, the author argues that laboratory experiment method is learning method that appropriate to be used to teaching salt hydrolysis.

The instruction procedures in conducting chemistry laboratory experiment must be concerned, it should at least containing of:

1. The purpose of experiment has to be clear and specified,
2. The equipment and materials which is used in experimental must be explained in details,
3. The steps in the procedures must be easy to follow in observation and data collection,
4. The raised question to be answered has to be relevant to the experimental that could be help to control the students,
5. Typical report to be done by students after finishing an experiment
6. The discussion and suggestion raised related to the laboratory experiment.

Laboratory experiment method is often used because it has the advantages by this following:

a. Students are trained to use the scientific method in facing any problems, so it is not easy to believe in something that has not been true.
b. Students are more active thinking and doing in trying to reach the truth or proof from a theory he had learned.
c. Students find the practical experience and skills in using the experimental tools.
d. Students proved themselves the truth of a theory.

In addition, laboratory experiment method also contain some weakness, among others:

a. This method requires many facilities equipment and materials are not always easy to obtain and expensive.
b. This method requires thoroughness, tenacity and patience.
c. Each experiment is not always deliver the expected results because there may be certain factors that are beyond the reach or control capabilities. (Djamarah and Zain, 2002)

According to Handoyo (in Sugondo, 1988) learning activity classified the or called students' intelectual learning, as this description : First, testing. When the teacher giving the matter, the teacher is possibly involve the students' intellectual by testing and explorating the situation in order to abstraction and finding. Abstraction means identify the essence from the form or structure from thething that has been known while finding means produce something that is new by using imagination, opinion or experiment.

Second, expressing. This activity hopes the students can produce the words, sentences, graph or table by using the symbol appropriate the problem condition. This is the learning process to construct the learning models from the problems that faced. Third, approving. If the students are succes to formulate something, they need tonprove based on the argument or structural reason. Fourth, applicating the problem. Concept and procedural that has been known needed to applicated to the new situation. In applicating , the students are possible to abstraction.

Fourth, finishing the problems. From the complex problem that faced but never has been finished, a student has to finish with the concept or theory also the procedure that has been mastered. Fifth, communicating. This activity is the changing of information between students, every students using the same symbol. The students have to get the chance to stated the their idea verbally and writely, comprehension and interpretation the idea to other students.

Learning activities that has been observed in this research during teaching and learning happen in the classroom those are: (1) give question, (2) answer the question, (3) give argument/suggestion, (4) doing experiment/doing discussion group, (5) write a note for observation result in experiment/discussion group result, (6) doing test/solve problems, (7) make conclusion from experiment /discussion group.

The chemistry topics of salt hydrolysis is categorized as a difficult subject to be taught to the students as the contents of the subjects are difficult to understand. Salt hydrolysis is one of the subject matter in the even semester of Grade XI Sciences. One of learning activities that suggested by BSNP (Badan Standar Nasional Pendidikan) in syllabus KTSP 2006 is to designing and doing experiment to determine salt hydrolysis. From that learning activity so teacher has to combine the theory and practice when teaching of salt hydrolysis.

2. Methods

As description before, the population of this research is all grade XI-Science class in SMA N 1 Lubukpakam, SMA N 2 Medan and SMA N 3 Medan which are having chemistry laboratory facilities. From each unit sample, taken 2 classes as a research target, one as experimental class that taught by laboratory experiment method and another as control class that taught by conventional method. In this research, there are 2 instruments those are test and non test. The instrument test is achievement test in the form of multiple choices and non test instrument is observation sheet. Both of the instruments were used in experimental class and control class. The achievement tests are arranged based on the hydrolysis topic that taught in the study including the properties of some salt which is hydrolyzed in water, the properties of salt which is hydrolyzed from the ionization reaction, the pH of salt solution that is hydrolyzed, the example of salt hydrolysis in daily life. The questions raised for each topic are distributed based on the grade, category, and difficulty level of the subjects. In the instrument that used for measuring student's activities having form observation sheet that has been filled by observer during teaching and learning process. There are 7 indicators, those are give

question, answer question, give suggestion or argument, doing experiment/doing discussion group, make a note for observation result in experiment/discussion group result, doing test/solve the problems, and make conclusion for discussion/experimental report. The research containing of students group treated with conventional method and experimental method.

Research design in this research by using *quasi experimental* with factorial design 2 × 2. This research is done in experimental design and divided into two groups namely control group which applying with conventional method and experimental group which applying laboratory experiment method. Pre test and post test are given to both of control class and experimental class before and after treatment and the students in each group would be taught with the same topic that is hydrolysis.

Implementing of learning has been done by researcher which is helped by observer and other teacher as an observer in the observation of the students' learning activity during the learning process. The steps in this research are:

1) Arranging the research instrument program like lesson plan for each group sample, the activity observation sheet of students in learning process and the test of learning achievement.
2) Giving the pretest in order to homogenize the data for two groups' sample, and then analyze.
3) Conducting the learning process based on lesson plan that has been arranged. During the learning process happen, the observers are active to observe and to write the student's activity in the observation sheet that is prepared.
4) Giving the post test with multiple choice test in order to determine the learning achievement of students for two groups sample.
5) Conducting the data tabulation and describing the research result data.
6) Doing the analysis requirement test (homogenity and normality test)
7) Doing the hypothesis test.
8) Giving conclusion.

3. Result and Discussion

The aim of this research to find out the student's activity and students' achievement that given the treatment by experiment laboratory methods. The result of that, can be seen at below descriptions.

Description of Students' activity

To find out the students' activity is used observation sheet that has been valid. The criteria of observation sheet, The Summary of the research result data can be seen at Table.1 below :

Tabel 1. The description of student's activity result data

SMA N 1 Lubukpakam						
Result	Control Class			Experiment Class		
	Meeting 1	Meeting 2	Meeting 3	Meeting 1	Meeting 2	Meeting 3
Averages	12.97	13.27	13.47	15.20	15.53	14.87
Deviation Standard	2.58	2.64	2.89	2.30	2.27	2.75
SMA N 2 Medan						
Result	Control Class			Experiment Class		
	Meeting 1	Meeting 2	Meeting 3	Meeting1	Meeting 2	Meeting 3
Averages	12.00	12.83	12.88	14.65	14.58	13.88
Deviation Standard	1.97	1.72	1.79	1.75	1.89	2.03
SMA N 3 Medan						
Result	Control Class			Experiment Class		
	Meeting 1	Meeting 2	Meeting 3	Meeting1	Meeting 2	Meeting 3
Averages	14.40	14.98	15.20	15.35	14.75	15.55
Deviation Standard	1.96	1.54	1.38	2.21	1.96	2.19

Based on table 1, the averages of students' activity in control for each meeting is increased from meeting 1 to meeting 3. The data in above table can be presented in figure 1.

Based on the above diagram, we can concluded that in control class for each meeting is increased from meeting 1 to meeting 3. It caused the discussion method that given in control so that it make students more active after giving the method. While in experiment class for each meeting is decreased from meeting 1 to meeting 3. It caused the laboratory experiment method that given in experimental class because he students' are active to observe the experiment.

Description of Students' Achievement

Students' achievement has been calculated by giving 20 instrument test before and after treatment that has been valid. The summary of the research result data can be seen at below table 3.

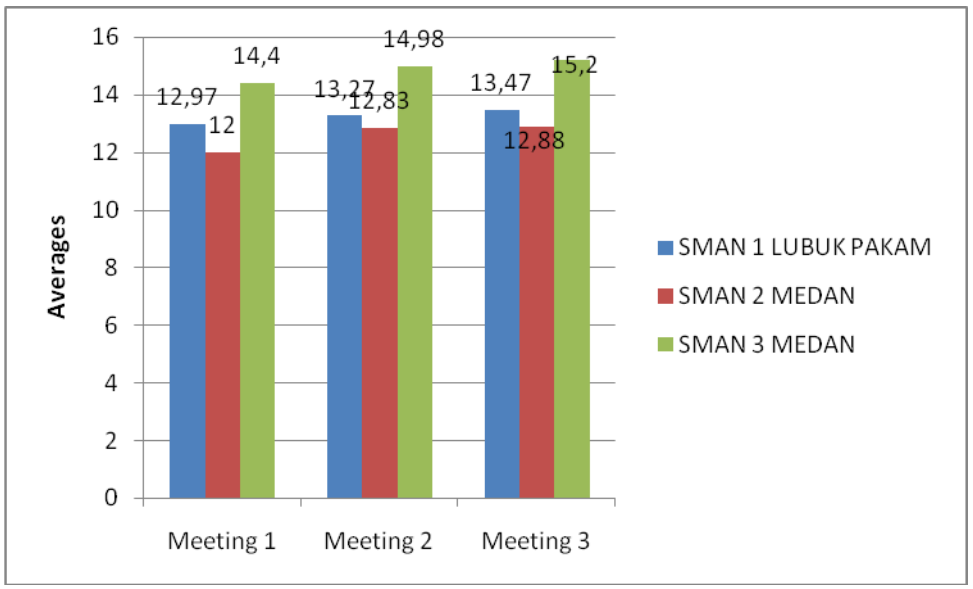

Figure 1. Comparison of students' activity between each school in experimental class

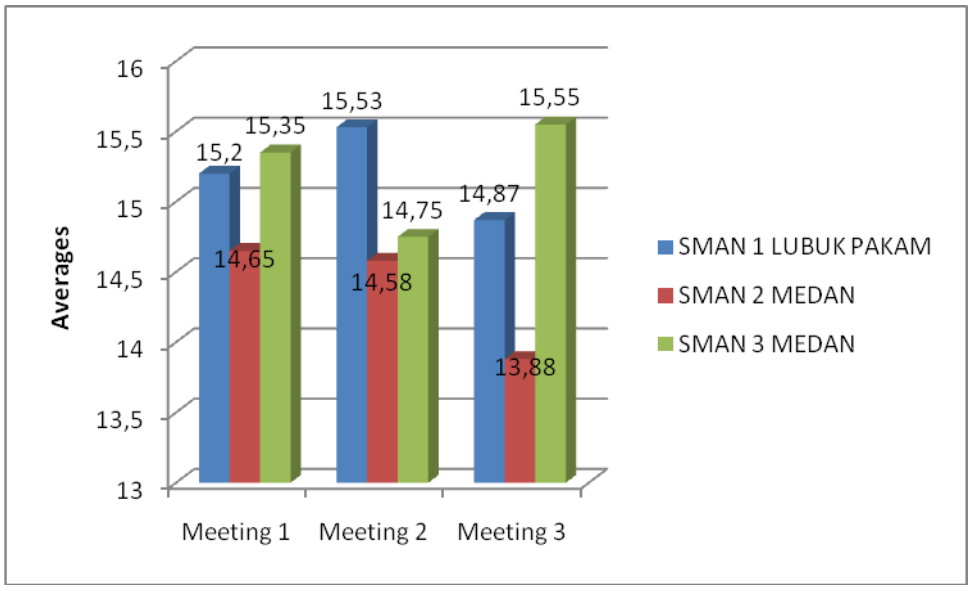

Figure 2. Comparison of students' activity between each school in control class

Tabel 3. The description of student's achievement result data

SMA N 1 LUBUKPAKAM						
Result	Control Class			Experiment Class		
	Pretest	Postest	N-Gain (%)	Pretest	Postest	N-Gain (%)
Averages	29.50	70	57	30.83	77.50	67.99
Deviation Standard	7.47	9.13	11.95	8.10	9.63	11.61
SMA N 2 MEDAN						
Result	Control Class			Experiment Class		
	Pretest	Postest	N-Gain (%)	Pretest	Postest	N-Gain (%)
Averages	29	69	57	30	73.38	62
Deviation Standard	6.28	8.16	11.00	6.50	8.35	8.77
SMA N 3 MEDAN						
Result	Control Class			Experiment Class		
	Pretest	Postest	N-Gain (%)	Pretest	Postest	N-Gain (%)
Averages	25	69	58	28	75	66
Deviation Standard	6.79	7.94	9.26	7.58	9.09	9.77

Based on Table 3, the averages in pretest for each class is similar, it means that the tthes students' for each school has same capabilities about the topic before giving the treatment. The data in the table can be presented in figure 3.

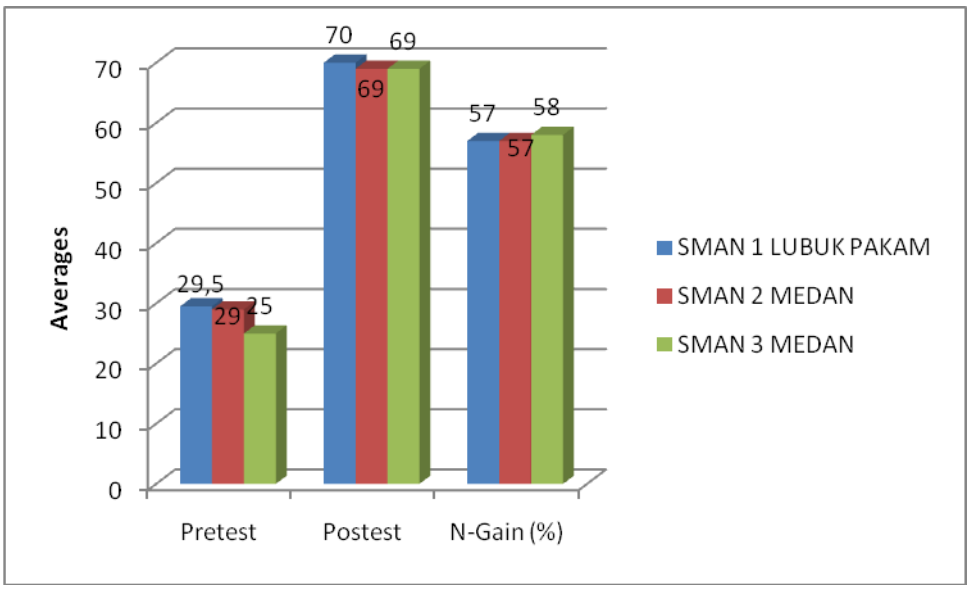

Figure 3. Comparison of students' achievement between each school in control class

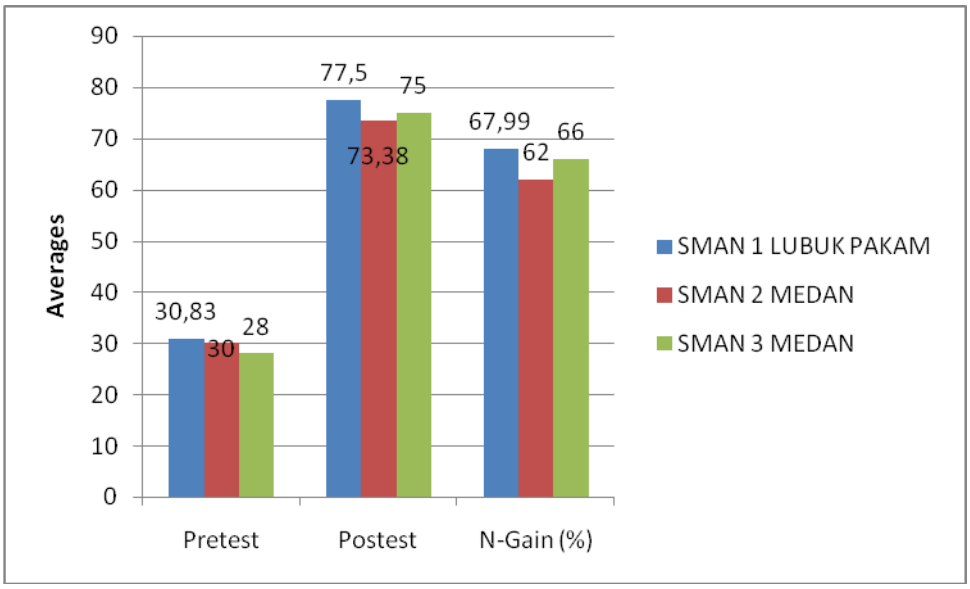

Figure 4. Comparison of students' achievement between each school in experimental class

From the above figure, we can see that the value pretest for each school has the similar value. It means that the students from each school has same capability before giving the treatment in teaching and learning process. The effectiveness percentage of student's achievement is 11.703 % from average gain of experiment class (64.60) and average gain from control class (57.04), while student's activity is 8.45 from average of experiment class (71) and control class (65).

4. Conclusion

Based on the research result in the discussion obtained the conclusions that (1) laboratory experiment method effective to be used to increase students' activities on teaching hydrolysis chemistry, (2) laboratory experiment method effective to be used to increase students' achievement on teaching hydrolysis chemistry, (3) There is correlation between two variables that is student's activities and student's achievement that is taught by laboratory experiment method. From the results obtained from this study, some suggestions had to be raised in order to the learning process on chemistry is effective in increasing of student's achievements, they are: It is suggested to chemistry teachers to use laboratory experiment method in order to increase students' achievement and students' activity on teaching Hydrolysis, it is suggested to other researcher in order to notice the relevant topic so that the research result for the next will be better and the activity of students will be increase, it is need to do the next research with other subject matter as an effort to increase education quality especially in chemistry subject.

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Application Method Of Inquiry In Improving Student Learning Chemistry Class XI SMA Sains In Jayapura City

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Abstract

This study aims to reveal the extent of the inquiry method can improve learning outcomes chemistry class XI SMA Se-Jayapura. Inquiry teaching method is a series of learning activities that emphasize the process of thinking critically and analytically to seek and find their own answer to a problem that is questionable thought process itself is usually done through a question and answer session between the teacher and the student The purpose of this research is the study of this entire SMA as the city of Jayapura namely SMA Negeri 1 Jayapura, Jayapura SMAN 2, SMAN 3 Jayapura, Jayapura 4 SMA, and SMA 5 Jayapura, as well as private high school that has at least two classes IPA IPA Two classes from each school were taken into the sample with the assumption that the sample has the same ability. Data analysis techniques (1) The data were analyzed based on the average value, (2) analysis of the problem of learning and mastery learning process; (3) Student learning outcomes expressed by an average score of student worksheets, and tests the end of each basic competence (KD), which consists of two KD each science subjects; (4) Minimum Criteria for completeness (KKM) based on the criteria that a student is said to be complete if the learning gain score ≥ 65 and a class is said to pass the study in the classroom when there are 85% of students have been thoroughly studied. Student study habits data were analyzed descriptively based on the average score. The results showed that (1) a method of inquiry to improve learning outcomes of students of class XI Chemistry as the city of Jayapura. (2) the method of inquiry is one model that can be used as a model for studying chemistry. Methods of investigation can be done individually, as well as with a group debriefing. Based on the minimum criteria for completeness (KKM), studied chemistry by using the method of inquiry showed that SMA 1 increased by 27.27% (KD1), and 25% (KD2); SMAN 2 increased by 23.68% (KD 1), and 57.18% (KD2); SMAN 3 increased by 74.20% (KD1), and 95.4% (KD2); SMA 4 increased 100% (KD1), and increased by 96% (KD2); 5 SMA increased 13.07% (KD 1), and 34.07% (KD2)

Keywords: Cooperative Learning Model, Methods of Inquiry, Learning Outcomes

1. Introduction

The problem of education is still a special attention by the government. Because the Education for All Development Index or the education for all (EFA) in Indonesia has declined each year. In 2011 Indonesia was ranked 69 of 127 countries and degenerate compared to 2010 which is at position 65 Index issued by UNESCO in 2011 is lower than Brunei Darussalam (34), and within four ratings of Malaysia (65). On the issue of education, the attention of the government still feels very minimal. This picture is reflected in the diversity of educational issues are more complicated. The quality of students is still low, less teaching professionals, the cost of education. The success of the learning process in the classroom can be seen from the learning activities and student learning outcomes.

Based on the results of preliminary observations and discussions with fellow teachers of high school chemistry class XI in Jayapura City were conducted in the learning activities in school that the results obtained studying chemistry for class XI is still low (average of 58), compared with the minimum completeness criteria (KKM), which is 65, although it has made various efforts by teachers to improve student learning outcomes learning outcomes are low presumably due to less active students in learning activities, whereas the concepts of science in class XI elusive 1st semester student learning activities that use the lecture method alone Though less precise method of teaching according Trianto (2007) is a teaching goal attainment can be seen from the change in behavior. According Nurhadi (2002), there are some differences in methods of inquiry with Conventional methods include:

Methods of Inquiry

1. Students are actively involved in the learning process.
2. Students learn from friends through group work, discussion, correct each other.
3. Learning and linked to real-life or simulated problems
4. Behavior built on consciousness itself.
5. language taught communicative model, the students were invited to use the language in a real context.
6. Pengtahuan humans have developed by itself in persons Humans create or construct knowledge by giving meaning and understand their experiences.
7. science was developed (constructed) by the man himself, while humans have always had a new perstiwa, then that knowledge is never stable, always evolving.
8. learning outcomes measured by brbagai way: learning process, works, performances, recordings, and other tests.

Conventional methods

1. Students are passive recipients of information
2. Students learn individually
3. Learning is very abstract and theoretical
4. Behavior built on the basis of habit
5. language taught by the formula described structural model to understand, then be trained.
6. Knowledge is a series of arrests against the facts, concepts or laws that are beyond the human self.
7. Truth is absolute and final knowledge.
8. learning outcomes measured by the test.

Based on the above background, it is to improve students' learning activities and learning outcomes of scientific inquiry method of selection method used is based on considerations other than its advantages, this method also is one of the ways recommended in the curriculum level education unit (SBC) in high school with this research expected to obtain results as desired.

2. Research Methods

This study entire SMA as the city of Jayapura namely SMA Negeri 1 Jayapura, Jayapura SMAN 2, SMAN 3 Jayapura, Jayapura 4 SMA, and SMA 5 Jayapura, as well as private high school that has at least two classes IPA IPA Two classes of tiap- each school was taken into the sample with the assumption that the sample mean has the same ability. Working procedures in this study is a cycle of activity that consists of several cycles. Each cycle includes planning, action, observation and reflection, as shown in Figure 1

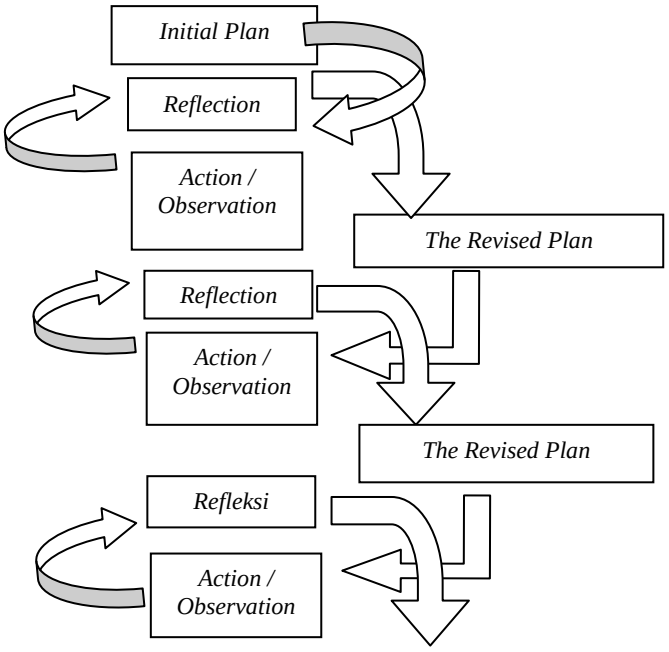

Figure 1, Classroom Action Research Spiral (Tiurlina, 2011)

Data analysis techniques (1) The data will be analyzed based on the average value, and presentation in tabular form; (2) Analysis of the learning process and ketutasan problem of learning; (3) Student learning outcomes expressed by an average score of student worksheets, and tests the end of each basic competence (KD), which consists of two KD each science subjects; (4) Minimum Criteria for completeness (KKM) based on the criteria that a student is said to be complete if the learning gain score ≥ 65 and a class is said to pass the study in the classroom when there are 85% of students have been thoroughly studied. Student study habits data were analyzed descriptively based on the average score Keberhsilan action is an increase in the quality of learning.

3. Results and Discussion

Overall learning is carried out in this study using the conventional method and the method of inquiry In general, the results of statistical analysis to the conventional method and the method of inquiry. In some schools there are students who have not yet reached the KKM although already uses the method of inquiry is due to be konvensioanal it does not work, this is caused by (1) the old paradigm still looks strong teacher, learning activities are still dominated by teachers who do not give a chance to the students to think itself in discovering new concepts, (2) Students are not familiar with the method of inquiry learning.

One of the things that is very influential on learning achievement at school is learning motivation Learning motivation is highly correlated with the results of the study, so that a variety of methods are used to increase students' motivation in school if the student's motivation can be improved, it can be expected that student achievement will also be one increased to improve student motivation is to make or change the method of teaching.

Cycle I (KD 1)

Based on the analysis of data KD I, the results are as follows: the average value of student learning outcomes Se-schools throughout the city of Jayapura for Chemistry subjects are: the average value is 64.85 Chemistry subjects (conventional method) and 74.84 (inquiry method).

Cycle II (KD 2)

Based on the analysis of data KD I and KD 2, the results are as follows: the average value of student learning outcomes across the Se-city school subjects Chemistry Jayapura to the average value is 65.50 (conventional method) and 77.20 (method inquiry).

Based KD 1 and KD 2 in chemistry subjects showed improvement in student learning outcomes by using the method of inquiry seen from the average value in the whole school overall Se-Jayapura. In the research method of inquiry is helpful and motivating students in the learning process, because in this method the students actually trained independently in finding their own concepts in solving a problem.

4. Conclusions and Recommendations

4.1 Conclusion

From the research it can be concluded that:

1. Inquiry method can improve student learning outcomes chemistry class XI Science as the city of Jayapura.
2. Based on the minimum completeness criteria (KKM), Chemical methods of inquiry indicates that SMA 1 increased by 27.27% (KD1), and 25% (KD2); SMAN 2 increased 23.68% (KD1), and 57.18% (KD2); SMA 3 increased 74.20% (KD1), and 95.4% (KD2); SMA 4 increased 100% (KD1), and increased 96% (KD2); 5 SMA increased 13.07% (KD1), and 34.07% (KD2);

4.2 Suggestions

1. Recommended to teachers of chemistry to be able to use the method of inquiry in learning more chemistry in order to improve student achievement.
2. Methods of inquiry in this model using a model of cooperative learning. It is suggested to science teachers to be able to apply the method of inquiry learning model that others ..

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Effect of Dehydration in Making Time Permanent Supply Aedes aegypti Larvae

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ABSTRACT

The preparation is essential in the preparation of a permanent identification. It is said that in the making of permanent preparations such stocks through the long process of breeding eggs form larvae, fixation, dehydration, clearing and mounting adhesive entelan. Through research on the effects of dehydration during a time of 1 minute, 2 minutes, 3 minutes and 4 minutes of time is expected to be more efficient in the preparation of a permanent preparations. This study aims to determine whether there are differences in the effect of dehydration time on making permanent preparations of larvae of *Aedes aegypti*. Microscopic observations performed with the parameters clarity and completeness of the larval morphology of larvae. Clarity of the affected larvae of time while the clearing process completeness is affected larval morphology of time immersion fixation and dehydration process and sampling using a spatula. Effect of dehydration did not produce a significant difference between time 1 minute, 2 minutes, 3 minutes and 4 minutes. It can be said the quality of the preparation preparations through dehydration of the best treatment is to take 5 minutes. For further research, preparation of a permanent can be done using a clearing treatment with various concentrations rise in order to obtain a clearer morphology.

Keywords: *Aedes aegypti* larvae, larvae permanent preparations, the effect of dehydration time of 1 minute, 2 minutes, 3 minutes and 4 minutes.

1. Background

In making permanent preparations, it can not be separated from the dehydration process by using alcohol-rise for insect body. This process has utility as a solution that is able to clean the process by 10% formalin solution that used in the fixation process and eliminate the remnants of fat. In addition, the protein content of the body of the insect is not soluble in aqueous solvents, either saline, acidic, alkaline or alcohol (Andiko, 2009). In making permanent preparations *Aedes aegypti* larvae through dehydration techniques using multilevel alcohol concentration of 30%, 50%, 70%, 80% and 96% for 5 minutes per level. If we have to create a permanent preparation requires quite a long time. For more efficient, the other research has been determined whether there are significant dehydration time in making permanent preparations *Aedes aegypti* larvae, with the same quality and the standard time used is five minutes (Dion, 2002). In addition, the reason for the use of a low alcohol concentration to high so that the process is not too rapid dehydration which will damage the mucosa (soft tissue) and so does not cause artefack that would interfere with the diagnosis. While the concentration used for processing of body tissues typically use alcohol-rise of 70% for 30 minutes, 80%, 90% and 96% for 1 hour (Mahmud *et al*, 2010). Another theory says the concentration of ethanol used for tissue dehydration immersed in a concentration of 70%, 95% and 100%. Ethanol ascertain the amount of dehydration, which makes it as an option for the electron microscope specimen processing. For the soft tissue is recommended that treatment begin using alcohol concentration of 30% (Bancroft, *et al*, 2012). This study aimed to investigate the influence of dehydration time in making permanent preparations with clarity and completeness of morphological parameters of *Aedes aegypti* larvae.

2. Material and Methode

The research was conducted by experiments and the experimental design used in this study is a statistical group comparisons. *Aedes aegypti egg* were used in this study was obtained from Loka Litbang P2B2 Ciamis, West Java. This egg has been bread in warm water on 5-6 days into the third instar larvae. In making permanent preparations *Aedes aegypti* larvae were fixed in 10% formalin for 24 hours The dehydration process using alcohol-rise 30%, 50%, 70%, 80% and 96% starting from the time of 1-5 minutes is done gradually from low concentration to high concentration. After that process, the next step was clearing using xylol for 2 minutes. The quality of each treatment has been observed by panelists that had skill on entomology observation. The statistical analysis Kruskal Wallis was used to compare the results.

3. Result and Discussion

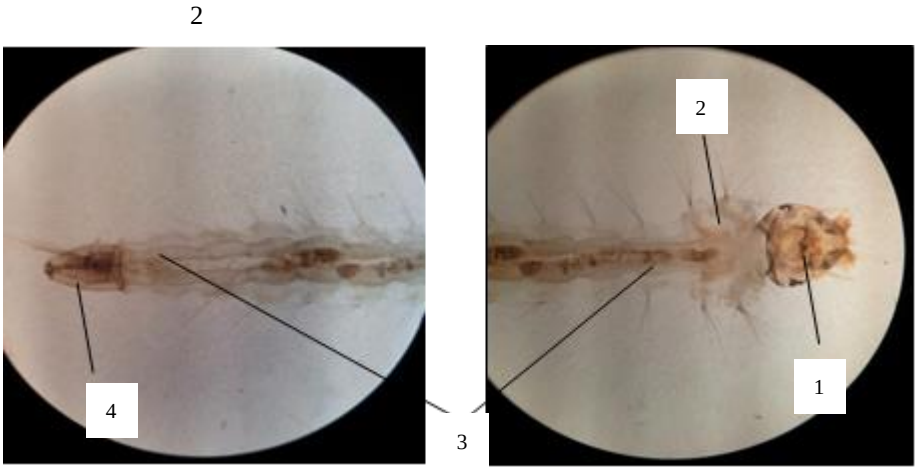


Fig.1 (a) The treatment of 1 minutes in dehydration process, posterior body of *Aedes aegypti* larvae (b) The treatment of 1 minutes in dehydration process, anterior body of *Aedes ae* 1 larvae

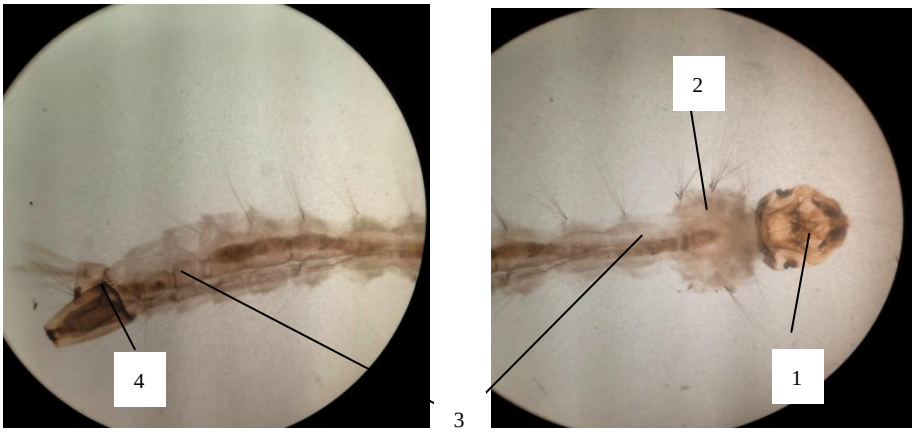


Fig.2 (a) The treatment of 2 minutes in dehydration process, posterior body of *Aedes aegypti* larvae (b) The treatment of 2 minutes in dehydration process, anterior body of *Aedes aegypti* larvae

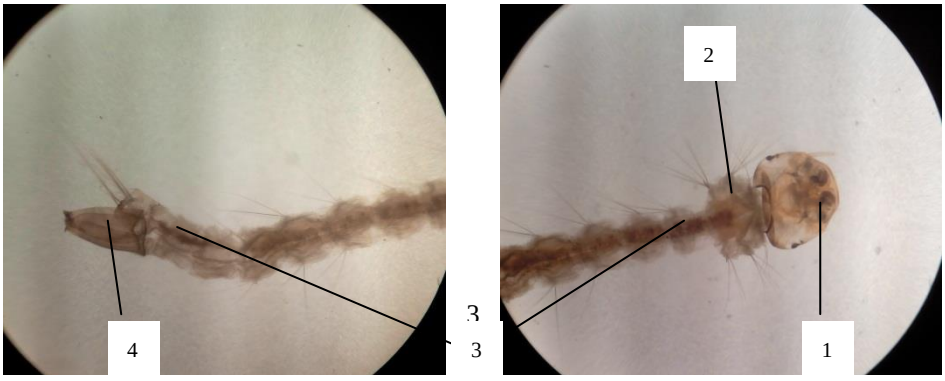


Fig.3 (a) The treatment of 3 minutes in dehydration process, posterior body of *Aedes aegypti* larvae (b) The treatment of 3 minutes in dehydration process, anterior body of *Aedes aegypti* larvae

The factor that influenced in making permanent preparations

Based on the results, it was obtained average of several panelists that the treatment of dehydration time there is no significant difference between time 1 minute, 2 minutes, 3 minutes and 4 minutes. But it was look at the difference in time of 5 minutes (as a control). The absence of a significant difference to the treatment of dehydration process time proving that the standard and quality of the preparations through a dehydration

process can not be influenced by a shorter time. Exoskeleton in Arthropods is used as a muscle attachment. The coated chitin exoskeleton (complex polysaccharides), which is a wax material that can reduce water loss. Chitin layer is called the cuticle. The existence of chitin on the outside of the body causes the growth of these animals is limited (Tarigan, 2012). Therefore, researchers conducted a gradual dehydration process of the concentration of 30%, 50%, 70%, 80% and 96%, so that the larvae are not shocked when the body is inserted into the solution.

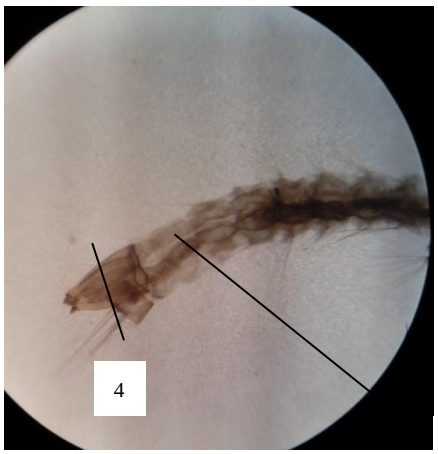
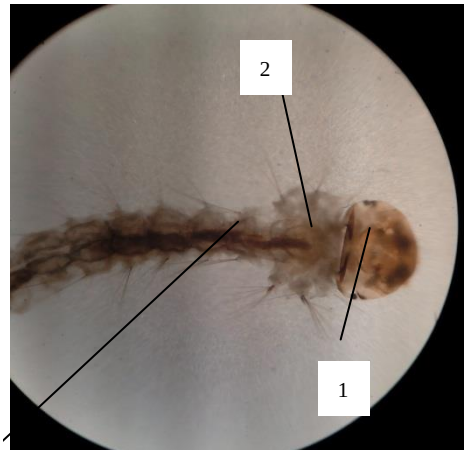


Fig.4 (a) The treatment of 4 minutes in dehydration process, posterior body of *Aedes aegypti* larvae



(b) The treatment of 4 minutes in dehydration process, anterior body of *Aedes aegypti* larvae

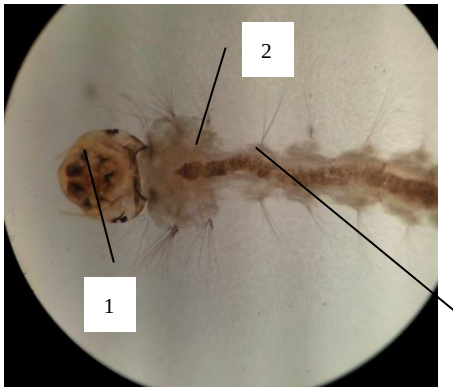
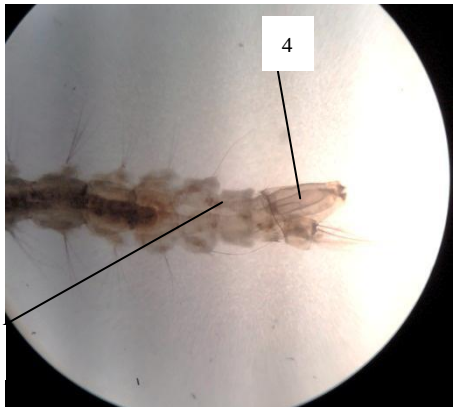


Fig.5 (a) The treatment of 5 minutes in dehydration process, anterior body of *Aedes aegypti* larvae



(b) The treatment of 5 minutes in dehydration process, posterior body of *Aedes aegypti* larvae

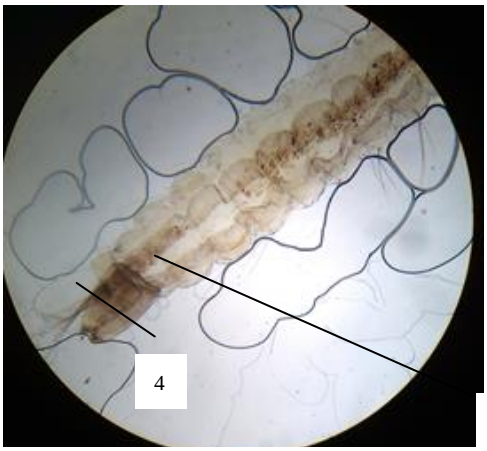
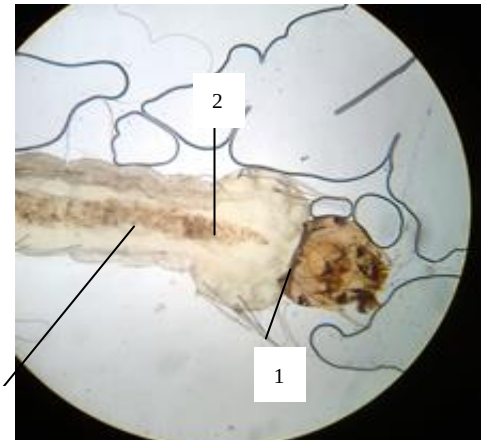


Fig.6 (a) Preparation with egg white mounting , posterior body of *Aedes aegypti* larvae (Yuliana, 2014)



(b) Preparation with egg white mounting , anterior body of *Aedes aegypti* larvae (Yuliana, 2014)

Notes :
1 : cephal of larvae
2 : thorax of larvae
3 : abdomen of larvae
4 : siphon

After pre-testing using dehydration treatment on the concentration variation there is damage to the morphology of the larvae in the ventral brush after being treated at a concentration of 96%. To produce clear preparations, in every solution of alcohol and xylol should be made to get 20 new permanent preparations. The effect of incomplete morphology *Aedes aegypti* larvae can occur due to the use of a spatula when the larvae move from one solution to another solution. In addition, based on the observations by Yuliana (2014), the process of mounting using adhesive variation of egg white as an alternative entelan. The egg white used in this research were egg white chicken, duck, and quail. After observed that the morphology of larvae increasingly clear (transparent) compared with entelan although done with the same treatment using the treatment time in the dehydration process. After it having obtained the average of several panelists to variations in egg white glue that there is no significant difference between egg white chicken, duck, and quail. However, it can be said the best average on the use of adhesive quail egg whites in this research.

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Separation Of Alkaloid Compounds From Fruit Of Mahkota Dewa (*Phaleria macrocarpa* Boerl.) Using Liquid Chromatography

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Abstract

High Performance Liquid Chromatography (HPLC) is a modern separation method that could be used for separating, purifying and determining the composition of chemical compound. Alkaloid is one of secondary metabolite compounds which has function as natural medicine. Mahkota Dewa (Phaleria macrocarpa Boerl.) is usually used by the native as traditional medicine. This research aimed to isolate and separate alkaloid compounds from fruit of Mahkota Dewa using HPLC method. Fruit of Mahkota Dewa was cut into small pieces, dried, then macerated with methanol. Concentrated methanol extract was acidified with hydrochloric acid to pH 2-3 then extracted with dichloromethane-water (1:3). Acidic water fraction then basified and extracted again with dichloromethane-water (1:3). Each of fractions was always tested with Dragendorff reagent and thin layer chromatography to prove the existence of alkaloid. Separation using HPLC was done for basic dichloromethane fraction using C_{18} (RP-18e) column, isocratic elution with mobile phase 10% acetonitrile and 90% sodium dihydrogen phosphate in water (pH 3) for 20 minutes. Alkaloids in basic dichloromethane fraction were found six components. Conventional column chromatography was prepared in order to get purer components , using silica gel G_{60} and 2.5% gradien of chloroform-methanol as eluent. Fraction 3 of 41 fractions, which assumed containing standard alkaloid, was fractioned by conventional column chromatography again using ODS and methanol-water (7:3) as eluent. Separation by HPLC was done again for fraction 2 and 3 of 20 fractions. The results showed that alkaloid in Mahkota Dewa could be separated using HPLC and obtained two components in fraction 2 and fraction 3 with good resolution but according to the retention time, those alkaloids are not the standard alkaloid used (atropin).

Keywords : Alkaloid, mahkota dewa, liquid chromatography

1. Introduction

Mahkota dewa (*Phaleria macrocarpa* Boerl.) is a tropical plant that originated in Papua are widely used as pharmaceuticals. Since ancient times, many of which utilize community Indonesia plant mahkota dewa to treat a variety of diseases ranging from mild to severe disease disease. Lately, the Crown of God have become popular and widely sold commercially in drugstores, pharmacies and hospitals. Mahkota dewa even has become a prima donna as a versatile plant drugs (Harmanto, 2002).

According to Gotawa et al. (1999) in the fruit skin mahkota dewa contained compounds such as alkaloids, secondary metabolites saponins and flavonoids in the leaves while contained alkaloids, saponins as well as polyphenols. According to Lisdawati dkk. (2007) the fruit meat containing lignan compound who also belong to the Group of polyphenols compounds. Moreover according to Simanjuntak (2008) also obtained the compound of fatty acids, steroids, Benzophenone Glycoside and carbs in fruit of mahkota dewa.

The presence of alkaloid in the fruit of the mahkota dewa is one of the important reasons for these plants can treat various diseases. Based on research conducted by Ramadani (2010), alkaloid in mahkota dewa fruit can be separated by column chromatography method and thin-layer chromatography, and obtained a total alkaloid levels defined by UV-Vis spectroscopy methods in fruit og mahkota dewa is approximately 0,0037%. Research by using high performance Liquid Chromatography method (HPLC)) has not been made.

2. Materials and Methods

The sample used in this study is the fruit of mahkota dewa, maroon colored, about 3 years old and is not a defect of the subdistrict Sukajadi, Bandung.

Isolation alkaloid done by method maceration and extraction, thin layer chromatography (TLC) conducted using plate of silica gel GF_{254} and ODS, column chromatography done with silica gel G_{60} and ODS in the laboratory analytical chemistry majors chemical fmipa unpad. Separation alkaloid with HPLC done with means HPLC Hewlett Packard series 1100 in research lab majors chemical FMIPA unpad.

3. Results and Discussion

Qualitative Test

Chloroform amoniakal added to the crown of the fruit of the gods. It is meant to free an alkaloid of the form of its salts because according to cordell (1981), mostly alkaloids in nature, being in the form of its salts bound with an organic acid. By the presence of ammonia and an alkaloid be free from its salts forming an alkaloid free later carried away by chloroform in phase organic. Next difiltrasi with cottony to deprive of residue a sample of being pulverulent and to take phase yet and course. Into phase organic then added a solution of sulphuric acid 2 n to reshape salt an alkaloid that is soluble in water. Phase water acid tested the existence of alkaloidnya with reagent dragendorff and reagent wagner.

Positive test with reagent dragendorff reagent and wagner characterized by formation of feculence brown until yellow. It is caused by the reaction between nitrogen atoms in compound alkaloid with metal contained in reagents the form complex compounds.

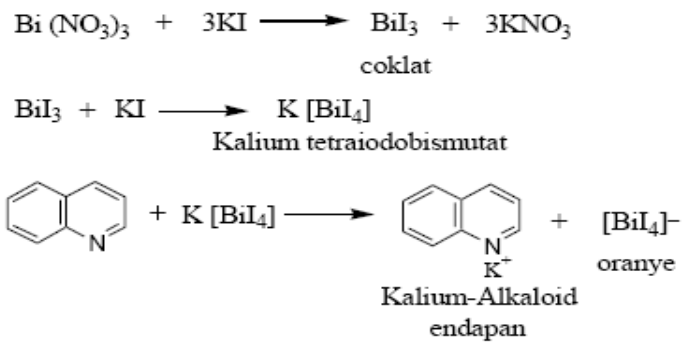


Figure 1. Reaction of Alkaloids in Dragendorff Reagent

Table 1. Qualitative test results of alkaloids in fruit of mahkota dewa.

Pereaksi	Hasil
Dragendorff	+ (endapan coklat tua)
Wagner	+ (endapan kuning muda)

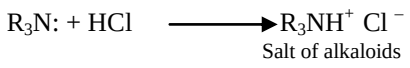
Explanation: (+) contained alkaloid
: (-) did not contain alkaloid

Isolation of Alkaloids

As much as 153,7 grams of fruit samples that have been dried Kumpulan and crushed by means of maceration extracted using solvents of methanol. The process of maceration is used to extract the compound because of natural materials with soaking herbs will take place the contact sample sample and solvent long enough, and with terdistribusinya organic solvents that are continuously into plant cell resulting in pressure difference between inside and outside the cell, leading to breaking of walls and cell membranes. Secondary metabolites which are in cytoplasm will be dissolved in organic solvents, and the extraction of compounds would be perfect because it can set a long soaking is done (Djarwis, 2004). In addition, maceration is carried out at room temperature so as to avoid degradation by high temperatures.

Methanol extracts concentrated with rotary evaporator Buchi R-200 at a temperature of 40 C. Heating is done at a temperature of 40 C to avoid degradation compounds due to high temperatures. Concentrated extracts obtained by maceration results 32,30 grams. Concentrated extracts was tested again the existence of alkaloidnya with reactant Dragendorff. Test results declared concentrated extracts containing alkaloids.

Into an extract concentrated added an acid solution of chloride of 1 % to ph 2.68. Has been said that most an alkaloid in nature are in the form of a salt attached to an organic acid. The existence of hydrochloric acid this will replace the position of an organic acid which binds an alkaloid when acidifying to be done. So that it will turn them into a salt an alkaloid that is soluble in water. Any reaction that occurs is as follows (fessenden & fessenden, 1986):



Subsequently extract with partitioned dichloromethane-water. It aims to eliminate acid-organic acids as well as other freelance organic compound that is insoluble in water, which will be distributed into the organic phase (dichloromethane) acid. Salt alkaloid himself would be distributed in the phase of acidic water. Dichloromethane would acid be phase at the bottom because dichloromethane heft type that is greater than the water i.e. 1,318 g/mL (25 C). Acid water fraction and fraction dichloromethane re-tested the existence of

alkaloidnya acid with reactant Dragendorff. from the test results can be known that contain acid water fraction alkaloids, whereas the fraction acid does not contain an alkaloid dichloromethane.

Acid water fraction is then dibasakan by the addition of ammonium hydroxide until pH 9,88. It aims to release alkaloid from the form of baths so obtained are free, with the reaction of alkaloids as follows (Fessenden & Fessenden, 1986):

$$R_3NH^+Cl^- + NH_4OH \longrightarrow R_3N: + NH_4^+Cl^- + H_2O$$

Alkaline water fraction then partitioned with dichloromethane to alkaloids into dichloromethane phase, whereas the alkaline salt ammonium chloride that form would be terekstraksi into the water phase. Faction bases and dichloromethane water fraction alkaloidnya with alkaline tested the existence of Dragendorrf reagents. From the test results can be noted that in the base containing the alkaloid fraction of dichloromethane, whereas in the fraction of water base does not contain alkaloids. Faction bases concentrated so retrieved dichloromethane extract concentrated base as much as 77.3 mg.

Identification of Alkaloids using TLC

For dichloromethane extract concentrated TLC is done using silica gel plate GF_{254} as stationary phase and klorofom-methanol (9.5: 0.5) as phases of motion, resulting in 5 stains. Stain the four equal to compound alkaloid with Rf standards i.e. atropin 0.76. The fourth is alleged to be as stain atropin, which later will be described with the separation of liquid chromatography using high performance.

Fractionation using Conventional Column Chromatography

Column chromatography is carried out using stationary phase silica gel G_{60} measuring 70-230 mesh, whereas motion phase used is klorofom and methanol with elusi bergradien 2.5% (first column chromatography).

Fraction column chromatography proceeds by as much as 39 accommodated fraction. Factions obtained later demonstrated with TLC.

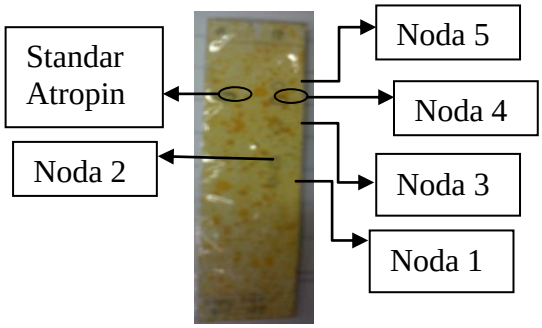


Figure 2. TLC chromatogram of concentrated alkaline dichloromethane extract silica gel stationary phase with G_{60}, mobile phase chloroform and methanol (9.5: 0.5).

Results from TLC fraction in chromatography columns-fraction, obtained that target alleged there were only on the fraction of atropin 3. To explain the alleged that the fraction 3 contained atropin, next 3 fraction separated by high-performance liquid chromatography

Dichloromethane extracts bases and concentrated the fraction 2-5 on the first column chromatography and separated by then proved HPLC. Before you do, phase separation with HPLC motion used to be screened first. This filtering is done with paper filter with a pore size of millipore 0.5 m, which aims to separate the phases of motion of particles and prevent the growth of microorganisms dopants that can ruin a quiet phase column.

In addition air dissolved in phase motion also omitted so as not to present top disruptive kromatogram on air separation. Dissolved air removed with sonifikasi process using sonikator. Addition of dissolved air also can be removed with an inert gas such as helium flow.

HPLC done for separation of alkaloids is done using reverse phase C18 column namely (RP-18e), phase motion 10% acetonitrile and 90% 0.05 M potassium phosphate dihidrogen in water (pH 3), the rate of 1.0 mL/min, temperature column 27.5 C (ambient), UV detectors at 210 nm wave length and injection volumes of 20 µL.

0.05 M KH_2PO_4 solution with pickled solution of phosphoric acid 10% to reach pH 3.00. Phosphoric acid is used because it is a weak acid salt forming of KH_2PO_4 solution that has a pH buffer is stable. Setting pH be 3 done because according to the literature, an alkaloid compound terelusi faster at low pH. This is due to the protonated amines being more polar at low pH, so it is not stuck in the C_{18} column (RP-18e) are nonpolar.

To further purify the alkaloid compounds in the fruit allegedly atropin, Kumpulan re-do fraksinasi with column chromatography. Column chromatography is done for 3 by using the phase fraction of silence and motion phase methanol DOS and water (7: 3) in isokratis. This second column chromatography is carried

out by reverse phase columns, unlike the first column chromatography using a normal phase-column. It is intended to make it easier to separate the compounds on the mass fraction 3 had very little and also so that separation can take better. Selection of phase motion done with TLC plate using DOS.

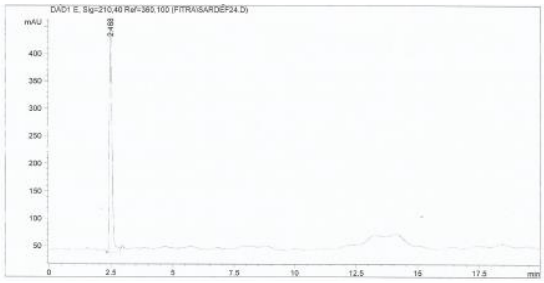


Figure 3. Chromatogram alkaloid compounds HPLC standard (atropin) with phase motion 10% and 90% acetonitrile KH_2PO_4 0.01 M in water (pH 3), isokratis, elusi column RP-18e, injection volume, flow rate 20 µL of 1.0 mL/min, UV detector at a wavelength of 280 nm.

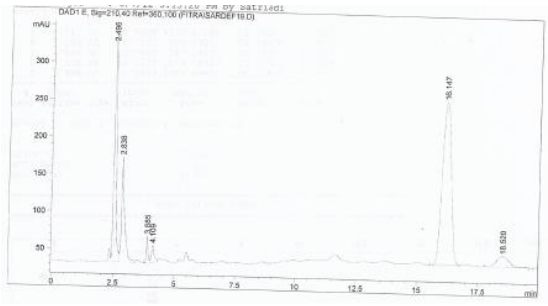


Figure 4. Chromatogram dichloromethane extract with HPLC base motion phase acetonitrile 10% and 90% 0.05 M KH_2PO_4 in water (pH 3), isokratis, elusi column RP-18e, injection volume, flow rate 20 µL of 1.0 mL/min, UV detector at a wavelength of 280 nm.

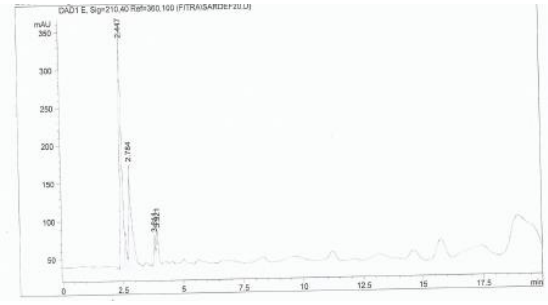


Figure 5. Chromatogram HPLC faction 3 first column chromatography proceeds with phase motion 10% and 90% acetonitrile KH_2PO_4 0.01 M in water (pH 3), isokratis, elusi column RP-18e, injection volume, flow rate 20 µL of 1.0 mL/min, UV detector at a wavelength of 280 nm.

Faction-fraction column chromatography results both remained separated with KCKT.

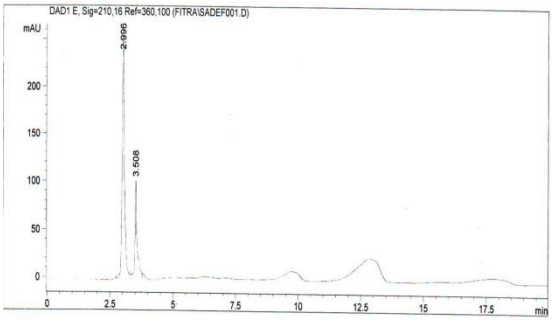


Figure 6. Chromatogram HPLC faction 2 second column chromatography proceeds with phase motion 10% and 90% acetonitrile KH_2PO_4 0.01 M in water (pH 3), isokratis, elusi column RP-18e, injection volume, flow rate 20 µL of 1.0 mL/min, UV detector at a wavelength of 280 nm.

4. Conclusion

From the research that has been done, we can conclude some of the following:

1) Alkaloid compounds in mahkota dewa could be separated by HPLC method, the separation condition as follows:
   Mobile phase: 10% acetonitrile and 90% solution of 0.05 M KH2PO4 in water (pH 3)
   Column: C18 (RP-18e)
   Flow rate: 1.0 mL / min
   Detector: UV 210 nm
   Injection volume: 20 mL
2) The number of components that can be separated alkaloid compounds are six components in the extract concentrated. Performed fractionation by column chromatography to obtain a purer alkaloid compounds are four components in the fractions after the chromatography column and the first two components in both fractions after column chromatography.
3) One of the alkaloid compounds contained in Phaleria not an alkaloid compounds (atropine) are used as standard

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Determination Of Chrom Hexavalent (Cr(VI)) from Electroplating Waste Using Spectrophotometry

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Abstract

With the rapid industrial activity, various types of heavy metals and organic waste generated can become a serious problem for health and environment. Waste of heavy metals Cr (VI), which is one type of hazardous waste, can be derived from the metal coating industry (electroplating). One of the electroplating industries that generate the chromium waste Chromium hexavalent (Cr^{6+}) levels electroplating waste was determined with spectrophotometric method to decide feasible waste water treatment. The analyzes were performed by using UV-Vis Spectrophotometer DR 2800 with the help of reagents Chromaver 3 (1.5 difenilCarbazyde) to form a complex reaction that produces the purple color and read at a wavelength of 540 nm. The results of analysis of waste samples determined to be eligible waste reduction processes and coagulation-flocculation. Coagulant used for the formation of sediment / sludge Chrome can use coagulants PAC (Poly Aluminum Chloride) or Alum / $KAl(SO_4)_2.12H_2O$. Measurement of waste samples from levels of chromium hexavalent (Cr^{6+}) were reduced with sodium Metabisulphite - $Na_2S_2O_5$ concentration and same volume as the coagulant PAC and Alum results obtained reduction with efficiency values unchanged at 99.87% and 99.89% with the average difference of the two coagulants amounted to 0.02%. And all processed waste samples were not exceed 0.1 mg/L so it were feasiblewaste according to the standards set by the goverment.

1. Introduction

There is a rapid development of industries in Indonesia and for the good and the bad there also arises problems. The good news is that there are many field of occupations so it can absorbs quite many employers and eventually decreases the unemployment and there is a chance to introduce a new development in technology in industries. Then also come along the flipside that there are wastes produced by this process.

Society has put an increasing attraction toward the problem of pollution in these recent years. Many problems concerning this pollution have surfaced. The rapid development of industries is one of the cause of the decrease of environment quality. The handling of this environmental pollution is important andin its connection with the development of area with environmental awareness it must be balanced with the efficient pollution control.

In general, these big industries have their own waste installations (IPAL) so the pollution that is caused by these industries almost all is contained and well-handled. But in reality, there are many big industries that haven't yet handled their wastes in accordance with the standard of industrial wastes. (Kristanto, P, 2002).

With the fast grows of industry these days, the metal and organic wastes can cause a serious problem for health and environment. The waste of heavy metal Cr(VI), that is one of the dangerous waste that comes from paint, electroplating, and leather tanning industries. Natural occurance of Chromium oxide are Cr(VI)*chromium hexavalent* and Cr(III) or*chromium trivalent*. Cr(VI) is easily dissolves in water and forms *divalent oxyanion* that is chromic(CrO_4^{2-}) and bichromic ($Cr_2O_7^{2-}$). High level of toxicity of Cr(VI) makes chrom is dangerous for all organisms at > 0,01ppm. Cr(VI) is carcinogenic and can cause an irritation to human skin. While the toxicity level of Cr(III) is much lower compared to Cr(VI), about 1/100 times, so to manage the waste of Chrom then Cr(VI) first must be reduced to Cr(III). And also Cr(III) is easily coagulated and absorbed by organic and inorganic compounds in neutral pH or alkaline(Slamet et al, 2005).

One of the industries that produced chromic wastes is electroplating industry. In this research, it has been determined the concentration of Chromium hexavalent (Cr^{6+}) from the electroplating waste that has been through the process of coagulation PAC dan alumina.

2. Literature

Waste

Waste is a byproduct from municipal, society, industry, land-water, surface-water, and all the waste and can be formed gas, dust, liquid, and solid.From these wastes, there are hazarduous and toxic waste and is called *Bahan Berbahaya dan Beracun (Limbah B3)*.

Definition of *B3* waste according to BAPEDAL 1995 is every byproduct (waste) from a production process that contains bahan berbahaya dan beracun (B3) because of the *toxicity*, *flammability*, *reactivity*, dan

*corrosivity*and the amount and concentration direct or indirect can cause a damage, gives an environmental pollution and can cause a problem in human health.

Water Waste Management

Polluting agents in water can be removed by physical and chemical process. Methods of physical processing are:

- Flocculation
  A good Ffocculation can be done by giving a horizontal and vertical agitation. The purpose of agitation is to give a biggerflocs, and also to prevent sediments settles at the bottom. To improve the size of the flocs, thickening agents can be added to the murky water. To form a group of particles that settle, a rapid agitation can be done for about 20-30 minutes that can cause the stack of particles unite to form a bigger particle.
- Sedimentation
  Sedimentation is a method to clean the water where water is passed to the tank for a certain amount of time and the speed of the water flows is adjusted so the heavier particles will settle immediately.
- Filtration
  Filtration is a way to clean the water with a help of sieving method. Filtration consists of layesof sand and pebbles with a variation in diameter from the smallest to the biggest. Water will flow throught the filter while suspended particles inside will stick to the sands.

While the chemical method is coagulation. Coagulation is a mechanism where colloidal particles. Coagulation is a mechanism where colloidal particles with negative charges are neutralized until the neutral charges get close to each others and stick to each others and form a flocc. To improve the size of colloidal particles with chemical reaction followed by uniting the flocs and absorption.(Setiadi, H. 1997).

Reaction of chromiun hydroxide sedimentation:

$$Cr_2(SO_4)_3 + 6\ NaOH <====> 2\ Cr(OH)_3 + 3\ Na_2SO_4$$

dissolve — Not dissolved

Poly Aluminum Chloride (PAC) to settle chromium hydroxide and other heavy metals.

$$2Cr(OH)_3 + Al_2(OH)xCl_{6-x} <====> Cr_2(OH)_xCl_{6-x} + 2\ Al(OH)_3$$

Not dissolved — PAC — slurry Cr^{3+}

This process is called coagulation, that is the forming of microfloccs (a small lump of sedimentaion). After forming this microfloccs, acid or alkaline is added to the waste until the pH reach neutral or slightly alkaline (7-9). Because the sedimentation that is formed is so small, then it takes a longer time to separate water and sediment, then at this stage flocculant is added in the form of polymer that serves as a binder to bind or to form macrofloccs, so it can speed the process of separating sediment and water.

Factors that effect flocculation process

Flocculation process in water is affected by pH, turbidity, type of coagulant, temperature, and the mixing to obtain the optimum condition.

1. pH
   pH is one of the important factors that affects coagulation process. According to many experts, for every area, there is a certain value of water pH that can cause a good coagulation. (Riyadi, W. 2009).
2. The effect of temperature
   In a low temperature the reaction rate is much slower and the water has a higher viscosity so the floccs is hard to settle. Water with the high turbidity needs a large dose of coagulant. A dose of coagulant for each turbidity unit is high then there is a higher possibility to collide with each other. A dose of coagulant that is low can cause an imperfect collision between particles. Neutralization of electric charges is not perfect and the formed floccs is only sparse and turbidity raises. Overdose of coagulant can cause a side effect to the particle so the turbidity raises.
3. Mixing
   A good coagulation is also determined by mixing. This mixing is necessary so the collision between particles to neutralization is perfect, there are two stages of mixing to produce a turbulent current in the waste processing. The goal of this fast mixing is to distribute coagulant evenly and to make a faster collision rate between coagulant particle and turbidity particles. Second stage of the development of floccs is done with a slow mixing.
4. Effects of dissolved salt in water
   Natural water usually contains salts, organic or inorganic in a different composition. The effect of those salt will vary according to the kind of ions and concentration. The higher the oxidation state of the ion then the bigger the influence to coagulant and flocculation (Setiadi, H. 1997).

Spectrophotometry

Spectrophotometry is an analytical method for a chemical compound based on its ability to absorb a ray of light. Spectrophotometry is based on Lambert-Beer law. According to Lambert law, if monochromatic light is passed through the transparent medium, the rate of the decreasing of the light intensity is proportional to the increase of the thickness of of absorbing medium. According to Beer law, if the light is passed through solution then the intensity of monochromatic light decreases as the concentration of the absorbing material increases.

Sistematically the combination of the Lambert and Beer Law can be written as:

$$A = a.b.c$$, (Day, R.A & Underwood, A.L. 1999)

where A : absorbancy (Abs)
a : absorpsivity
b : cell thickness (cm)
c : concentration(g/l)

One of the spectrophotometry is UV-VIS spectrophotometry that is a combination of UV spectrophotometry and Visible spectrophotometry, using two different light source: UV and visible source of light. For spectrophotometry sistem, UV-Vis is the most abundant and most popular in the field. The use of this method is for the transparent sample or coloured sample. (Riyadi, W. 2009).

Spectrophotometry UV-Vis mainly is used to perform quantitative measurement but it also can be used for qualitative measurement. The use of it in quantitative measurement is based on Lambert-Beer Law. The wavelength that is used to perform quantitative analysis usually is the wavelength where the compound gives the highest absorption to provide the greatest accuracy. The optimum absorption for measurement with UV-Vis Spectrophotometry is in the range of 200-800 nm (Day, R.A & Underwood, A.L. 1999).

Composition of Spectrophotometry :

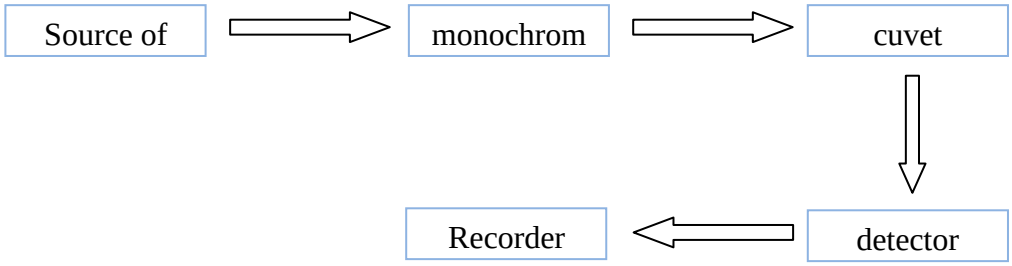


Figure1. Diagram-Block for Spectrophotometry(Day, R.A & Underwood, A.L. 1999)

3. Materials and Methods of Research

Equipment :

- UV-Vis Spectrophotometer DR 2800
- 100 mL Volumetric Flask
- 2 mL Volumetric Pipette
- Dropper
- Stirrer
- Filler
- Water Bottle
- 100 mL Beaker Glass

Materials :

- Universal pH Indicator
- H_2SO_4 (sulphuric acid) 20 %
- $Na_2S_2O_5$ (Natrium metabisulphite)
- NaOH
- PAC (Poly Alumunium Chloride)
- Tawas / $KAl(SO_4)_2.12H_2O$
- Polymer Dust(Multifloc A-108)
- Chromium wastes
- Tissue
- reagent chromaver 3 (1.5 diphenilcarbazide)

Methods in this research is experimental and the diagram of this research can be seen in Figure 2.

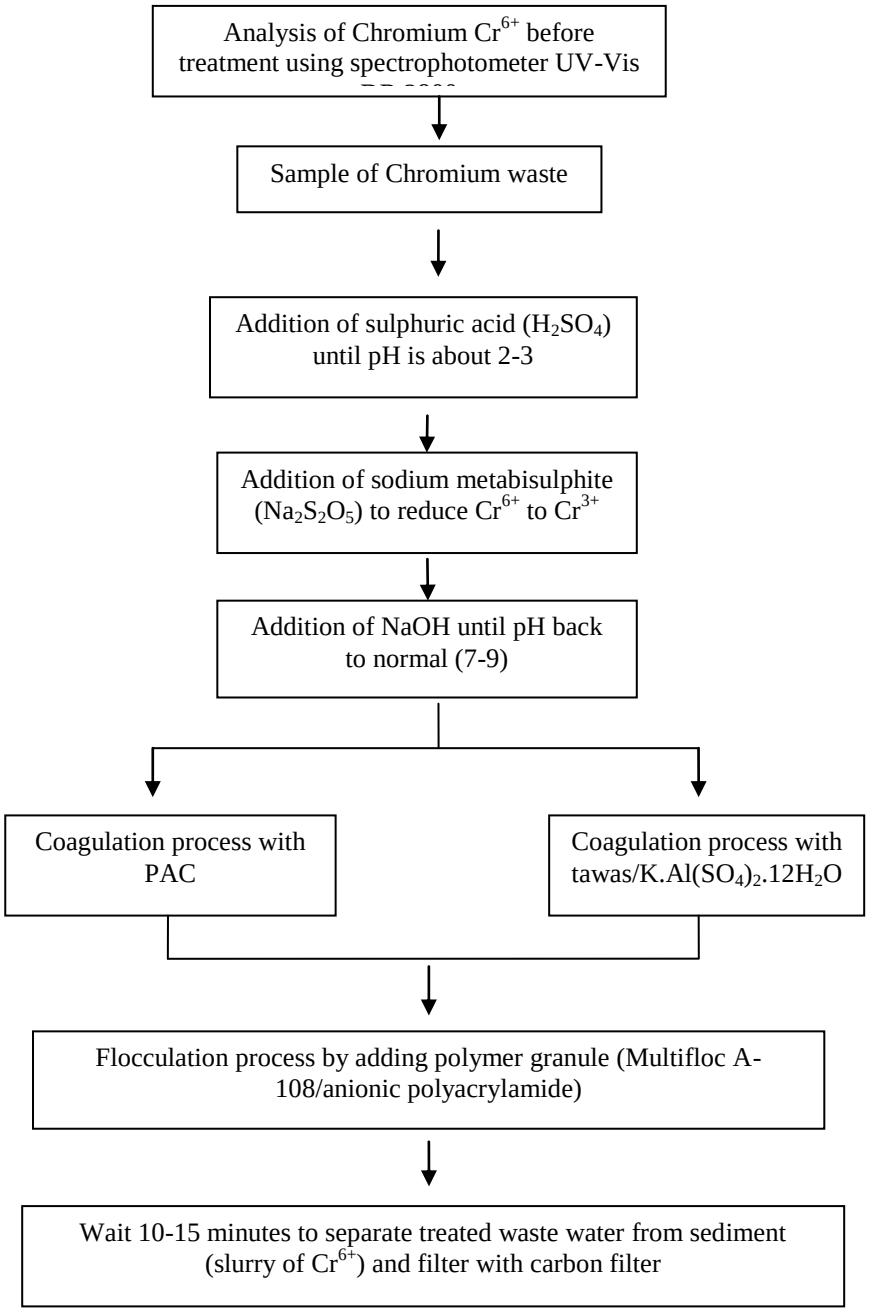


Figure 2.Flowchart of Research

Research Procedure

Determination of Initial Chromic (Cr^{6+}) Concentration Before Treatment

A. Preparation of sample of Chromium wastes with 50x dilution
   1. Preparing sample of Chromium to the 100 mL beaker glass
   2. Pipetting 2 ml sample of Chromium waste using measurement pipette and transferring it to 100 mL volumetric flask and diluting it with aqua DM until the border and homogenizing it.

B. Preparing sampe Chromium that is already diluted 50X.
   1. Preparing a clean cuvette for wastes sampling
   2. Transferring 10 mL waste water sample with 50X dilution to the cuvette to the border mark and adding 1 pack of reagent chromaver 3 (1.5 diphenilcarbazide) so all content is dissolved (5 minutes)

C. Preparing blank
   1. Preparing clean cuvette for blank

2. Transferring 10 mL of waste water sample that has been 50X diluted to the second cuvette.

D. Reading Sample with Spectrophotometer DR 2800
1. Turning on Spectrophotometer DR 2800, choose menu reading sample:
2. Setting the reading of Spectrophotometer DR 2800 to the diluted solution.
3. Transferring blank to cuvette hole, placing the line to the right. Push nol (in monitor it can be seen 0.00 mg/L Cr^{6+}). Transferring sample to the cuvette hole with line facing right and push"read" (in monitor it can be seen the concentration of Chromium hexavalent)

Determination of Chromium hexavalent (Cr^{6+}) after treatment

A. Treatment of wastes using coagulant PAC (Poly Alumunium Chloride)and Tawas / $KAl(SO_4)_2.12H_2O$
1. Preparing wastes of Chromium in two 500 mL beaker glasses
2. Turning on strirrer to mix, and check initial pH of waste using universal pH indicator (it should be around 2-3)
3. If initial pH is>3, add 20% sulphuric acid until pH is 2-3
4. Add sodium metabisulphite (concentration of 0,1 Kg/L) until the solution changes colour from yellow to green.
5. Adding NaOh (concentration of 0.1 Kg/L) until pH is 8-9.
6. Adding poly alumunium chloride (concentration of 0.1 Kg/L) until floccules are formed
Adding tawas$KAl(SO_4)_2.12H_2O$(concentration of 0.1Kg/L)until floccules are formed
7. Adding 0,2 gram of polymer granule(Multifloc A-108/anionic polyacrylamide)until flocs are formed
8. Checking end pH and adjusting the waste water until its pH is neutral
9. Waiting for 10 minutes to settle the sediment
10. Wasting water sample that has been treated is separated from the slurry
11. Filtering the treated waste water by passing it through the carbon filter

B. Preparing water sample of treated chromium waste by using coagulant PAC and tawas
1. Preparing a clean cuvette for waste sample
2. Transferring 10 mL of treated waste water to the cuvette until the mark and add one pack of reagent chromaver 3and wait until all the reagent is dissolved (about 5 minutes)

C. Preparation of blank of treated chromium waste water using coagulant PAC and tawas
1. Preparing a clean cuvette for blank
2. Putting a 10 mL treated waste water to the second cuvette

D. Reading sample with Spectrophotometer DR 2800
1. Turning on Spectrophotometer DR 2800, choose menu sample read for Cr^{6+}(Chrom hexavalent)
2. Putting blank to the cuvette hole with line facing right. Push nol (in monitor it can be seen 0.00 mg/L Cr^{6+}). Transfer sample to the cuvette hole with line facing right and push read (in monitor it can be seen the concentration of Chromium hexavalent)

4. Result And Discussion

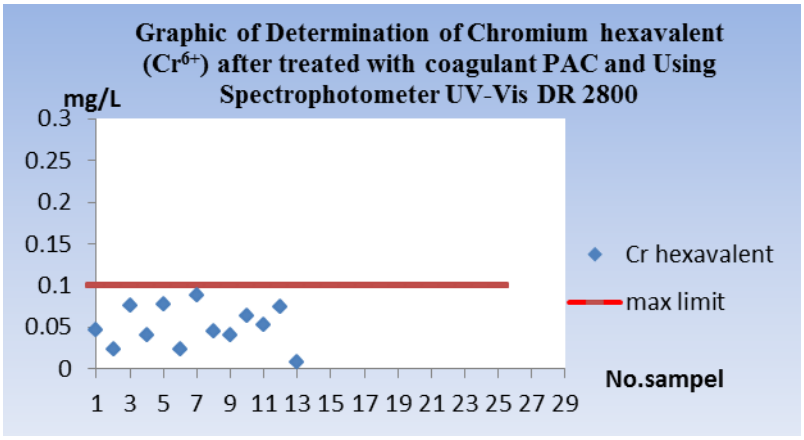
Data of determination of waste sample and concentration of Chromium hexavalent (Cr^{6+}) that is reduced by Sodium Metabisulphite – $Na_2S_2O_5$in the same concentration and volume with coagulant PAC dan Tawas can be seen in table and graphic below:

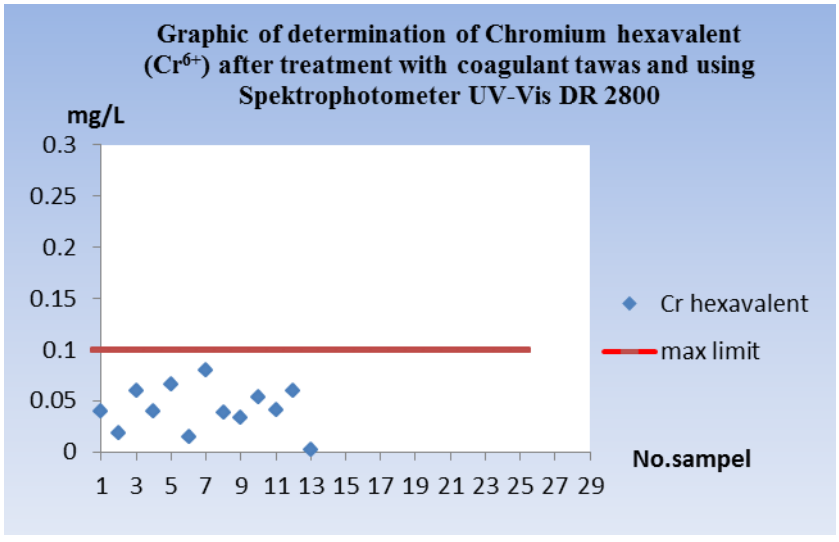
Table 1. Result fo determination of Cr^{6+}that is treated with coagulant PAC and Using SpektrophotometerUV-Vis DR 2800

No	Before treatment (Cr^{6+} mg/L)	After treatment (Cr^{6+} mg/L)	Reduction efficiency (%)	Information
1	36.754	0,047	99.87	Disposable
2	33.624	0,023	99.93	Disposable
3	54.212	0.076	99.86	Disposable
4	31.244	0,041	99.87	Disposable
5	60.221	0,077	99.87	Disposable
6	35.054	0,024	99.93	Disposable
7	33.237	0,088	99.73	Disposable
8	63.331	0.045	99.93	Disposable
9	30.569	0,041	99.86	Disposable
10	42.232	0,064	99.85	Disposable
11	40.125	0,052	99.87	Disposable
12	32.289	0,074	99.77	Disposable
13	72.539	0.008	99.99	Disposable
Average of Reduction efficiency			99.87	Disposable

Table 2 Result of Determinaton of Treated Cr^{6+} with coagulant tawasUsing Spectrophotometer UV-Vis DR 2800

No.	Before Treatment (Cr^{6+} mg/L)	After Treatment (Cr^{6+} mg/L)	Reduction Efficiency (%)	Information
1	36.754	0,040	99.89	Disposable
2	33.624	0,019	99.94	Disposable
3	54.212	0.06	99.89	Disposable
4	31.244	0,040	99.87	Disposable
5	60.221	0,066	99.89	Disposable
6	35.054	0,015	99.96	Disposable
7	33.237	0,080	99.76	Disposable
8	63.331	0.038	99.94	Disposable
9	30.569	0,033	99.89	Disposable
10	42.232	0,054	99.87	Disposable
11	40.125	0,041	99.89	Disposable
12	32.289	0,060	99.79	Disposable
13	72.539	0.002	99.99	Disposable
Average of Reduction Efficiency			99.89	Disposable





According to the graphic above, we can conclude that the waste water from the treatment can be removed safely and follows the standard of disposable water.

5. Conclusion And Suggestion

From the result, the determination of waste sample from concentration of Chrom hexavalent (Cr^{6+}) yang direduksi dengan Natrium Metabisulphite – $Na_2S_2O_5$with the same concentration and volume from coagulant PAC dan Tawas, it gives the same result that is 99.87 % and 99.89 % with the difference between average of efficiency is 0.02%. and all the concentration of Chromium hexavalent (Cr^{6+}) waste samples do not exceed 0,1 mg/L and can be disposed according to SK Gub. KDH TK.1 Jabar No. 6 Th.1999 and Kep-51/MENLH/10/1995 about liquidwaste of electroplatingindustry.

PAC (Poly Alumunium Chloride) that is used as coagulant in a process of sedimentation in the treatment Chromium waste water can be displaced by tawas/ $KAl(SO_4)_2.12H_2O$ that is quite cheap and easy to obtain in market.

Analysis of wastewater should be done in every batch of waste to avoid pollution of chromium hexavalent in a large dose that can exceed the standard concentration of chromium in waste that is set by the authority.

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Myristicin Isolation Of Nutmeg (Myristica fragrans hout) With Hexane Extraction Method

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Abstract

Isolation of pure compounds myristicin process begins with the extraction process of nutmeg. Extraction carried out is using maceration method using soxchlet with hexane. The fraction results are tested by TLC to find the existence of myristicin. Fractions containing myristicin then tested using column chromatography to separate myristicin of other compounds that are not expected/ impurity. Myristicin is not free from impurities, then tested using VLC. After that, samples were regarded purely collected and evaporated then measured its purity concentration using GC-MS analysis instrument. Based on analysis by GC MS Myristicin with another name benzodioxole 1.3, 4 methoxy 6 has a percentage of 74.07% purity.

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Keywords: Nutmeg, myristicin, hexan extraction

1. Introduction

Nutmeg is widely used by public mainly for seasoning. Besides being used for cooking, this nutmeg can also produced nutmeg oil. Nutmeg oil is one of the essential oils that are widely exported in Indonesia. Indonesian nutmeg oil exports in 2011 recorded at 400 tons with a value of USD 24 million [1]. Nutmeg oil is widely used in medicine formulas, perfume, drinks, detergents, aromatherapy, etc. According to International standards[2], Nutmeg oil is produced from the distillation of nutmeg Myristica fragrans or type known as Pala Banda. Type the nutmeg widely cultivated and processed in the Moluccas, North Sulawesi, Aceh, West Sumatra, and Java[3].

Besides exporting nutmeg oil, Indonesia also exports nutmeg mainly from North Sulawesi and Moluccas. Nutmeg which is exported to the United States and Europe, beside being used as a spice and oleoresin, are also distilled as essential oil [4].

According to Dorman *et al*[5] the main components of nutmeg oil are terpenes, terpene alcohols and phenolic ethers. Monoterpene hydrocarbon component is the main component of nutmeg oil consists of β-pinene (23.9%), α-pinene (17.2%), and limonene (7.5%). While the ethers of phenolic components primarily miristicin (16.2%), followed by safrole (3.9%) and methyl eugenol (1.8%). Furthermore, Dorman *et al.*, (2004)[5] states there are 25 components identified in the oil of nutmeg (some 92.1% of total oil) were obtained by distillation (hydrodistillation) using a refiner.

Myristicin is fenilpropen, from natural organic compounds in small amounts in the essential oil of nutmeg and other spices such as parsley and fennel with smaller amounts. Myristicin solubility in water can not happen, but this myristicin soluble in ethanol and acetone.

Myristicin very helpful in the prevention of tumor formation, and can be used in the export of fish fainting technique so that the condition of the fish is always fresh during the trip. In addition, the ability to prevent the occurrence of liver toxicity of carbon tetrachloride in rats is another thing that adds to the appeal of myristicin. Myristicin found in nutmeg oil can be toxic, easily absorbed in other media in nutmeg oil and has a very sharp scent. In addition, myristicin can be used as an anesthetic and a mixture of certain drugs in the pharmaceutical field. This substance is an agent that is hallucinogenic and toxic poisoning which can lead to excessive dosage [6].

This study aims to isolate the compound myristicin from nutmeg oil using hexane extraction method to obtain the purity levels of these compounds in order to assess the potential value added therein and development opportunities.

2. Materials and methods

2.1 Isolation of myristicin compound

Nutmeg used in this study were obtained commercially from CV. Zanzibar Tasikmalaya. The nutmeg seed is separated from the shell and then mashed. Nutmeg powder is then weighed 1000 grams, and then extracted with soxchlet using 1 Liter of hexane and soaked in nutmeg powder for 3 days. Then the extraction will be concentrated by means of evaporatedResults of concentration mixed with 96% ethanol up to 300 ml and stir until homogeneous and allowed to stand a few minutes to form two distinct phases. Trimiristin (fat)

will not dissolve in 96% ethanol while miristicin be united and dissolved in 96% ethanol. The soluble portion (miristicin) is taken and separated for Thin Layer Chromatography (TLC) with eluent n-hexane: chloroform = 3: 2 were compared with miristicin from reference. After that, further analyzed with column chromatography methods for purification by using hexane and ethyl acetate as a gradient using silica gel 60G, then the result will be stored in vials bottles and labeled.

2.2 Purity test for the isolated

To test the purity of the results of column chromatography is analyzed using TLC with eluent hexane and chloroform in the ratio 2:3. If the results of the column chromatography has been separated myristicin from other compounds it will be marked by the appearance of one stain on TLC chromatogram results, thus indicating that myristicin was pure enough. If the TLC results are still a lot of stain the samples then it's still need to be purified further. However, when the results have been quite pure then further analyzed by using GC-MS to see the purity of myristicin.

3. Results and discussion

3.1 Isolation of myristicin compound

Isolation of pure myristicin compounds process begins with the extraction nutmeg process. Extraction is carried out using maceration method using soxchlet with solvent hexane. Meat nutmeg powder (simplicia) put in soxchlet then soaked by hexane while heated at 750C for three days until all the simplicia dissolved by hexane were marked with colored translucent droplets. After that, the extraction was evaporated using a rotary evaporator until that solvent in the extraction results disappear (approximately 1 day). The final result of the extraction is obtaining the hexane fraction which is then tested further using thin layer chromatography (TLC) to find the existence of myristicin.

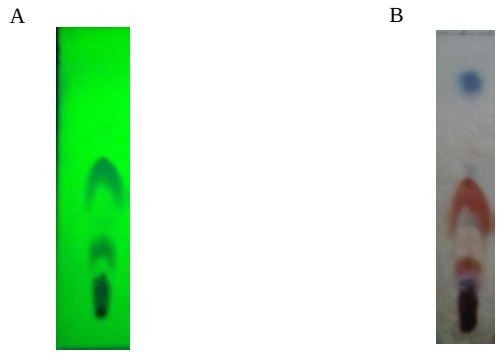

Fig.1 Visualization results of soxhletasi extraction method using TLC Remarks: (A) Under 254 UV, (B) After the sights of vanillin sulphate spotting given.

Based on Figure 1 in the hexane fraction contained myristicin compounds, it's just still impurities there in the fraction. Isolation followed by column chromatography.

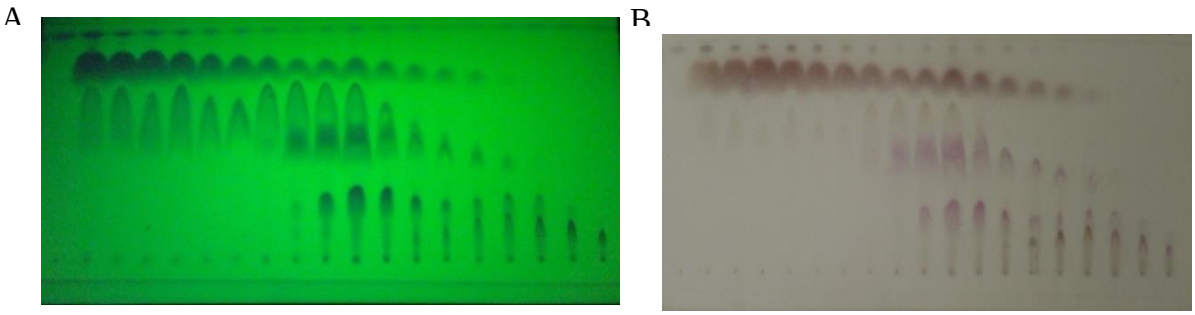

Fig. 2 Visualization results column chromatography silica gel 60 (particle size: 0.063 to 0.200 mm)
Remarks: (A) Under 254 UV, (B) After the vanillin sulphate spotting sights given.

Fractions containing myristicin then tested further by using column chromatography to separate myristicin of other compounds that are not expected/ impurities (Fig.2). Because based on the results of the column

chromatogram obtained myristicin not free of impurities then the test followed by vacuum liquid chromatography (VLC) (Fig.3). The differences in VLC and the chromatography column is the silica gel pore size used, the size of the pores in chromatography column are larger.

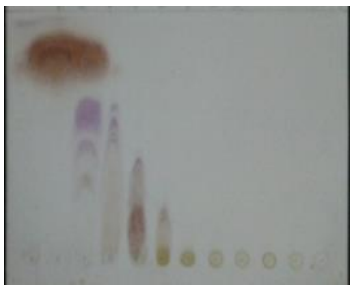

Fig. 3 Visualization results of vacuum liquid chromatography. Remarks: After the vanillin sulphate spotting sights given.

Based on the TLC results an Rf hexane fraction of 0.5 were obtained. Samples that have been considered pure (Fig.4) then measured its purity concentration using GC-MS analysis instrument (Fig.5).

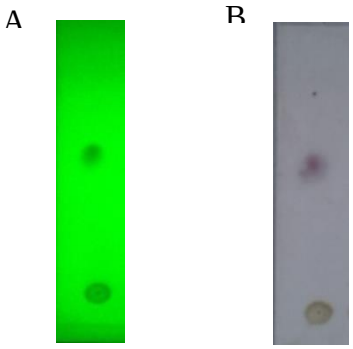

Fig. 4 Myristicin from hexane fraction Ket: (A) Under 254 UV, (B) After the vanillin sulphate spotting sights given.

3.2 The purity test of the isolated myristicin compound

Analysis using gas chromatography mass spektrofotometry (GC MS) aims to determine the purity percentage of the myrsiticin compounds isolated. Based on Figure 5 below, can be seen the presence of multiple peaks. The figure shows the results of the GC MS analysis of myristicin compound obtained from isolated using hexane extraction method. Each peak that appears have a vary retention time and concentration. In addition, the most striking data is the presence of the highest peak that dominates among other peaks. The peak is the peak of the myristicin compound.

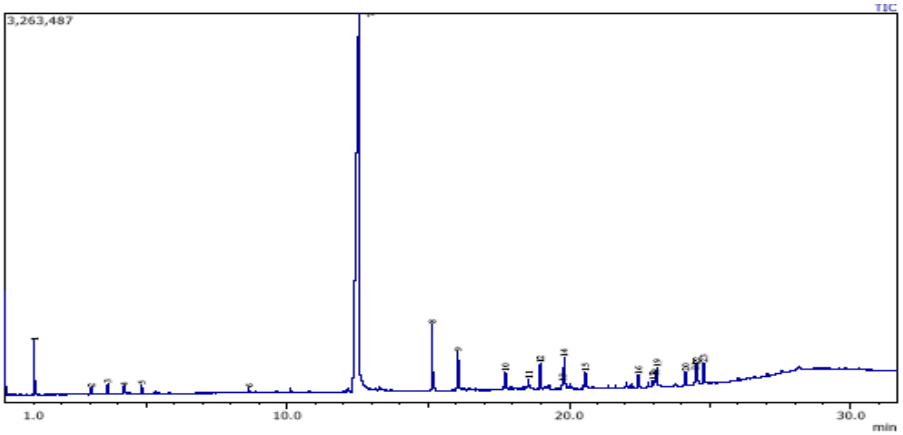

Fig. 6 GC-MS curve of Hexane Fraction

The following Table.1 containing compounds and the percentage contained of isolates from the myristicin compound isolated. Myristicin with another name 6-allyl-4-methoxy-1,3-benzodioxole has an area percentage of 74.07% and followed by other compounds with a much smaller area percentage. The results of the GC MS analysis showed the percentage of myristicin quite good when compared with the results of the analysis of GC MS based on Essam et al journal, 2012, which has a myristicin area percentage of 97% [7]. Some things that could cause these differences include the method and origin of different nutmeg used so as to give different results. Nutmeg plants located in the Indonesia area are certainly has a special characteristics that can not be found in nutmeg plants located in Saudi Arabia areas. It is influenced by different environmental and climatic conditions in each country or place.

Table. 1 Percentage of compound content from hexane fraction GC-MS results.

Peak	R.Time	Height	Area	Area%	Peak A/M	Report TIC Name
1	1.031	449052	417037	1.53	0.93	Aceton
2	3.044	53349	86023	0.32	1.61	.alpha.-Thujene
3	3.625	84574	159265	0.59	1.88	.beta.-Phellandrene
4	4.209	61881	121908	0.45	1.97	.ALPHA. TERPINENE
5	4.835	67849	123297	0.45	1.82	1,4-Cyclohexadiene, 1-methyl
6	8.640	37449	59830	0.22	1.60	1,3-Benzodioxole, 5-(2-propenyl)
7	12.529	3101311	20130666	74.07	6.49	6-allyl-4-methoxy-1,3-benzodioxole
8	15.143	546230	1167289	4.30	2.14	Tetradeconoic acid, methyl ester (CAS)
9	16.060	305660	686895	2.53	2.25	Tetradeconoic acid, ethyl ester (CAS)
10	17.740	138078	314107	1.16	2.27	Hexadecanoic acid, methyl ester (CAS
11	18.564	57157	107095	0.39	1.87	Hexadecanoic acid, ethyl ester (CAS)
12	18.974	204075	429487	1.58	2.10	1N-Naphthol[2,1-b]pyran, 3-ethenyldo
13	19.762	56371	121189	0.45	2.15	9,12-Octadecadienoic acid (Z,Z)-, methyl
14	19.823	254170	722517	2.66	2.84	9-Octadecadienoic acid (Z)-, methyl ester
15	20.574	127130	322589	1.19	2.54	Ethyl Oleate
16	22.458	104008	233009	0.86	2.24	1-Naphthalenepropanol, .alpha. –ethen
17	22.973	35509	65825	0.24	1.85	6.beta.Bicyclo[4.3.0]nonane, 5.beta.
18	23.054	74724	172216	0.63	2.30	1,4-Dimethyl-8-isopropyldenetricyclo
19	23.110	147692	354792	1.31	2.40	9,19-Cycloergost-24(28)-en-3-ol
20	24.133	112939	290102	1.07	2.57	Citronellyl propionate
21	24.475	114143	298833	1.10	2.62	2,6-Octadien-1-ol,3,7-dimethyl-,propenyl
22	24.525	155161	391406	1.44	2.52	Di-(2-ethylhexyl)phthalate
23	24.776	165803	401058	1.48	2.42	2-hexylfuran
		6454315	27176435	100.00		

4. Conclusion

Hexane extraction method can isolate the myristicin compound from nutmeg oil with the acquisition of 74.07% myristicin purity.

Acknowledgements

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Adsorption of Carbon Black Using Chitosan in Deinking Process

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Abstract

The adsorption of Carbon black using chitosan made from shell of shrimp has been investigated. The study was conducted to obtain the adsorption capacity of chitosan. Experiment were carried out as function of weight dosage of chitosan (0.5, 1, 1.5 and 2 g) and concentration of carbon black (20, 40, 60, 80 and 100 ppm). The intial concentration of 1000 ppm was diluted to 100 ppm. Samples as 100 mL inserted into erlenmeyer and added a number of chitosan. The mixture was filtered with Whatman 40 and residual concentration of carbon black analyzed using UV-Vis spectrophotometer. The results shows that the condition of adsorption is archieved at weight doses of 1.5 g.

*Keywords:*Adsorption, Chitosan, Carbon Black, Deinking

1. Introduction

Deinking process is one of the important process in recycling paper. The process of deinking involves ink particle dislogment from the fiber surface and the separation of dispersed ink from fiber suspensions by flotation. The news paper consist of carbon black as pigment ink printing. This ink is physically bonded to the fibers and expensive to remove by conventional method. Adsorption is considered an effective and economical method to remove dyes from wastewaters.[1,2,3]. Adsorption and interaction between added surfactant and the solid surfaces of ink pigment and pulp, the fundamental mechanism of flotation deinking has been reported. The increasing surfactant concentration can cause calcium adsorption to decrease (calcium exclusion effect), probably due to covering up of negative adsorption sites on the surface.[4,5]. It has been reported that many different types of adsorbents are effective in removing color from aqueous effluents. It is now recognized that adsorption using low-cost adsorbents is an effective and economic method for decontamination. Biopolymers have received a great deal of attention due to the fact that they represent renewable resources and more environmental friendly than commercial materials. Natural polymeric materials are gaining more and more interest for application as adsorbents due to their biodegradable and non-toxic nature. Chitosan offers an interesting set of characteristics, including non-toxicity, biodegradability, biocompatibility, and bioactivity. Chitosan is prepared from chitin by deacetylating its acetoamide groups with a strong alkaline solution. This is the most abundant biopolymer in nature after cellulose. As shown from Figure 1, chitosan has three types of reactive functional groups, an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively. Its advantage over other polysaccharides is that its chemical structure allows specific modifications, especially at the C-2 position. These functional groups allow direct substitution reactions and chemical modifications, yielding numerous useful materials for different domains of application.[6] Chitosan is known as an ideal support material for enzyme immobilization because of its many advantages such as its hydrophilicity, biodegradability, biocompatibility and anti – bacterial property.[7] Chitosan has been widely used as an adsorbent for transition metals, organic species and for dye waste removal from aqueous solutions, due to the presence of the amino ($-NH_2$) and the hydroxyl (-OH) groups on chitosan chains which serve as the coordination and reaction sites. [8,9,10,11] The high proportions of amino functions in chitosan have been found to provide novel adsorption properties for many metal ions[12,13,14] and organic dyes[15,16,17,18,19,20] . The deacetylated amino groups in chitosan can be protonated and the polycationic properties of the polymer are expected to contribute to the charged interactions with a model dye, methyl orange, which is an anionic azo dye. The aim of this study is to investigate adsorption capacity of carbon black using chitosan in deinking process. In this study, chitosan was used as adsorbent to remove carbon black in deinking process.

2. Material and Method

2.1. Materials

Material that were used are Carbon black (Cemani Toka), NaOH (Merck), HCl 37% p.a (Merck), Sodium Dodesyl Sulfat (SDS), Waste paper (old news paper), and aquadest.

Instruments that were used include glass aparatus, analytical scale, thermometer, pH meter, hotplate stirrer, spectrophotometer UV-Vis, and spectrofotometer FTIR.

2.2 Experimental method

Adsorbent

The isolation of chitosan consist of three steps, deproteinization, demineralization and deacetylation. The shells of fresh shrimp were thoroughly and repeatedly washed in water and sun dried. The raw material was completely immersed in 4% NaOH solution (w/v) and boiled for two hour for deproteinization. After cooling, the alkali was drained off and washed repeatedly with ionized water to obtain neutral pH. The contents were added 1M HCl and allowed to act for one hour to remove $CaCO_3$. The acid was decanted and repeatedly washed with water. Deacetylation process is carried out by adding 40% NaOH solution at 100^0C for 3h. The NaOH was quickly drained off and the content was repeatedly washed with water and filtered in order to retain the solid matter, which is the chitosan. The sample were then left uncovered and oven dried at 110^0C for 6 hour.

Adsorbate

Carbon Black was from Cemani Toka, and used without chemical purification. The stock solution was prepared by dissolving 1mg of carbon black in one liter of distilled water and added 20 ml surfactant solution (SDS). The experimental solutions of desired concentrations were obtained by successive dilutions with double distilled water.

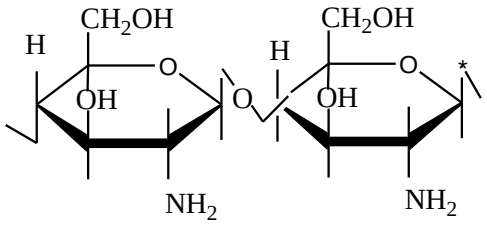

Fig. 1. Chemical structure of chitosan

Characterization of the adsorbent

Characterization of chitosan using spectrophotometer FTIR to determine functional groups of chitosan in the range of 4000-400 cm^{-1}. To determine the ash content, the standar ASTM D2866-94 was used. Some physicochemical properties of chitosan are given in Table 1.

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Pulp slurry preparation

The waste paper is put into pulper with a large quantity of water and broken down into a slurry during 15 min and 45^0C. Most of the water containing dispersed ink (carbon black) is drained off from the pulp through slots or screens with 1% consistency. Chitosan was added (with different dosage 0.5, 1, 1.5, and 2 g) in order to adsorb carbon black from cellulose fibers.

Adsorption experiments

A stock solution of carbon black was prepared by dissolving 1 mg of carbon black in 1 L of distilled water. The desired concentrations ranging from 20 to 100 ppm were obtained by dilution.
For each adsorption experiment, the pulp slurry that contain chitosan (with different dosage) was filtered with Whatman 40 and residual concentration of carbon black analyzed using UV-Vis spectrophotometer. The residual concentration was determined from a constructed calibration curve by measuring the absorbance at λ_{max} = 223 nm using UV-Vis spectrophotometer.

3. Result and Discussion

3.1. Characterization of the adsorbent

Figure 2 represents the FTIR spectra of chitosan (400-4000 cm^{-1}). The wide band at 3476 cm^{-1} shown in the spectrum is attributed to stretching vibration of hydroxyl group of chitosan. The band at 3725 cm^{-1} is due to stretching vibration of N-H groups. The band at 2952 cm^{-1} is assigned to C-H stretching vibration of

polymer backbone. The other band at 1484 cm^{-1} is due to C-H bending. The bands observed at 1622-1673 cm^{-1} is due to C-O stretching, 1373 cm^{-1} correspond to (-NH stretching vibration). The presence of two bands, one at 1144 $cm^{-1}$and another at 1069 cm^{-1}, probably indicates stretching vibrations of C=O groups.

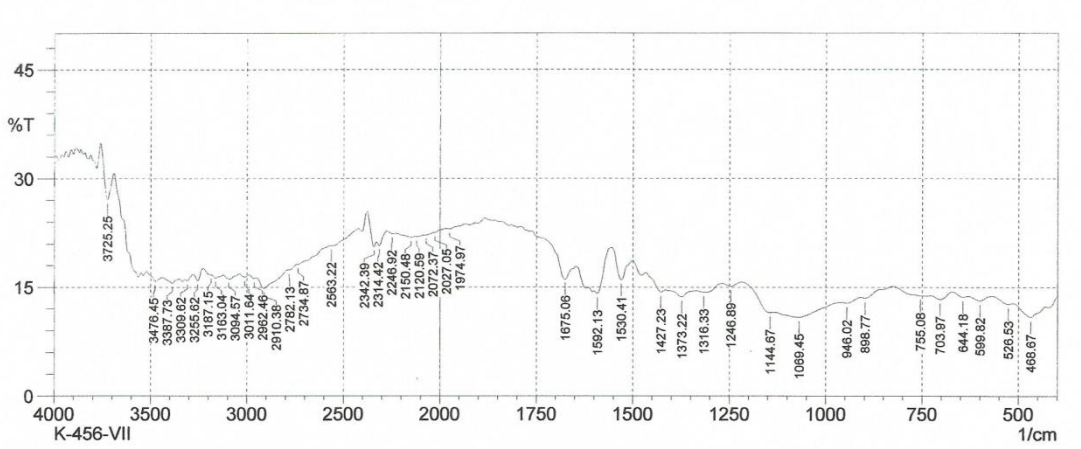

Fig 2. FTIR of chitosan

Table 1. Physicochemical characteristics of chitosan

Characteristic	Value
Moisture content (%)	0,063
Ash Content (%)	27,61
Deacetylation (%)	74,95

3.2. Effect of adsorbent dosage

The effect of adsorbent dosage of chitosan (varied from 0.5 to 2 g) on the removal of carbon black solution is shown in Fig. 3. The percentage removal of CB increased from 57% to 72% as the adsorbent dosage increased from 0,5 to 1,5 g. In this study, adsorption capacity increase from with increase in the dosage of chitosan from 0,5 to 1,5 g. An increased in the chitosan dosage lead to an increase in the adsorption capacity of the CB on chitosan. This result is expected because of the increased adsorbent surface area and availability of more adsorption sites caused by increasing adsorbent dosage. When the adsorbent was increased to 2 g, the adsorption is decrease, this due to saturation of active sites which do not allow further adsorption to take place. The result show that the optimum concentration of chitosan was 1,5 g. The mechanism of CB adsorption on chitosan is complex. It primarily involves the surface physical adsorption on biosorbent, but there exists the possibility of a covalent binding of dye on the substrate that occurs simultaneously with the exchange process. In general, dye adsorption on an adsorbent material may be assumed to involve four steps, of which film diffusion (diffusion of dye through the boundary layer to the surface of the adsorbent) and pore diffusion or intraparticle diffusion (transport of dye from the surface into the pores of the particle) are essential for establishing the mechanism.[18,22] However further research is necessary to study the kinetics and adsorption mechanism of carbon black onto chitosan.

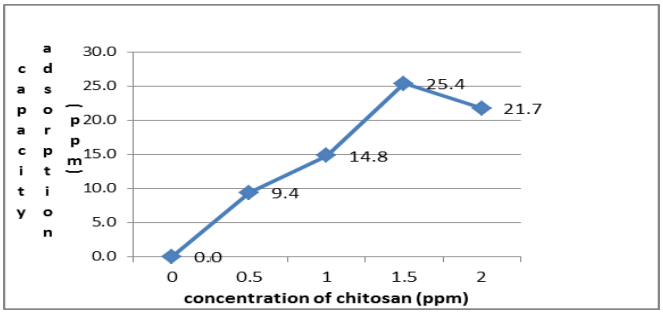

Fig. 3. adsorption of CB on chitosan at different concentration adsorbent

4. Conclusion

Chitosan could be used as a biosorbent to adsorb and to remove the carbon black in deinking process. The synthesize of chitosan involved three main stages as deproteinization, demineralization, and deacetylation. It characterized by using Fourier Transform Infrared Spectroscopy (FTIR). The capacity adsorption of carbon black onto the chitosan at weight doses of 1.5 g.

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Synthesis of Mg/Al Hydrotalcite by Sol-Gel and Coprecipitation Methods

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Abstract

Hydrotalcite compounds are layered materials. Mg/Al Hydrotalcite compounds were prepared by using two different synthesis methods, on the one hand sol-gel and the other coprecipitation. Sol-gel hydrotalcite compounds were synthesized by using two different aluminium precursors, ie aluminium nitrate (HTG1) and aluminium acetylacetonate (HTG2). Coprecipitation method was using aluminium nitrate precursor (HT). The magnesium precursor always magnesium nitrate. In the present work all hydrotalcite compounds synthesized with ratio molar Mg/Al = 2. The solids were characterized by X-ray diffraction, infrared spectroscopy, nitrogen physisorption and scanning electron microscopy. It is found that only HT was formed be hydrotalcite compound.

Keywords: coprecipitation, hydrotalcite, sol-gel method

1. Introduction

Hydrotalcite is an interesting material to study the process of synthesis for diverse usage benefits. In the field of catalysts, hydrotalcite serve as a catalyst for hydrogenation reactions, polymerization, and be used as catalyst supports CeO_2 and Ziegler-Natta. In the industrial field, hydrotalcite used for refractory materials, molecular filters and ion exchange. Hydrotalcite can also be used in medicine as antiacid, antipeptin or stabilizer. In addition, the hydrotalcite can be used as an adsorbent, such as chlorine ion capture and purification of waste water containing anions, both organic and inorganic (Cavani *et al.*, 1991).

Hydrotalcite is a layered double hydroxides or an anionic clays as they have positively charged layers, due to a partial substitution of a divalent metal by a trivalent one (Sommer *et al.*, 2009). Hydrotalcite preparation is done by various methods include coprecipitation (Zhao *et al.*, 2003), urea hydrolysis and microwave irradiation (Yang *et al.*, 2007), hydrothermal, sol-gel (Guiterrez *et al.*, 2009), the activation steam and solvothermal (Sun and Hu, 2011). Synthesis method is one of the factors in shaping the surface properties of layered double hydroxides.

The combination of sol-gel method with a microwave during gelation and crystallization process can increase the surface area of hydrotalcite (Othman *et al.*, 2009). Synthesis method can result material properties. Wang *et al.* (2008) compared the method of sol-gel and coprecipitation on barium hexagonal ferrite synthesis ($BaFe_{12}O_{19}$), it shows that sol-gel method can not anticipate the formation of intermediate crystalline phases such as γ-Fe_2O_3, α-Fe_2O_3, $BaCO_3$ and $BaFe_2O_4$ because $BaFe_{12}O_{19}$ did not establish. In other hand, coprecipitation method can form directly precursor of amorphous hydroxide $BaFe_{12}O_{19}$ without forming intermediate crystalline phases. Synthesis $BaFe_{12}O_{19}$ using coprecipitation technique is is easier to control the resulting material products than the sol-gel method at low temperature (Wang *et al.*, 2008). The other research of synthesis $ZnAl_2O_4$ shows that sol-gel method produces materials with surface area and pore volume wider and more homogeneous than coprecipitation method (Valenzuela *et al.*, 1997).

The synthesis method on each material gives a different effect. This study will compare synthesis method of Mg-Al hydrotalcites using sol-gel and coprecipitation method. The advantage of this modification is determine optimum method for produce Mg-Al hydrotalcites.

2. Materials and Methods

2.1 Materials

Magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$, Merck, PA), aquabidest, aluminium nitrate nonahydrate ($Al(NO_3)_3.9H_2O$, Merck, PA), aluminium acetylacetonate ($C_{15}H_{21}AlO_6$, Merck, PA), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), dillute HCl, dan amonium hydroxide (NH_4OH) were used for the synthesis of hydrotalcite samples.

2.2 Synthesis of Mg-Al Hydrotalcite

2.2.1 Sol-gel Method

Mg-Al hydrotalcite with Mg/Al=2 were prepared by the sol-gel method (Gutierrez *et al.*, 2009). Magnesium nitrate hexahydrate (0,66 mol) and aluminium nitrate nonahydrate (0,33 mol) were dissolved in ethanol containing a small amount of HCl. The solution was refluxed at 70°C with constant stirring. The pH of the mixture was adjusted at 11.5 with NH4OH. Then, 0.5 mol of water was added. The mixture was stirred and refluxed until the gel was formed (± 2 hours). Finally, the samples were dried at 70°C for 24 hours. This sample was denoted to HTG1. This procedure was repeated using differ aluminium source, i.e. aluminium acetylacetonate, this sample was denoted to HTG2.

2.2.2 Coprecipitation Method

Hydrotalcite samples were prepared by co-precipitating (Zhao *et al.*, 2003). The first solution (A solution) containing $Mg(NO_3)_2.6H_2O$ and $Al(NO_3)_3.9H_2O$, dissolved in deionized water at Mg/Al molar ratio of 2:1. The second solution (B solution) containing appropriate amounts of sodium hydroxide–sodium carbonate was added on a certain ratio so that the final $Al/CO3^{2-}$ molar ratio equals to 2 and the pH of the final solution was kept at 8.5±0.3. Two solutions were added dropwise at different rate to keep the pH of the slurry in the range of 8.5±0.3. Then the mother liquid was poured into a closed vessel with inner liner of teflon(polytetrafluoroethylene) and aged at 110 °C for 12 h. The precipitate was separated by filtering and washed with deionized water, and then the precipitate was dried at 393 K overnight. This sample was denoted to HT.

2.3 Characterization

The X-ray diffraction technique (XRD) was carried out using a *X-Ray Diffraction Phillips Expert*. The diffractometer employing CuKα radiation to generate diffraction patterns from powder crystalline samples at ambient temperature. The CuKα radiation (λ = 1.5405 Å) was generated at 40 kV and 30 mA in the range of 2θ = 1.5 to 40° with a scan speed of 0.02°/sec. Fourier transform infrared (FTIR) spectroscopy was done on a Shimadzu *Instrument Spectrum One* 8400S spectrometer, with 2 cm^{-1} spectral separation, at 20 °C. Nitrogen sorption Quantachrome Instruments was used for the analysis of pore structure. A sample of 0.4 g of a preheated 250 ºC for 2.5 hours and cooled to adsorption and desorption of nitrogen at a temperature of 77 K. The surface morphology and particle size of solid samples were determined by scanning electron microscopy (JEOL SEM Model JSM-5800LV).

3. Result and Discussion

Powder X-Ray Diffraction Characterization

Figure 1 shows the XRD pattern of the Mg-Al hydrotalcite samples. For Mg-Al hydrotalcite by Co-precipitation method sample (HT), the diffraction peaks appear at 2θ =11.70, 23.34 and 34.93° which assigned to hydrotalcite structure. The diffractogram pattern of HT had similar results with the diffractogram of hydrotalcite synthesized by Kloprogge *et al.* (2002), Zhao *et al.* (2003), Sharma *et al.* (2007), and Mokhtar *et al.* (2012). For Mg-Al hydrotalcite by sol-gel method samples, the diffractogram shows the different pattern with hydrotalcite structure, it means that the synthesis of hydrotalcite by sol-gel method is not succesful. For HTG1 samples, the highest diffraction peaks appear at 2θ =28.95° which attributed to the $Mg(NO_3)_2.2(H_2O)$ peaks due to incomplete dehydration of $Mg(NO_3)_2.6H_2O$ (Sulaiman *et al.*, 2013). The highest diffraction peaks for HTG2 samples appear at 2θ =22.56° which assigned to the organic compounds from acetylacetonate (Prinetto *et al.*, 2000). The XRD patterns of sol-gel method samples assigned to the unreacted organic precursors. Hydrotalcites phase was only detected when the reflux time increased up to 6 h. These results showed that in the sg synthesis procedure the reflux not only allowed to enhance the crystallinity, but was also necessary to complete the hydrolysis reaction, which thus appeared as the limiting step (Prinetto *et al.*, 2000).

Table 1. Comparation of fuctional group

Hydrotalcite	υ OH (cm^{-1})	δ H_2O (cm^{-1})	υ NO_3^- (cm^{-1})	υ Al – O (cm^{-1})	υ Al–O–Mg (cm^{-1})	υ Mg – O (cm^{-1})
HT	3456.44	1627.92	1381.03	678.94	455.20	393.48
HTG1	3448.72–3140.11	1635.64	1381.03	671.23	416.62	354.90
HTG2	3425.58–3155.54	1627.92	1381.03	671.23	416.62	354.90

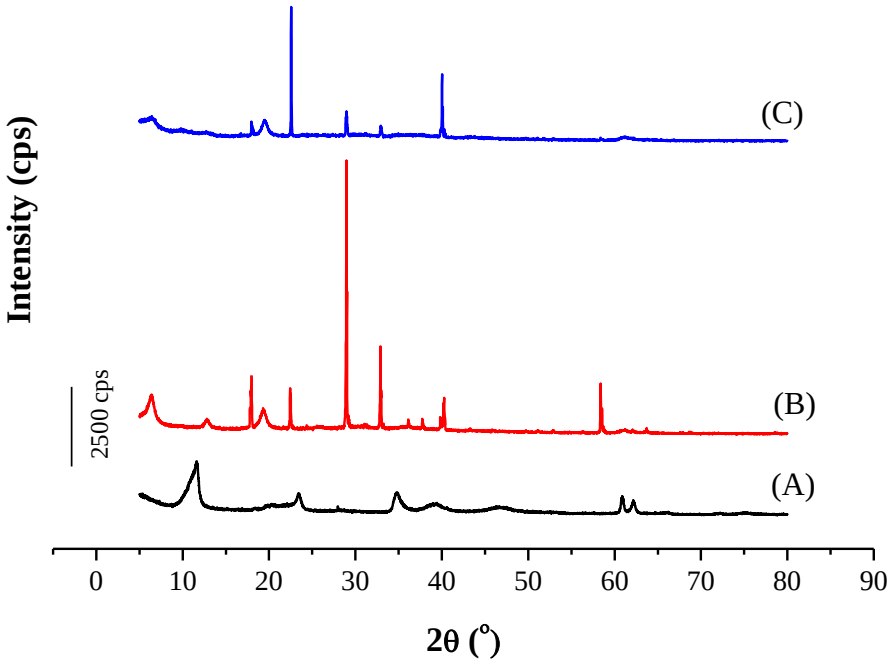


Figure 1. The XRD pattern of hydrotalcites, (A) HT; (B) HTG1 and (C) HTG2

Infrared Characterization of Hydrotalcite

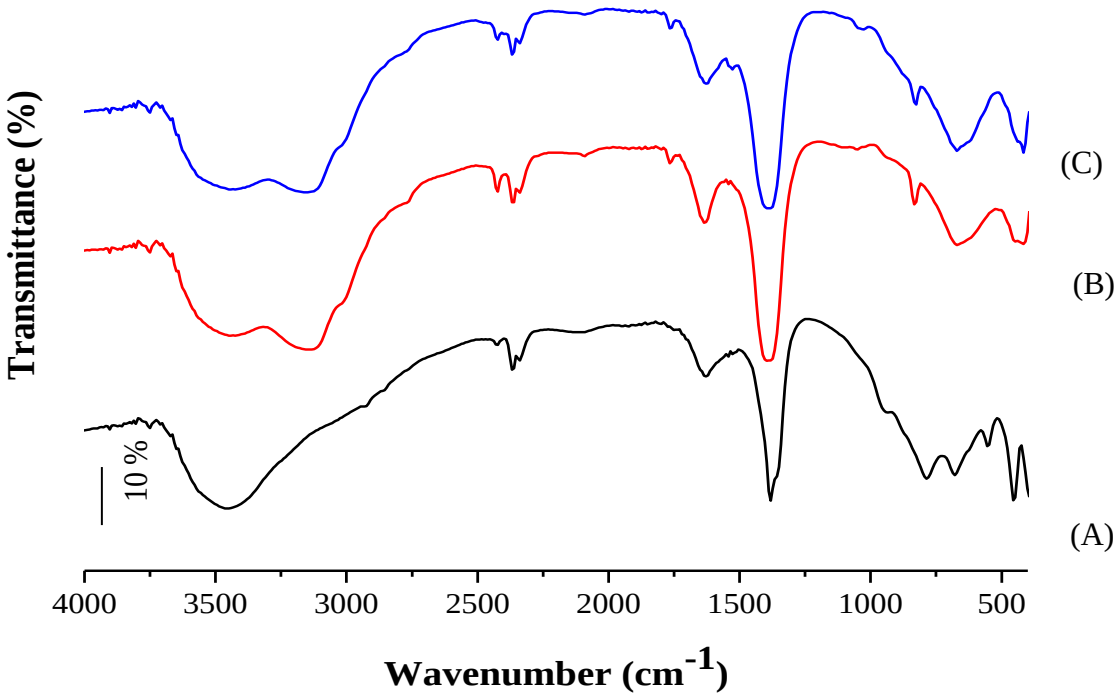


Figure 2. IR-spectra data of hydrotalcites, (A) HT; (B) HTG1 and (C) HTG2

The IR-spectra data of hydrotalcites is given in Table 1. In HTG1, υ_{OH} streching vibration due to water molecule got split and appeared at 3448.72 and 3140.11 cm^{-1}. The HTG2 sample shows broad absorption at 3425.58 cm^{-1} with shoulder at 3155.54 cm^{-1}, respectively. The corresponding of δ_{H2O} peak was observed at 1627.92 cm^{-1} (HT and HTG2) and 1635.64 cm^{-1} (HTG1). Presence of intercalated NO_3^- was supported by FTIR band at 1381.03 cm^{-1} for HT, HTG1 and HTG2. The regions below 700 cm^{-1} show Al – O, Al – O – Mg and Mg – O.

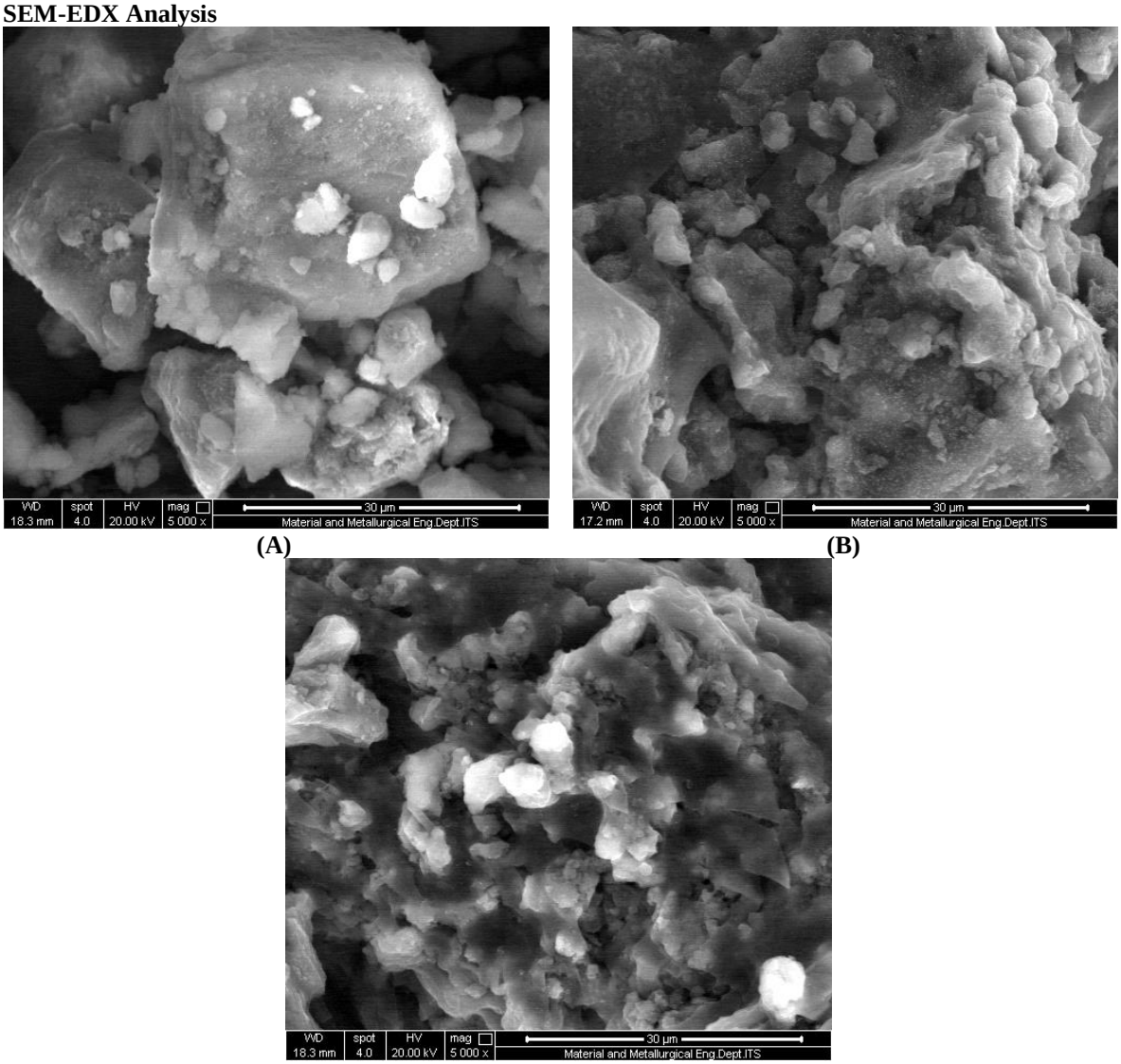
Isotherm N_2

Nitrogen adsorption-desorption isotherms is physical adsorption used to determined the pore distribution and the total surface area of a solid (Haber *et al.*, 1995). The results of the surface analysis of the hydrotalcites samples are shown in Table 2. The surface properties for HT and HTG-1 samples are similar in the range of 60 m^2/g, this is because the similar source of Mg and Al. HTG-2 samples has different surface area, its about 7 m^2/g due to the different of Al sources. HTG-2 used aluminium acetylacetonate for Al sources. This is the smallest of surface area from hydrotalcites samples.

Table 2. The results of surface area analysis from prepared hydrotalcite samples

Sample	Surface Area (m^2/g)
HT	58.053
HTG1	67.909
HTG2	7.535

SEM-EDX Analysis



(A) **(B)**

(C)

Figure 3. SEM Image of Hydrotalcites, (A) HT; (B) HTG1 and (C) HTG2

The particle morphology obtained by SEM is compared in Figure 3. All samples of hydrotalcites show similar morphology of particle were formed as an accumulation of nano particles aggregates. The morphology is similar to the amorphous γ-AlOOH. The composition of the observed particles on the surface material was determined by EDX. The result of elemental analysis of prepared hydrotalcite samples is listed in Table 3.

Table 3. Result of elemental analysis by using EDX

Element (% Wt)	HT	HTG1	HTG2
O	54.89	59.02	54.57
Mg	24.87	8.67	8.63
Al	16.64	4.31	4.42
C	-	2.55	8.52
N	-	25.46	23.87

The ratio of magnesium and aluminum content for HT was still lower of the theoretical value its caused by condition pH during synthesis of hydrotalcite. The HT samples were synthesized at pH 8.5, resulted the molar ratios was 1.49. The molar ratio of prepared hydrotalcite with the synthesis pH 8-9 is around 1.5-2.3 (Wang *et al.*, 2012). The ratio of Mg/Al from hydrotalcite by sol-gel method samples, HTG1 and HTG2, respectively were 2.01 and 1.95. These ratio are the same with theoretical value but the weight percentage of Mg and Al lower than HT samples, due to the final products are not hydrotalcites like material. It also be confirmed by XRD pattern. The hydrolysis of Mg-Al source didn't occur and completely because reflux process didn't take sufficient time. The existence of carbon (C) and nitrogen (N) are another proof that hydrolysis of Mg-Al source didn't occur and completely. The weight percentage of carbon from HTG2 samples was higher than HTG1 because of HTG2 samples used aluminium acetylacetonate as Al source which has more carbon than aluminium nitrate nonahydrate.

Conclusions

Mg/Al hydrotalcite was successfully prepared using co-precipitation method but the synthesis of hydrotalcite by sol-gel method were not succesful. The chemical properties of prepared hydrotalcite was affected by molar composition and showed by diffraction pattern, infrared spectra, isotherm N_2, morphology structure, and elemental analysis as well. The XRD patterns of sol-gel method samples assigned to the unreacted organic precursors, due to the reflux process didn't take sufficient time.

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The Chitosan Synthesis And Characterization Of *Tutut* Shell (*Bellamya Javanica van den Bush*) as Anti Microbial

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Abstract

The used of anti microbial agreed accurately will sustain the age reserve and ensure the product safely. It requires the substances as natural anti microbial in order not to endanger the health. In this research which chitosan used as anti microbial, goldfish synthesized from *tutut* shell (*Bellamya javanica van den Bush*). The population of *tutut* shell which used in this research is from restaurant in Tasikmalaya. The population of fish is live goldfish from the seller in Cikurubuk Market Tasikmalaya. Chitin and Chitosan synthesized from *tutut* shell (*Bellamya javanica van den Bush)* by using Hong K. No method[1]. Chitin and chitosan which is successful synthesized will be characterized and the result includes the test of water content, ash content, nitrogen content and used as anti microbial in goldfish. The identification of anti microbial functions to identify the concentration and immersion time optimally. The variation of chitosan concentration which is used 0.5%, 1% and 1.5%, whereas the variation time of conservation of goldfish that is 6 hours, 8 hours, 10 hours and 12 hours. The result of the research shows that the chitosan has 7.81% water content, 37.195% ash content and 4.92% nitrogen content. Whereas the test result of anti microbial chitosan solvent of goldfish shows the optimum concentration which used is 1 % with the time immersion is 6 hours.

Keywords : Chitosan, goldfish, anti microbial

1. Introduction

Tutut (Bellamyâ javanica van den Bush) are classified as members of the Mollusca phylum animals that have a soft body and a hard protected shell. Currently *Tutut* shell is not used by humans, and become a thrown away waste. Many people assume that the shell *Tutut* can not be used for something useful. In *Tutut* shell contains 80% calcium contained in Calcium Carbonate ($CaCO_3$) or calcium alkaline.

One of the alternative efforts to use Tutut shell waste that has a value and usability of Tutut waste into products of high economic value is the processing into chitin and chitosan. Chitin is a biopolymer composed by units of N-acetyl D-glucosamine binds b (1-4) are most often found in nature after cellulose. Chitosan is a compound result of deacetylation of chitin, is composed of units of N-acetyl glucosamine and N glucosamine. The presence of reactive amino groups at the C-2 atom and the hydroxyl group at atom C-3 and C-6 on chitosan helpful in the extensive application as a preservative and stabilizer fishery food products color, as flocculants and assist the process of reverse osmosis in water treatment, additives and preservatives for agrochemical products and seeds [2,3].

Fish is one food product that is very easy to damage. Fish spoilage occurs immediately after the fish are caught or die. In tropical conditions of temperature, fish decompose within 12-20 hours depending on the species, tool or by arrest. Cooling process will extend the shelf life of fish. At a temperature of 15-20 ºC, the fish can be kept to about 2 days, at a temperature of 5 ºC fish can survive for 5-6 days, whereas at a temperature of 0 ºC can survive to reach 9-14 days, depending on the species of fish[4].

Fish processing needs to be done to make it more durable so that fish can still be consumed in a good condition. Basically goldfish preservation aims to prevent bacterial decay into the fish. The use of appropriate anti-microbial can extend shelf life and ensure the safety of food products, it is necessary for anti-microbial material alternatives of natural ingredients that are not harmful when consumed and can inhibit the growth of microbes in the product that serves to inhibit damage to food due to microbial activity. In materials that exhibit anti-microbial activity further identification is needed to determine the anti-microbial active component, the concentration and the time required for optimum results required to inhibit or kill microbes target. By preserving the economic value of the fish will be much longer than if it is not doing a preservation.

This study aimed to examine the effectiveness of chitosan in inhibiting the growth of microbes on goldfish that can extend shelf life in fresh condition.

2. Materials and Methods

2.1 Purification of chitin

Chitin purification is done by using the Hong method[1] a way that has a clean shell Tutut smoothed to obtain a size of 60 mesh. Then performed deproteination process, demineralization and depigmentation.

2.2 Preparation of chitosan

Chitin is added with NaOH 50% with a ratio (1:10) was stirred while heated 100 ^{0}C for 30 minutes. After chilling, it's filtered and washed until neutral and dried at 65 ^{0}C. This product called chitosan. Furthermore, the active groups of chitosan were characterized using Infrared spectrophotometry (IR)[1]. Characterization used to distinguish chitin and chitosan are stoichiometric water content, ash content, N content and degree of deacetylation.

2.3 Characterization of chitosan

1) Testing the water content using the AOAC method to heat.
   Samples of 1-2 g put in an oven at a temperature of 100-105 °C for 1-2 hours. Then cooled in a desiccator for approximately 30 minutes and then weighed, repeat until constant weight.
2) Testing ash content.
   A sample of 2-5 g heated in the furnace until a whitish gray while stirring. Then krus and ash is cooled in a desiccator for approximately 30 minutes. After it's cold, ashes weighed.
3) Determination of N content using the macro-Kjeldahl method.
   A sample of 1 g was added to the kjeldahl flask. Then added 7.5 grams of K_2SO_4; 0.35 grams of HgO and 15 mL of concentrated H_2SO_4. The mixture was heated in a Kjeldahl flask in a fume hood until it stops fuming. Heating continues until the liquid becomes clear and then cooled. Then added 100 mL of distilled water in a Kjeldahl flask cooled in ice water and some Zn plates; 15 mL of 4% K_2S and gradually add 50% NaOH solution of 50 mL. Furthermore Kjeldahl flask fitted immediately on distillation tool. Distillate is collected in erlenmeyer which has been filled with 50 mL of 0.1 N HCl standard solution and 5 drops of red methyl indicator. Do distillation until the distillate is mixed with about 75 mL. Then the obtained distillate is titrated with a standard solution of NaOH 0.1 N.
4) Analysis of degree of deacetylation. The degree of deacetylation is the percentage of acetyl groups were successfully removed during the process chitin deproteination, chitin which was given treatment by adding 50% NaOH which caused hydrolyzed acetyl group of the acetamide group of chitin. The degree of deacetylation can be determined from the absorption spectrum of IR spectroscopy with a baseline method. The highest peak is recorded and measured from the base line selected. Comparison of wave numbers between the amide absorption band (1655 cm-1) with a hydroxy absorption band (3450 cm-1).

2.4 The application chitosan as antimicrobial.

Media used for the growth of microbes is PCA (*Plate Count Agar*) were made by about 7 grams of PCA dissolved in 500 mL erlenmeyer with the addition of 400 mL of distilled water. Then sterilized by autoclave at a temperature of 121 ^{o}C for 15 minutes. While the number of goldfish microbial testing using the Total Plate Count (TPC) method after soaking in chitosan solution.

To search for the optimization of chitosan as antimicrobial materials, the chitosan used be varied in concentration by dissolving chitosan (w/v) into acetic acid 2% (v/v). To determine the optimal concentration of chitosan in storage, goldfish samples soaked in a solution of chitosan with some variation of concentrations (0.5, 1 and 1.5%). Comparison between fish with chitosan solution is (1: 1) is 1 kg of fish in a 1 L solution of chitosan. As for seeing the most optimal storage time is done by soaking the goldfish by using the optimal concentrations that have been previously obtained by variation of the storage time (4, 6, 8, 10 and 12 hours). Storage is done with the variation of time until the ISO safety limits set for the number of microbes in frozen fish is $5x10^5$ cells/mL.

3. Results and Discussion

3.3 Purification of chitosan

Deproteination. Into a 250 mL round bottom flask containing 120 g of *Tutut* shell powder added 1200 mL of 3.5% NaOH solution at a ratio of 1:10 (w / v), and then heated with stirring for 2 hours at a temperature of 65^{o}C. Once cool, filtered and neutralized with distilled water. The solid obtained was dried in a 60 ^{o}C oven until dry. Deproteination stage is a process of separation binding proteins and chitin in the *Tutut* shell. Reagents used were 3.5% NaOH (1:10 w/v) at a temperature of 64-66 °C for 2 hours. At this stage, the protein will be separated and form a soluble Na-proteinat and is lost during the process of washing and screening [5]. From this deproteination process yield as much as 91.56% was obtained

Demineralisation. Tutut shell powder from deproteination is added to a solution of 1 N HCl at a ratio of 15: 1 (v/w) in a 500 mL round bottom flask and then reflux at 40 °C for 30 minutes and then cooled. Once cool, the solution was filtered and the solid was neutralized with distilled water, then dried in a 60 ^{o}C oven. This stage aims to remove the minerals contained in the *Tutut* shell. Solvents used were 1N HCl, the solvent can dissolve Ca^{2+} binding as $CaCO_3$ and $Ca_3(PO_4)_2$ to produce a bond $CaCl_2$ soluble in water so as to produce chitosan with a lower mineral. Demineralization is generally done using other acids such as HCl or H_2SO_4 on certain conditions. Effectiveness in dissolving calcium HCl is 10% higher than the H_2SO_4 [6]. Then the heating process is carried out at a temperature of 44 °C for 1 hour until the froth is reduced and the solution changed

color to yellow turbid. Randemen obtained from the demineralization are 30.85%, it indicates that there are a lot of mineral lost during the demineralization.

Depigmentation. 0.315% NaOCl solution was added into the demineralized powder at a ratio of 10: 1 (v/w) in a 250 mL round bottom flask. Then reflux for 1 hour at 40 °C, then the solids filtered and neutralized with distilled water. The solid from neutralization results was dried in an oven at a temperature of 80 °C to constant weight. Chitosan obtained are identified using an infrared spectrophotometer instruments. Basically leaching residue and dye done during the process of neutralization or washing using distilled water after the deproteination and demineralization process. However, to obtain chitosan with white color should be added a solution of bleach which is NaOCl (sodium hypochlorite) with a concentration of 0.315%. Depigmentation process aims to produce a white color on chitosan thus providing an attractive appearance on chitosan products obtained[7]. Randemen obtained during the process of depigmentation is equal to 28.87%.

The next stage is the process of deacetylation, this process is the transformation of chitin into chitosan. Deacetylation reaction function to decide the acetyl group attached to the nitrogen in the compound structure of chitin to enlarge amine compounds on chitosan. At this stage of deacetylation use a strong alkaline solution such as NaOH 50%, this is high concentration produce optimum conditions on the transformation process of the acetyl group bonded to the nitrogen atom forming the amine group[8]. The use of NaOH with a high concentration (> 40%) will increase the degree of deacetylation and resulting depolimerasi in termination of the double bond between the carboxyl group to the nitrogen atom[8]. Randemen obtained on deacetylation process is as much as 14.98%.

As a result, reported that 7.81% of water content was generated from chitosan. This value was lower than 10% as a requirement due to related with durability. A higher ash content was generated and it was refers to high mineral content. In *tutut* shell, mostly consist of calcium carbonate and calcium phosphate in small quantities or other mineral which is depend with their habitat.

The ash content was related with soluble factor, viscosity. Determination of ash content is very important for efficiency demineralization process. A higher of ash content could be indicated that demineriläsation process has not been perfect. Albes lesbani studied that in shell of crab was produced 15.25% and it has been shown that ash content was lower than in *tutut* shell which is contain 37.195% respectively.

Harry Agusnar[9] reported that Nitrogen content from squid bone was 7.4 meanwhile from chitosan was 4.92%, it shown that from *tutut* shell the value was lower than squid.

The Application of chitosan as antimicrobial

The use of chitosan from *Tutut* shell waste (*Bellamyâ javanica van den Bush*) as an alternative antimicrobial goldfish preservation using the ALT method resulting that soaking goldfish in chitosan can reduce the number of microbes. The number of goldfish microbes before immersion is 19,000 colonies/mL, whereas for the amount of goldfish microbial after immersion with 0.5% chitosan solution was 18,000 colonies/mL and by using a chitosan solution of 1.0 and 1.5% number of microbes that grow <2,500 colonies/mL. Soaking using 2% acetic acid solvent as a blank obtained the number of microbes that grow as much as 2,800 colonies / mL. (Table 1)

Table 1. Number of total carp (goldfish) plate numbers before and after immersion

Sample	The number of colonies for each dilution				The number of colonies/mL
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
Before Soaking	85	42	67	1	19.000
Chitosan 0,5%	118	60	2	9	18.000
Chitosan 1,0 %	6	2	2	6	< 2.500*
Chitosan 1,5%	17	6	4	2	< 2.500*
Control of acetic acid 2%	10	6	28	0	2.800
Negative control (-)	0				0

The research method of ALT on gold fish eith variations perpetuity immersion 6, 8, 10 dan 12 hours with 1,0% chitosan concentration listed in table 2 of result calculation the amount of ALT method is as following :

In general, effectiveness of antimicrobial has exponential related with concentration. There is no significally effect even continuous increase of concentration . The Afinity antimicrobial behavior of chitosan was depended by weight molecule and acetilation degree and it seen as linierly related[10].

Irianto[11] reported, that chitosan has function group of amina with positive charge and reactive, and so could react with negative charge in electronegative site from cell of bacteria. The mechanism of chitosan as antimicrobial by the destruction of bacteria cell wall and then into abnormal form and produced an enlarged pores wall. This causes the wall cell could not regulate the exchange of substances into the cell, then the membrane becomes demaged and lysis, the activity was inhibited will die.

Tabel 2. The Total Number of plate based on variations perpetuity goldfish immersion

Treatment	Dilution												The number of colonies/mL
	10^{-1}		10^{-2}		10^{-3}		10^{-4}		10^{-5}		10^{-6}		
	I	II	I	II	I	II	I	II	I	II	I	II	
6 hour immersion	3	1	0	0	0	0	0	0	0	0	0	0	< 2500*
8 hour immersion	27	25	18	9	0	0	0	0	0	0	0	0	2.600
10 hour immersion	48	41	39	32	10	5	0	0	0	0	0	0	8.000
12 hour immersion	237	211	75	77	28	26	16	7	0	0	0	0	33.000

Typographic symbol * : expressed as the number of bacteria is estimated to be less than 250

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Besides that, amina which is consist of free electron could react with Ca from cell wall and Mg from ribosome to form covalent bond, which could lead of leakage of intracellular constituent and make a damage of intraceluler system of bacteria and it can causes a death.

Conclusion

Chitosan could applied as antimicrobial in fresh fish. The optimize condition from microbe test have been gained < 250 cell/ mL from 1 % of chitosan solution which contact time 6 hours respectively.

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Antibacterial Potency of AC Kuningan's Sweet Potato (*Ipomea batatas*) on E*escherichia coli* Bacteria

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Abstract

AC Kuningan sweet potato (*Ipomea batatas*) is an endemic plant in Kuningan regency that becomes the biggest comodity for sweet potato farmers, *Ipomea batatas* is a foodstuff that rich of vitamin and mineral consisting of beta-carotene, vitamin B1, vitamin C, calcium, phosphorus, zinc, kalium and natrium. Beta-carotene contained in *Ipomea batatas* is useful as an antioxidant to protect the body tissue from negative effect of free radical and to improve the immunity (natural antibiotic). The objective of the research is to discover the antibacterial AC Kuningan's sweet potato extract activity from n-hexane fraction, chloroform and ethanol. The antibacterial activity testing was conducted by applying paper disc diffusion. The microorganism test used *Escherichia coli* (atcc 25922) bacteria. The data were analyzed statistically using one-way annova method. The result revealed that the highest inhibitory potency with various numbers on bacterial testing was shown in a concentration of 1000 µl/ml for ethanol extract and N-hexane extract, 2000 µl/ml and 500 µl/ml for chloroform extract in a low category up to strong.

Keywords: Antibacterial potency, Escherichia coli, Inhibition, Ipomoea batatas L

1. Introduction

The research of characteristics and potency towards minor food commodity uitilization is still few compared with main food commodity such as rice and soybean. Sweet potato (*Ipomea batatas*) is categorized as food commodity which its utilization is still limited (gklinis, 2004). Sweet potato is an Indonesian foodstuff which has fine potential nutrition and good bioactive component, yet has not been utilized optimally since the limitation of people's knowledge on its advantages.

Kuningan is a regency in the east of Ciremai Mountain and producing rice plants and sweet potatoes. Therefore, the excavation of superior local products potency is needed to achieve the national food security. One of the main tuber products in Kuningan is the AC (Anak Ciremai) sweet potato that has a high potencial productivity.

The Anak Ciremai sweet potato has higher vitamin A and beta-carotene compositions than spinach. The protein composition in it can reach 20 – 25% and it has a fat, carbohydrate, and energy. This sweet potato has a high calorie, reaching 123 calories/100 grams. Sweet potato is a kind of foodstuff that rich of vitamin and mineral including beta-carotene, vitamin B1, vitamin C, calcium, phosphorus, zinc, calium and natrium. From nutritional value, every 100 grams of sweet potato consists of 34 calories; 1.7 grams of protein, 0.5 grams of fat, 0.8 mineral and 45 mg of calcium (Shodikin, *et al*, 2009). For nutrition possessed, sweet potato is able to increse immunity system. The beta-carotene in it is useful as an antioxidant which is able to prevent free radicals and a heartattack as well as various cancers, especially prostate cancer. The objective of the research is to discover the potency or antibacterial activity from AC Kuningan's sweet potato powder extract from various solvents (ethanol fraction, choloroform fraction, and n-hexane) by applying paper disc diffusion method in inhibiting the growth of *Escherichia coli*.

2. Substances and Methodology

2.1. Devices and Substances

Devices used were autoclave, *rotaryevaporator* Buchi, incubator, refrigerator, test tube, micropipettes 20-200 µL, 100-1000 µL and other supported devices. The substances needed were a white AC's sweet potato (Iphomea batatas) gained from PT Galih Estetika, Kuningan- West Java, ethanol 96%, chloramphenicol, DMSO 1.25%, Natrium Agar (NA) medium and Nutrient Borth (NB) medium. The organism tested for antibacterial test was *Escherichia coli* (ATCC 25922) from Microbiology Laboratory collection of Biology Department, FKIP, University of Kuningan.

2.2. Extraction of Sweet potato Powder (Harborne, 1996)

The 1000 grams of sweet potato powder was macerated with 90% ethanol solvent in 24 hours and was covered with alumunium foil. Having finished macerating, the powder was then filtered by filter paper which resulted filtrat and pulp. The filtrat gained was concentrated using *Rotary evaporator device in* 50^{o}C degree until resulted the ethanol extract of sweet potato for 17.27 grams. That extract was divided into three, 5 grams for antibacterial extract ethanol testing, 5grams of sweet potato extract was fracted by chloroform solvent

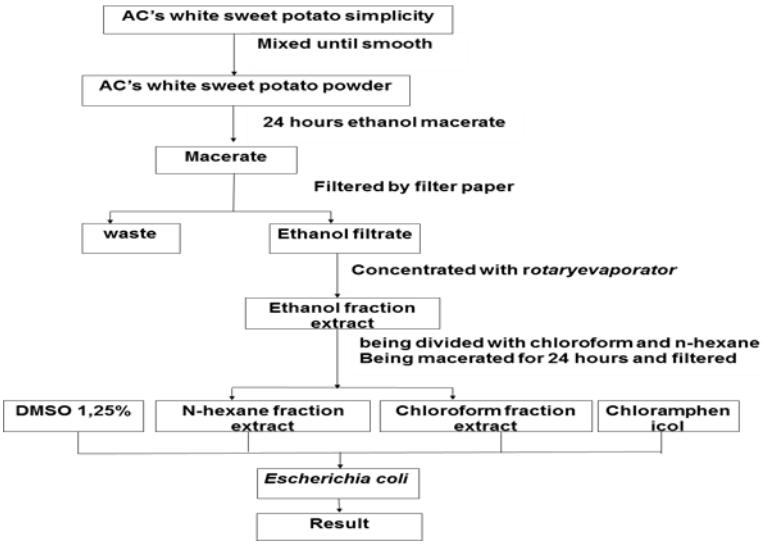
resulting 1.63 grams of chloroform, and 5 grams for n-hexane fraction resulting 0.72 grams. The extract used for testing the antibacterial activity was 0.01 grams.

2.3. The Activity of Sweet Potato Powder Antibacterial Extract by Paper Disc Diffusion Method (Modification of Kirby-Bauer in Nugraheny, 2011)

The intracellular extract and chloramphenicol dissolved in DMSO with concentration of 2000, 1000 and 500 pm (mg/l). The sterile paper disc which has been spilled by sweet potato and chloramphenicol extracts were stored on agar medium and incubated for 24 hours. The petri dish that has been filled with bacteria test and antibacterial compound were then poured into an incubator for 24 hours with 30°C. The inhibition zone formed in petri dish was measured by measuring the diameter of clear zone which was not passed by the bacteria around paper disc. The size of inhibition diameter formed by sweet potato (Ipomea batatas) extract can be compared to the chloramphenicol clear zone diameter (positive control), so that the size of inhibition potency can be known by using formula as follow.

$$\frac{\text{Diameter of tested inhibitor}}{\text{Diameter of control inhibitor at same concentration}} \times 100\%$$

The extraction method and testing of antibacterial activity can be seen in a picture 1.



Picture 1. The extraction method and antibacterial activity testing

3. Findings and Discussion

3.1. Inhibition potency towards E. Coli bacteria tested

The inhibitory potency of AC Kuningan's sweet potato extract in each concentration towards E. coli bacteria tested can be seen in table 1.

Tabel 1. The inhibitory potency of AC Kuningan's sweet potato extract in each concentration towards *E. coli* bacteria tested

Tested bacteria	Extract fraction	Inhibitory potency (%) in each concentration (ppm)		
		500	1000	2000
E. coli	Ethanol	24,69	28,13*	18,38
	Chloroform	56,93*	6,25	33,91
	n-hexane	21,94	70,21*	54,31

In general, from table 1, the highest inhibitory potency is shown with various percentage for three AC Kuningan's sweet potato extract fractions towards bacteria test. In 1000 ppm concentration of ethanol extract, the antibacterial activity was gained with 28.13%, 70.21% in n-hexane, while the biggest inhibitory potency in choloroform extract is shown in 200 ppm concentration with 56.94%. This means that the choloroform extract fraction has a different ability in inhibiting the growth of *E. coli* compared to n-hexane and ethanol extracts.

3.2. Minimum Inhibitory Concentration (*MIC*)

Based on the testing activity in AC Kuningan's sweet potato powder extract, the minimum inhibitory concentration can be determined by comparing the clear zone diameter in each extract towards controlled

clear zone diameter. The minimum inhibitory concentration is determined by comparing the negative control (DMSO) and positive control (chloramfenicol). Table 2 reveals the data of inhibitory zone diameter of each extract in bacteria test.

Table 2. *inhibitory diameter of three fractions in AC Kuningan's sweet potato extract towards E. coli bacteria*

Extract Fraction		Inhibitory diameter towards *E. coli* (mm) in each concentration (ppm)		
		500	1000	2000
Ethanol	Mean	5*	**6.75**	3.17
	Positive control	20.25	24	17.5
	Negative control	4.25*	7.25	1
Chloroform	Mean	**7.8***	2	**7.8**
	Positive control	13.7	32	23
	Negative control	3*	0.5	3
n-Hexane	Mean	3.4*	**16.5**	10.7
	Positive control	15.5	23.5	19.7
	Negative control	2*	2	2

According to table 2, it can be explained that the size of clear zone diameter, especially in positive control occurred for 500 ppm concentration (lowest concentration from various concentration tested). This Chloramphenicol is a proprietary medicine that causes the possibility to inhibit bigger than the treatment.

The minimum inhibitory concentration percentage is gained if it is only compared to the negative control (DMSO) for ethanol extract, in 500 ppm concentration. the minimum inhibitory concentration of chloroform extract is in 500 ppm concentration and n-hexane extract is in 500 ppm concentration. Based on the fact above, the antibacterial substance in AC Kuningan's sweet potato extract sample that has a high activity is an extract fraction in 500 ppm concentration with extract concentration of 12.5 µg.

Commonly, the inhibitory zone diameter tends to improve along with the improvement of extract concentration. However, it does not occur in this research. In ethanol extract, the biggest inhibitory diameter is respectively in a concentration of 1000 ppm (6.75 mm), 500 ppm (5mm), and 2000 ppm (3.17 mm). for choloroform extract, the biggest diameter is serially in concentration of 2000 ppm (7.8 mm), 500 ppm (7.8 mm), and 1000 ppm (2 mm). for n-hexane extract, the biggest diameter is continuously in concentrations of 1000 ppm (16.5 mm), 2000 ppm (10.7 mm), and 500 ppm (3.4 mm). It is in line with Mpila (2012) which states that the inhibitory zone diamter does not always increase comparable with antibacterial concentration, it possibly occurs since the differences in diffusion speed of antibacterial compounds in agar medium, types of antibacterial compounds and concentration can give the different inhibitory zone diameter in certain range of time. Besides of that, it is assumed since the bacteria have a non-genetic mechanism of resistance which menas the bacteria in a rest condition (metabolic inactivation). If the bacteria returns to be active, it indicates that that the bacteria is sensitive towards he antibacteria like it did before (Meyer, *et al* 1982); dulger, *et al* 2004). In addition, it is also suspected that the extract concentration is increased so the inhibitory antibacterial active compound is increasing supported by compounds that encourages stronger activity which can improve bacterial growth faster that inhibitory bacteria.

The inhibitory zone diameter measurement result shows that ethanol and chloroform extracts of AC Kuningan's sweet potato have weak to moderate inhibition towards *E. coli*. Meanwhile, the n-hexane extract has strong inhibition towards *E. coli* bacteria. This determination is according to Davis and Stout (1971 as cited in Dewi, 2010: 11) which reports that provision of antibacterial power are formed as follow: regional inhibition is 20 mm or categorized as very strong, regional inhibition of 10-20 mm is strong, 5-10 mm is moderate, and 5 mm and less is weak. *E. coli* bacteria is categorized into negative gram bacteria which has more complex structure and has three layers; lipoprotein, middle layer of lipopolysaccharide that hinders of bioactive substance in antibacteria to come, and inner layer or peptidoglycan with high lipid substance (11-12%). Outer membrane consists of phospholopid (inner layer), and lipopolysaccharide (outer layer) formed of lipid A which is nonpolar (Jawetz *et al*, 2005). Negative gram bacteria especially in *E. coli* has more lipid, few peptigoglycal, bilayer as selective compounds resistence that comes in and out the cell and causes toxic effects). This is causing antibacterial compounds in sweet potato is more difficult to enter the cell. Types of solvent used can also become the factors of inhibition, if we compare between ethanol extract and chloroform extract which have weak and moderate inhibition towards n-hexane extract which has strong inhibition, because the antibacterial compound in n-hexane is predicted to be more dissolved.

The ability of Ipomoea batatas in inhibiting the growth of bacteria depends on the concentration and types of active compounds dissolved in an extract. The AC putih Kuningan's powder antibacterial extract activity is assumed because there is beneficial compound substances such as vitamin B, fat, magnesium and phenol. Accrding to a source of PT Galih Estetika (2013), substances in sweet potato with AC putih variety consits of magnesium, mangan, phosphor, fat, and vitamin B. Prescott (2002) explains that the competence of an antimicroba substance in omitting the ability of microorganism to live depends on the concentration of

antimicroba substances. The inhibition of growth is not only influenced by concentration but also types of antimicroba substances.

Antibacterial activity is also determined by work spectrum, performance, *MIC* and potency in *MIC*. An antimicroba is categorized having a high activity if *MIC* occurs in low level but has a strong inhibition (Nurlaelah, 2012: 348-362).

3.3. Anova testing Analysis

The result of ANOVA test (Table 3) shows that three AC putih Kuningan's sweet potato powder extract can inhibit the growth of *E. coli* bacteria.

Table 3 ANOVA analysis in three extracts treatments towards *E. coli*'s growth

Variety Sources	F_{hit}	F_{tab}	1. Sig. level (0.5 %)
Treatment	9.532	3.08	Significant

The following analysis test used Student- Newman-keuls model (table 4) and gained the significant result for n-hexana extract, it means that the extract concentration has shown a different effect in inhibiting the growth of *E. coli* bacteria.

Table 4. Student-Newman-Keuls

	Extract	N	Subset for alpha .05	
			1	2
Student-Newman-Keuls [a,b]	Ethanol	36	4.97	
	Chloroform	34	6.15	
	n-Heksan	36		10.11
	Sig.		.347	1.000

The table 4 reveals the significant number for ethanol and chloroform are in the same subset which indicates both extracts have the same effect, while n-hexane extract is in a second subset which has bigger number than ethanol and chloroform. It indicates that the substance of antibacterial active compound is predicted in n-hexane extract.

Conclusion

Antibacterial activity from three AC putih Kuningan extracts is revealed with highest inhibitory potency with various numbers in bacteria test (*E. coli*) generally is shown in 1000 ppm concentration from sample test except for chloroform extract is in 500 ppm concentration. The value of minimum inhibitory concentration for three extract fractions towards bacteria test is gained in 500 ppm concentration. Based on statistical analysis, it is referred that n-hexane extract has a highest antibacterial activity.

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Application of Trunk of Heart-leaved Moonseed Extract as an Intercalating Agent of DNA Visualization

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Abstract

Berberine from extract of heart-leaved moonseed trunk could interact with DNA through Hydrogen and van der Waals bonds. The purpose of this study is avoiding of Ethidium bromide as a DNA intercalating agent for visualizing DNA. The most suitable extraction method to extract Berberine from trunk of heart-leaved moonseed is ultrasonic method with Methanol : HCl (1:1). The most optimum concentration of Berberine as a loading dye component is 10 ppm and 0,05 % Eosin. Extraction method and solvent variation didn't affect to the quality of DNA bands, therefore, combination of Methanol : HCl (1:1) and maceration method shouldn't use.

Keywords: Berberine; heart-leaved moonseed; Agarose gel electrophoresis

1. Introduction

DNA fragments could be separated by electrophoresis. DNA movement depend on its size, shape and charge. One of the medium that could support the DNA separation is a gel from Agarose. The DNA fragments which has been separated could be visualized by using Ethidium bromide (EtBr) and a UV trans-illuminator. The EtBr is an intercalator which has a strong fluorescent under UV light[2]. EtBr is a strong mutagenic, carcinogenic, and teratogenic compound. Based on the research, in its picomolar (10^{-12} M) EtBr caused mutation on *E. coli*. Because of this effect, EtBr should be substituted by another materials[7].

Trunk of heart-leaved moonseed contain picroretocid, alkaloid, palmitine, berberine, columbine, and etc which has been used as a vermifuge, wound healer, anti-piretic, and has a bacteriostatic to *S. aureus* and *E. coli*. The result of mutagenic test by using Ames method of the extract of trunk of heart-leaved moonseed is negative[5]. Berberine is a traditional herbal which has biological and pharmacological activities. The binding characteristic and molecular interaction between Berberine and DNA of Herring sperm has been studied and the result is the Hydrogen bond and van der Waals force plays as the main role in the reaction, therefore, ionic effects indicate there was an electrostatic force between them[3].

Based on those background, researcher would like to use the Berberine in extract of trunk of heart-leaved moonseed as a substitute for EtBr in a DNA visualization. DNA fragments which has been separated by Agarose gel electrophoresis would inserted by Berberine so the DNA fragment could be visualized under UV light. This method has an advantage that is no mutagenic, carcinogenic, or teratogenic compound were used.

The purpose of this study were optimizing the most suitable solvent and extraction method to extract Berberine from trunk of heart-leaved moonseed, determining the optimum concentration of Berberine as an intercalating agent in Agarose gel electrophoresis, and testing the extract of trunk of heart-leaved moonseed as a non-mutagen intercalator in DNA visualization.

2. Materials and Methods

2.1 Materials

Trunk of heart-leaved moonseed, liquid Nitrogen, Methanol p.a, hydrochloric acid (HCl 1N), distilled water, Glycerol, ashes filter paper, Agarose, TAE 5x solution (contain Tris-HCl 1M (pH 8,0), Ethylen diammin tetra acetic (EDTA) 0,5M (pH 8,0 M), Acetic acid (CH_3COOH)), Eosin, Ethidium bromide, dan *GelRed.*

2.2 Method

2.2.1 Optimization of Extraction of Trunk of Heart-leaved Moonseed Based on Variation of Solvent and Extraction Method

The trunk of heart-leaved moonseed was cleaned in water. It was crushed with liquid Nitrogen until make a good powder. The powder of trunk of heart-leaved moonseed was weighed and extracted with solvents and methods based on Table 1.

Table 1. Variation of Solvents and Extraction Methods

Variation	80% Methanol[1] (P1)	Methanol : HCl (1:1)[4] (P2)	Water (P3)
Maceration (M1)	P1M1	P2M1	P3M1
Ultrasonic (M2)	P1M2	P2M2	P3M2
Heat (M3)	P1M3	P2M3	P3M3
Microwave (M4)	P1M4	P2M4	P3M4

After the extraction finished, the solutions were filtered with ashes filter paper. Filtrates were concentrated in rotary evaporator at 60 ^{o}C. Evaporates were diluted in 5 mL of water and stored in 4 ^{o}C.

Concentration of Berberine in each extract was measured by Spectrophotometer which has been developed by Pundarikshudu and Dave[6]. 1 mL of trunk of heart-leaved moonseed diluted with Methanol until 5 mL. The absorbance was measured in Spectrophotometer at 259 nm. The absorbance was compared with Berberine standard curve of 6 ppm, 7 ppm, 8 ppm, 9 ppm, 10 ppm, 11 ppm and 12 ppm.

2.2.2 Determination of the Optimum Concentration of Berberine as Loading Dye Component in DNA Visualization

The standard loading dye consist of pure Berberine, Eosin and Glycerol in TAE 1x. Those materials were mixed based on Table 2.

Table 2. Loading Dye Composition

No.	Berberine Concentration	Eosin Concentration	Glycerol
1	50 ppm	0,01 %	20 %
2	10 ppm	0,01 %	20 %
3	50 ppm	0,05 %	20 %
4	10 ppm	0,05 %	20 %

The standard loading dye was mixed with DNA by 2:5. The mixture was being electrophoresis in 1% Agarose gel. The result was visualized in UV trans-illuminator and the bands were tested to 20 panelist by organoleptic rank method for their quality. The result of this step was compared with the electrophoresis result through EtBr intercalation and Gel Red intercalation. Loading dye composition which has the best rank were used for loading dye composition in next step.

2.2.3 Application of Extract of Trunk of Heart-leaved Moonseed as a Component of Loading Dye in DNA Visualization

Determined composition of loading dye was applicated to each trunk of heart-leaved moonseed extract. Trunk of heart-leaved moonseed extract used as substitute of pure Berberine. There were 12 composition of loading dye contain trunk of heart-leaved moonseed extract. Their qualities were tested by Agarose gel electrophoresis.

The Agarose concentration was 1 %. 0,4 g Agarosedissolved in 40 mL of TAE 1x Buffer. The solution was heated in microwave. The solution was poured into the template which has been applied by suitable comb.

Loading dye was mixed with DNA by 2 : 5. The mixture was loaded into the well of Agarose. The electrophoresis was run for 30 minutes in 100 V. After that, Agarose gel was visualized in UV trans illuminator. The DNA bands were observed.

3. Result and Discussion

3.1 Optimization of Extraction of Trunk of Heart-leaved Moonseed Based on Variation of Solvent and Extraction Method

The optimizations which have been conducted based on differences of solvent, those are80 % Methanol, Methanol : HCl (1:1), dan aquadest, and 4 extraction method, those are maceration, ultrasonic, heat, and microwave.

The result of the optimization was showed in Table 3. The highest concentration of Berberine was extract P2M2, which extracted by ultrasonic in Methanol : HCl (1:1) showed the concentration of Berberine was 5337,99 ppm or 22,72 mg Berberine per 1 gram simplicia. This method was the most optimum than another.

The frequency of ultrasonic wave was 20 kHz – 10 MHz. If the ultrasonic wave through the tissue, it would heat up which results in increased tissue temperature and then create the effect of cavitation, ie the formation, growth and rupture of bubbles in a liquid. Rupture of the bubble as it approaches the surface of the tissue, causing the tissue surface resistance, but if the pressure exceeds the bubble due to fractional surface resistance tissue, the tissue will be splitted so that Berberine would be easy to get out. The combination of ultrasonic method with solvent Methanol: HCl (1: 1) provide a beneficial effect, Methanol can dissolve substances of secondary metabolites, then HCl will keep the Berberine is stable in water to form a salt (Bendicho, C. and Lavila. I, 2000).

Extraction by maceration method using distilled water was the solvent extraction technique which was less efficient to use because it only managed to extract Berberine much as 5.36 mg per 1 gram of crude drugs or just Berberine concentration in the extracts obtained was 1103.44 ppm.

Table 3. Optimization Result of Extraction of Berberine from Heart-leaved Moonseed Based on Varies of Solvents and Extraction *Methods*

No.	Code	Berberine Concentration (ppm)	Berberine Extracted (mg/g)
1	P1M1	1175,13	5,63
2	P1M1	1175,13	5,63
3	P1M2	1282,65	5,61
4	P1M2	1282,65	5,61
5	P1M3	1705,09	8,15
6	P1M3	1705,09	8,15
7	P1M4	1991,83	9,75
8	P1M4	1991,83	9,75
9	P2M1	2421,94	11,57
10	P2M1	2421,94	11,57
11	P2M2	5337,99	22,72
12	P2M2	5337,99	22,72
13	P2M3	1705,09	7,33
14	P2M3	1705,09	7,33
15	P2M4	1418,35	5,83
16	P2M4	1418,35	5,83
17	P3M1	1103,44	5,36
18	P3M1	1103,44	5,36
19	P3M2	2350,25	10,05
20	P3M2	2350,25	10,05
21	P3M3	2665,16	13,10
22	P3M3	2665,16	13,10
23	P3M4	2421,94	11,92
24	P3M4	2421,94	11,92

3.2 Determination of the Optimum Concentration of Berberine as Loading Dye Component in DNA Visualization

The results of the analysis are shown in Figure 4 aims to compare the variation of the composition of Berberine-eosin and compare the composition variation with dye that has been used previously, namely EtBr and GelRed compounds and determine the composition of loading dye to be used in the qualitative phase of loading dye test using extracts brotowali . DNA bands in lanes 1-4 are DNA bands Berberine and eosin mixed with certain variations. Lanes 5-6 are blank, lanes 7-8 is a standard dye commonly used for visualization of DNA.

DNA band in lane 4 had the 1st rank with the average value of 1.2. DNA band in lane 4 is the result of electrophoresis using a loading dye composition Berberin 10 ppm and 0.05% Eosin. It has the brightest light intensity than another lane, however, DNA separation lane is not clean. This could be due to the composition of Eosin oversized 0.05%, so in addition to color DNA band, glowing radius Eosin give enough away. Loading dye composition Berberin-Eosin in lane 4 can be used for DNA identification that has 1 DNA band, and can be used for quantitative testing. Loading dye is not necessarily for DNA samples with multiple fragments because the glowing radius can interfere with the process of observation Eosin DNA tape.

DNA band in lane 1 is ranked second with an average value of 2.0. DNA band in lane 1 is the result of electrophoresis using a loading dye with Berberine composition of 50 ppm and 0.01% eosin. The DNA band intensity is light dimmer than the DNA bands in lane 4. DNA band in this lane was cleaner compared to lane 4. Loading dye in lane 1 can be used for identification of DNA which has 1 DNA bands, but not like loading

dye in lane 4, the loading dye in lane 1 cannot be used for quantitative assay using UV transiluminator, because the light intensity is less bright. For DNA identification test with a lot of tape, loading dye in lane 1 may not be used as the radius of eosin phosphorescence still interfering.

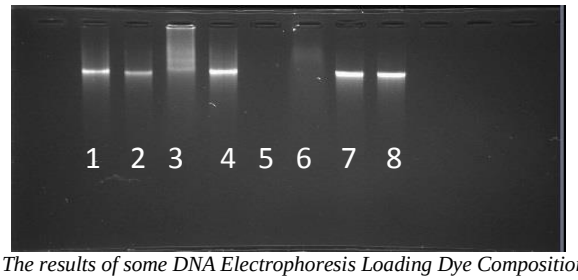

Figure 1. The results of some DNA Electrophoresis Loading Dye Composition

Specification:
Lane 1 : Berberine 50 ppm + Eosin 0,01 %
Lane 2 : Berberine10 ppm + Eosin 0,01 %
Lane 3 : Berberine 50 ppm + Eosin 0,05 %
Lane 4 : Berberine 10 ppm + Eosin 0,05 %
Lane 5 : Berberine 50 ppm + *Loading dye*
Lane 6 : Eosin 0.05% + *Loading dye*
Lane 7 : GelRed + *Loading dye*
Lane 8 : EtBr + *Loading dye*

DNA band in lane 2 is ranked third with an average value of 2.8. The DNA band has the dimmest light intensity among the other lane. DNA band in lane 2 is the result of electrophoresis using a loading dye composition Berberine 10 ppm and 0.01% eosin. Loading dye on this column can be used merely to determine whether or not the DNA in the sample, however, loading dye can be used for DNA consisting of several fragments due to lane 2 has lane cleaner separation. DNA band in lane 3 has the lowest rank compared to lanes 1, 2, and 4 with an average value of 4.0. The light intensity of DNA bands was quite bright but not a clean DNA band, so that the loading dye in this column cannot be used

Lane 5 is the result of DNA electrophoresis using 50 ppm Berberine and commercial loading dye commonly used for DNA by agarose gel electrophoresis. Lane 6 is the result of DNA electrophoresis using 0.05% eosin and with the same dye loading lane 5. Both lane showed that Berberine and eosin cannot fluoresces when used separately.

Having obtained the best DNA bands, namely lane 4, then the best DNA bands were compared with the dye used for the visualization of DNA and EtBr GelRed. A total of 15 panelists or 75% assume that the DNA bands generated by using a loading dye-eosin Berberine did not differ significantly with DNA bands generated by using EtBr staining and GelRed.

Based on survey results, 18 panelists or 90% prefer to use Berberine-eosin dye loading due to have several advantages, those are:

1. Loading dye of Berberine-eosin is safer than EtBr. Berberine-eosin dye loading does not have the nature of mutagens, teratogens or carcinogens such as EtBr. Because Berberine used is made from extracts of heart-leaved moonseed which has been through the Ames test with negative results.
2. Loading-dye of Berberine-eosin is cheaper than the GelRed and EtBr.

Utilization of loading dye Berberine-eosin still have shortcomings, namely, loading dye can not be used for various analytical purposes as well. Composition between Berberine with eosin dye loading should be set so that it can be used in accordance with the purpose of analysis to be performed.

Processing of statistical data on Table 4 shows the results that the concentration of Berberine affects the quality of DNA bands generated during the visualization process using UV trans-illuminator. The optimum concentration of Berberine in loading dye is 10 ppm and 0.05% eosin.

3.4 Application of Extract of Trunk of Heart-leaved Moonseed as a Component of Loading Dye in DNA Visualization

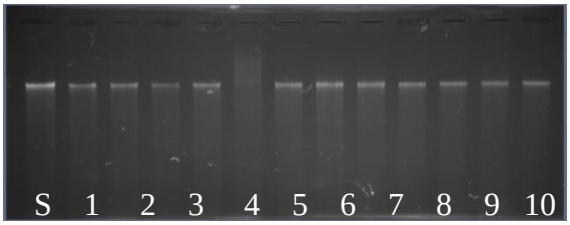

Figure 2. Electrophoresis of DNA Profiles using the Loading Dye Containing 0.05% Eosin and Trunk of Heart-leaved moonseed Extract 10 ppm

Specification:

Lane S = Standardof Berberine;
Lane 1 = P1M1; Lane 2 = P1M2;
Lane 3 = P1M3; Lane 4 = P1M4;
Lane 5 = P2M1; Lane 6 = P2M2;
Lane 7 = P2M3; Lane 8 = P2M4;
Lane 9 = P3M1; Lane 10 = P3M2;
Lane 11 = P3M3; Lane 12 = P3M4

Figure 2 is a profile of DNA electrophoresis using agarose gel loading dye with Berberine-eosin composition in lane 4 at Optimum Concentration Determination of Berberine as a Component Loading Dye, namely Berberine concentration of 10 ppm and 0.05% eosin. Berberine is used to extract brotowali replaced that have been obtained in the extraction process using 3 solvents and 4 kinds of methods

DNA bands obtained did not show significant differences. A total of 15 panelists or 75% consider the differences shown by the bands are not too different. There is a striking difference in the DNA bands in lane 5. Ribbon DNA produced very thin and dominated by eosin dye alone. DNA bands in lane 5 is the DNA bands were electrophoresed with the help of loading dye containing extracts brotowali using Methanol solvent: HCl (1: 1) by maceration method.

This is because the extraction method by maceration produce the type of polyphenol compounds are much more than the other way. Maceration causing compounds called polyphenols, which are not heat resistant participate extracted into the solvent methanol: HCl (1: 1). The polyphenol compounds will interfere with the DNA-binding process between Berberine-eosin by means of enveloped DNA and eosin Berberine so cannot interact with both optimally.

The results showed that the statistical data processing differences as well as differences in the time of extraction solvent extraction method does not provide a significant difference to the resulting DNA bands when visualization with UV trans-illuminator, however, to obtain a good DNA bands, preferably maceration method and solvent Methanol: HCl (1: 1) do not use the method of extraction.

4. Conclusion and Suggestion

4.1 Conclusion

The conclusions based on the research titled "Application of Trunk of Heart-Leaved Moonseed (*Tinospora crispa*) Extract as an Intercalating Agent of DNA Visualization" are:

1. The combination of solvents and extraction methods suitable to extract Berberine from trunk of heart-leaved moonseed is solvent extraction using methanol: HCl (1: 1) with ultrasonic method. This method can extract Berberine was 22.72 mg per 1 gram of simplicia or Berberine concentration in the extracts obtained at 5337.99 ppm.
2. The optimum concentration of dye loading Berberine as an appropriate component for visualization of the DNA was at 10 ppm with a concentration of 0.05% eosin.
3. Trunk of heart-leaved moonseed extract can be used as an ingredient in the process of DNA visualization using UV trans-illuminator with the addition of eosin. Eosin serves as an indicator dye electrophoresis and visualization. Differences solvents and extraction methods to extract Berberine from trunk of heart-leaved moonseedare not affect to the quality of the resulting DNA bands. The use of ultrasonic methods, microwave, and boiling solvent can be combined with 80% methanol, methanol: HCl (1: 1), and distilled water. Maceration method can be used with solvent methanol and 80% distilled water. Methanol solvent: HCl (1: 1) by maceration method should not be used, because the resulting DNA band is very thin.

4.2 Suggestion

The suggestions of this research are:

1. Purification or purification is necessary to extract trunk of heart-leaved moonseed in order to obtain a cleaner DNA band.
2. Further research needs to be done to determine the effect of a comparison between the number of A-T and G-C with interaction capabilities Berberin.
3. Further research needs to be done on the molecular interaction between DNA-Berberine-eosin.

Table 4 The Result of Ranking Test of Optimum Composition Berberine as a Component in the Visualization of DNA Loading Dye

Panelist	Rank			
	Lane 1	Lane 2	Lane 3	Lane 4
A	2	3	4	1
B	2	3	4	1
C	2	3	4	1
D	2	3	4	1
E	2	3	4	1
F	2	3	4	1
G	2	3	4	1
H	2	3	4	1
I	2	3	4	1
J	2	3	4	1
K	2	1	4	3
L	2	3	4	1
M	2	3	4	1
N	2	3	4	1
O	2	3	4	1
P	2	3	4	1
Q	2	1	4	3
R	2	3	4	1
S	2	3	4	1
T	2	3	4	1
Summary	40	56	80	24
Average	2.0	2.8	4.0	1.2

Specification:
Number 1 = The best DNA band; Number 2 = 2nd best DNA band; Number 3 =3rd best DNA band; Number 4 = 4th best DNA band. The best DNA band is the smallest average at all.

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Comparison Study of Three Different Polymerase Amplifying Breast Cancer HER-2 Gen For PCR-Based Diagnostic Applications Purpose

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Abstract:
Limitations number of diagnostic methods available for the breast cancer Patient service, could be one of the inhibiting factors to improve survival rate and quality of the cancer patient management. It required continuous effort in getting appropriate diagnostic methods to overcome the above problems. PCR-based diagnostic method is one way which could be developed. There were several factors which are supporting for the PCR, one of them was polymerase. There were several commercially available polymerases, each with different characteristics and superiority. This research study aims to compare three types of different polymerase in amplifying HER-2 gene. HER-2 gene is known as a cancer malignancy biomarker and as predictive factor for appropriate treatment. Three compared polymerases were from three different products. One of them is the KOD polymerase, whereas two others were Taq polymerase. Annealing temperatures were optimized for each polymerase. The results of this study showed, all of polymerase could amplified 148bp HER-2 gen, with different annealing temperatures. KOD polymerase showed optimum temperature annealing at 65^{o}C, other polymerase 55^{o}C, 60^{o}C respectively. The results of this study could serve as a source of polymerase characteristics information for HER-2 gen detections, analysis and diagnostic applications.

Key words: DNA polymerase, PCR, HER-2, Biomarkers, Diagnostics

1. Introduction

Treatment and management of patients is often not carried out properly as targeted, resulting in low life expectancy and low number survival of breast cancer patients. This can be occured due to the limitations of the diagnosis method to support those personal theraphy and medicine. Development and invention of new molecular diagnostic methods is needed as one way to overcome the above problems. According to the National Cancer Institute, the benefits of molecular diagnostics were as follows: 1. Applicable for cancer screening, 2. To provide information appropriate treatment, 3. To monitor the effectiveness of treatment, 4. To preadict a patients response to a particular drug used in theraphy. Polymerase Chain Reaction was a method that is widely used and still can be further explored for routine detection apply for public service. Based on data reported by a diagnostic companies Trovagen, (2014), the total market for molecular diagnostics range were from $ 20,2 billion on 2013, with almost half of that, $9,5 billion, is occupied by PCR.

Breast cancer took first place as a cancer type which affects to many Indonesian women patients with the incidence was 26 per 100,000 women, followed by cervical cancer was 16 per 100,000 women (hospital information system data, 2007). HER-2 or Human Epidermal Growth Factor Receptor 2 gene was a member of a group of genes that regulate cell growth. HER-2 breast cancer has a low survival rate. It is known that HER-2 overexpression were occurs in 20-30% of the total breast cancer patients (Clifford and Huddis, 2007; Pohlman et al., 2009). HER-2 overexpression would affected to increase cell division activity. In order to be active, the HER-2 protein must go through dimerization stage. Dimerization process does not only involve the extracellular domain itself, but also for the transmembrane domain. There are two motifs in the transmembrane domain of HER-2 which important for the dimerization process, Stenberg-Gullick and G *** G motifs. Point mutations in the transmembrane region also affected to the receptor dimerization stability (Fleishman et al 2002, Xie et al, 2000). Based on the protein modeling results, Fleishman et al. (2002) suggested that the dimerization of receptors on the HER-2 / Val (mutant) are stable in contrast to Ile (wild-type) whicht are not stable. Existence of valine, can stabilize the receptor active site, reducing the occurrence of endocytosis and accelerating of receptor recycling. Furthermore, HER-2 / Ile, will have an effect on reducing protein tyrosine kinases activity.

Because of its role, the HER-2 can be analyzed as a basis to find an appropriate treatment alternative for breast cancer patients. In this study a comparative study of three polymerase to amplify the transmembrane domain of HER2 for future diagnostic applications will be reported.

2. Method

Sample Collection Breast Cancer Network.

HER-2 gene was isolated from HER-2 IHC +3 breast cancer patients of Mohammad Djamil Hospital in Padang and Dharmais Cancer hospital.

Isolation of DNA genomic

The process of DNA extraction was performed using invitrogen kit. To confirm the DNA isolation result were using agarose electrophoresis performed with 1% agarose. DNA isolation results were not immediately used were stored at -20 ° C.

Optimization of PCR annealing temperature of each polymerase.

PCR process was performed using specific primers to amplify the HER-2 transmembrane region by using 3 different polymerase: Taq Polymerase I and II, KOD Polymerase. Optimization was done approximately at 45°C, 50°C, 55°C and 60°C annealing temperature for each polymerase. PCR products were confirmed in 1% agarose continue with sequensing method.

3. Results and Discussion

This study has received ethical clerance documents issued by the Ministry of Health Directorate General of Health Services Cancer Hospital Dharmais Indonesian National Cancer Center. As the genome source were acquired from breast cancer patient of the M. Djamil and Dharmais hospital. Genomic extraction were performed using Purelink mini kit from Invitrogen. 1% agarose electrophoresis results showed that cancerous tissue quite well as a source of genomic isolation. Furthermore, genomic DNA was used as a template to perform PCR.

The steps in performing the PCR can be divided into: denaturation, Primer annealing, Extension, Cycle Number, Final Extension. There are various variants of the PCR method used for diagnostic purposes. All processes must be initiated by a simple PCR for optimization of PCR conditions and the selection of the appropriate polymerase. Successful to get thick and PCR products specific to the targeted position was influenced by many things, including the polymerase used. Polymerase between one and the other has its own advantages and characteristics. In the Table 1 below, were shows the properties of the polymerase commercialized in the market.

Table 1: Properties of DNA polymerase (Takagi et al, 1997).

Property	Value for indicated DNA polymerase			
	KOD	*Pfu*	Deep Vent	*Taq*
Deduced molecular mass (kDa)	90.0	90.1	90.6	93.9
Optimum temp[a] (°C)	75	75	75	75
Optimum pH at 75°C[a]	6.5	6.5	7.5	8.0–8.5
Thermostability (half-life)[a]	95°C, 12 h; 100°C, 3.0 h	95°C, 6 h; 100°C, 2.9 h	95°C, 13.5 h; 100°C, 3.4 h	95°C, 1.6 h
3′-5′ exonuclease activity	+	+	+	–
Fidelity[b]	3.5×10^{-3}	3.9×10^{-3}	ND[c]	1.3×10^{-2}
Terminal transferase activity	–	–	–	+
Processivity (bases)[b]	>300	<20	<20	ND
Elongation rate (bases/s)[b]	106–138	25	23	61

In this study conducted PCR conditions refer to the recommendations of the commercial product supplied. PCR optimization was conducted after previous ensuring that targets band was obtained confirmed with sequencing. (Figure 1).

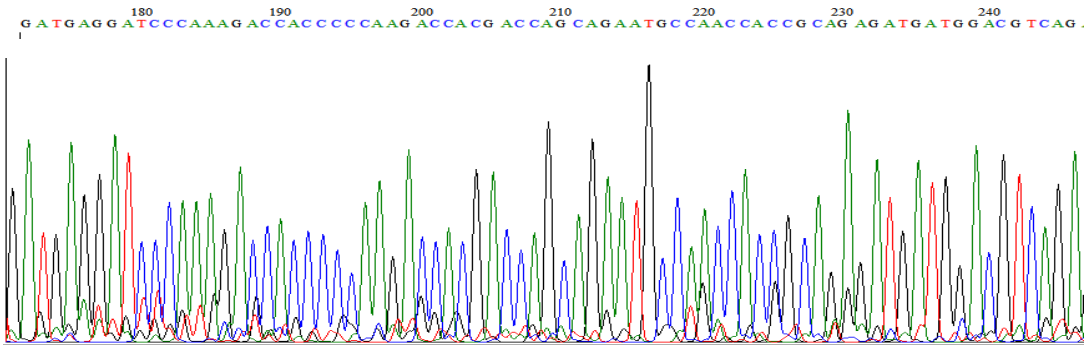
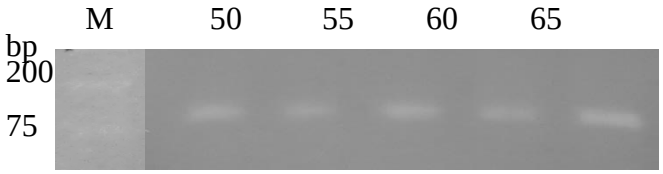


Figure 1. Sequensing of PCR target.

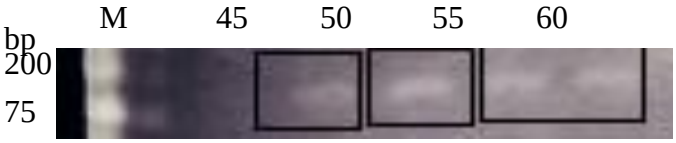
Here below can be seen the results of Three Different commercial Polymerase amplifying Breast Cancer HER-2 Gen. Observations was focused on the annealing temperature indicated by the thickness of the DNA bands.

A. Taq DNA Polymerase I



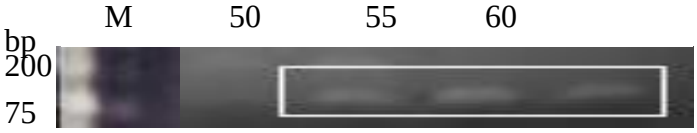
Optimal annealing temperature on the amplification of the HER2 gene encoding transmembrane domains using Taq DNA Polymerase I was 60 ° C, indicated by a single band is thicker than the other bands. As a positive control used pGEM-Teasy Her-2 to control the feasibility of PCR reagents and reactions used.

B. Taq Polymerase II



The optimum annealing temperature for Taq polymerase II activity that is used in this study was 55 ° C. There was no PCR product in the annealing temperature below 50^{o}C.

C. KOD Polimerase



KOD polymerase derived Pyrococcus sp. KOD1 strain. Compared to other polymerases, polymerase has high fidelity. Based on the results of electrophoresis on 2% agarose, thick single band can be seen at the annealing temperature of 65^{o}C, and no PCR product below 55^{o}C. While on the other annealing temperatures showed a non-specific band.

By comparing the above three types of polymerases, we can see that different Polymerase have different properties, which will give different characteristics and different pattern PCR result. The results of this study could serve as a source of polymerase characteristics information for HER-2 gen detections, analysis and diagnostic applications.

4. Conclusion

1. Different Polymerase have different properties, which will give different characteristics. KOD polymerase has an optimal annealing temperature of 60 ° C, Taq Polymerase I and Taq polymerase II shown annealing 55^{o}C, 65^{o}C for each respectively.
2. 2. The three polymerases can be used for diagnostic applications. Utilization for diagnostic applications purpose was conducted by paying attention to economic aspects in order to reduce the cost of diagnostic services.

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Peat Water Purification By Absorption With Baggase Charcoal

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Abstract

This research was aimed to use the bagasse Charcoal as an adsorbent to increase quality of peat water. Bagasse charcoal made by was carbonization at 300^{o}C for 2,5 hours and sieved 100 and 200 mesh. Absorption process was observed by contacting the bagasse charcoal with 100 ml of peat water for 30 minutes. The results of such intensity of color, turbidity and Fe metal content meets the standards PERMENKES RI "About The terms and Supervision QUALITY Water" no.416 / Menkes / PER / IX , respectively (1114 Pt-Co) (10.8 NTU), (0.148 mg / L), and (0128 mg / L) except pH.

Keywords: bagasse; carbonization; adsorption; peat water.

1. Introduction

Peat water is one of the many sources of water found in Riau because of the wide spread peat moss. Peat soil will produce peat water quality does not meet the physical requirements of the organoleptic quality of the water. Peat water has a brownish red color, high mineral salt and acidic pH in the range of 3-5. High color intensity on peat water caused by the presence of high dissolved organic matter, particularly in the form of humus acids and their derivatives[1].

Peat water has a character-specific, depending on the location, in terms of vegetation, soil types of the peat water is, the thickness of the peat, peat age, and the influence of weather[2].

If the peat water is used for prolonged consumption will have a negative impact on health. One effort that can be done to improve the water quality of the peat, which is the process of using charcoal adsorption bagasse as adsorbent. The ability of biomass bagasse adsorbent enhanced by activation of carbonization.

According to Husin [3], the chemical composition of bagasse consists of the presence of cellulose (37.65%), lignin (22.09%), pentosan (27.97%), SiO_2 (3.01%), ash (3.82 %), and the extract (1.81%). The content of cellulose and lignin, which has the potential to be converted into a carbon source that plays an important role in the adsorption process.

Bagasse as adsorbent has been used by researchers Ashabani [4] to reduce the iron content in well water. Bagasse was first activated with 0.1 M HCl adsorption efficiency reached 90.32%. Researchers Apriliani [5] have used the results of carbonization of bagasse charcoal as an adsorbent for heavy metals such as Pb, Cu, Cr, Cd with the highest adsorption efficiency respectively is 95.92%; 92.85%; 80.70%; and 58.98%. Power jerap bagasse charcoal has also been tested its effectiveness by Rinawanti [6] separately remediation magnesium, manganese, zinc, and nitrate in the leachate with efficiency, respectively for 86.50%; 80.67%; 73.86%; and 20.93%. Effectiveness based on the results of the analysis is higher than the use of bagasse fibers.

Based on previous studies, the use of bagasse charcoal to alter the characteristics of the peat water and improve water quality to be clean has never been reported. In this study, the adsorbent of bagasse charcoal was made on the condition of 300 ^{0}C temperature for 2.5 hours. The charcoal was then applied to the adsorption process of peat water..

This research studied the effect of variable adsorbent dose on the adsorption capacity of bagasse charcoal in improving peat water quality. Some water parameters analyzed, namely the intensity of color, pH, turbidity and Fe metal content.

2. Materials and methods

2.1. Chemicals

The materials used in this study were well dug peat water samples from the village of Long Rimbo, bagasse samples obtained from sugarcane juice seller in one area at the University of Riau, starch / starch, methylene blue (Merck), HNO_3 p (Merck), H_2SO_4 (Merck), HCl (Merck), KI (Merck), KIO_3 (Merck), I_2 powder (Merck), anhydrous $Na_2S_2O_3$ (Merck), Fe powder (Merck), akuabides.

2.2. Preparation Charcoal from Baggase

Samples of bagasse cleared from the husk, sugarcane dumped the rest of the existing water, washed thoroughly, drying in the sun ± 2-3 days. Further cut ± 1 cm and a blender to obtain a measure floured. The powder was sieved with a size of 100 <x <200 mesh. The powder is then heated in an oven at 105 ^{0}C until constant weight is obtained to remove the water content contained in the bagasse charcoal. The results of the sieve and then carbonized at 300 ^{0}C temperature conditions for 2.5 hours. Charcoal carbonization results are stored in a desiccator to maintain moisture.

2.3. Analytical methods for Peat Water

Adsorption process Bagasse charcoal carbonization results (condition 300 ^{0}C, 2.5 hours) with mass variations (0.5; 1; 1.5; 2) g adsorbed in 100 mL of water samples of peat. The mixture was stirred with a magnetic stirrer at a speed of 150 rpm for 30 minutes. After stirring was completed, the mixture is allowed to stand and allowed to ± 1 week so it can be completely settled. The filtrate obtained was analyzed by mass variation parameters and the peat water compared with clean water standards for the determination of the optimum absorption conditions. Results were analyzed and compared with the provisions based PERMENKES 416 / Menkes / Per / IX / 1990.

3. Result and discussion

Samples were coded according to the reaction conditions the adsorption process (Table 1) to make it easier to analyze the results of observation.

Table 1. Code each sample

Adsorben dose (gr)	Contact of time (minutes)	Code
0,5	30	$A_{0,5\text{-}30}$
1,0	30	$A_{1,0\text{-}30}$
1,5	30	$A_{1,5\text{-}30}$
2,0	30	$A_{2,0\text{-}30}$

3.1. Peat water parameters before adsorption. Before treated, peat water parameters analysis namely odor, color intensity, pH, turbidity, Fe content. Analysis of the overall parameters of peat water before adsorption process is shown in **Table 2**

Table 2. The results of the analysis of parameters of peat water samples before adsorption

Parameter	Result
color (Pt-Co)	2028
turbidity (NTU)	39,7
pH	3,79
Fe content (mg/L)	1,192

3.2. Peat water parameter analysis after adsorption

3.2.1 Intensity of Colors. Initial color of peat water before adsorption showed a high intensity of 2028 Pt-Co. Organoleptic, 11 respondents stated that the peat water reddish brown. Humic acids contribute to give a brown color. The presence of iron ions causes the water to a reddish color. Figure 1 shows the intensity of colors in the peat water after adsorption.

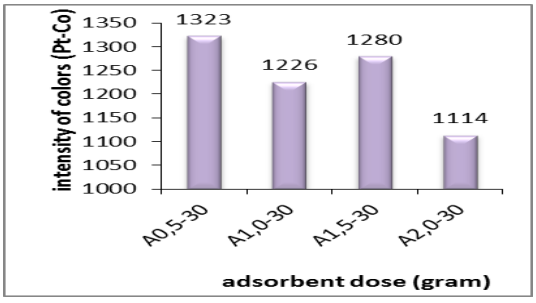

Figure 1. The intensity of the color of peat water after adsorption at each reaction condition (clean water standards: 50 Pt-Co).

Mass variations also affect the decrease in intensity of color. It can be seen that the removal efficiency of each parameter is obtained by using the highest mass of bagasse charcoal as much as 2.0 grams. The more doses of the adsorbent, the surface area will be increased so that the availability of active sites in the pores of the adsorbent more thus allowing an increase in absorption.

3.2.2. pH. The presence of organic acids were dispersed in peat of water , cause acidic pH tended . The results of the analysis of the peat water pH after adsorption can be seen in Figure 2.

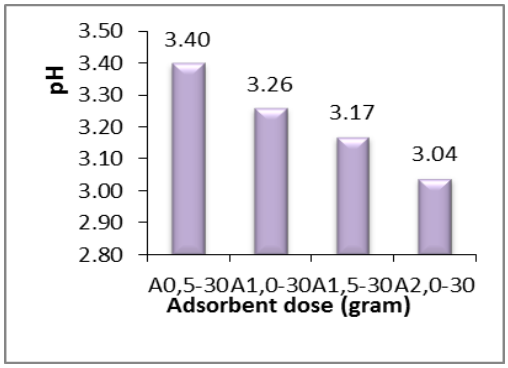

Figure 2. The pH value of peat water after adsorption (initial pH: 3.79; standard clean water from 6.5 to 9.0)

The overall pH of the peat water adsorption results do not meet clean water standards are set. The lower the pH value is affected by the acid sites on the surface of solids bagasse charcoal.

3.2.3.Turbidity The intensity of the initial turbidity of 39.7 NTU peat water. Turbidity is caused due to the presence of suspended particulates in the water in the form of organic matter decomposed peat [7]. The results of the turbidity parameter analysis of water samples of peat after the adsorption process can be seen in Figure 3.

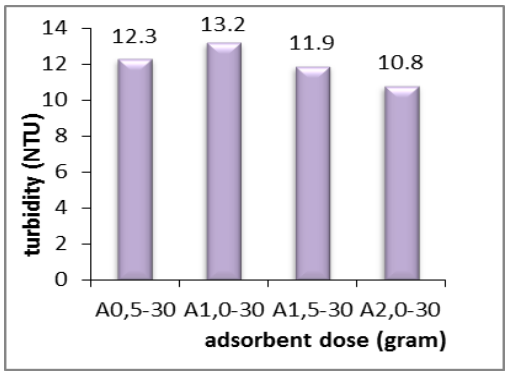

Figure 3. The value of peat after adsorption of water turbidity (25 NTU standard clean water)

Decrease in turbidity caused by peat adsorbed contaminants in water due to the pull of bagasse charcoal surface is stronger than the strong power hold in solution. The compounds are easily adsorbed usually has a smaller solubility values of adsorbent [8]. If the amount of particulates in the peat water decreases, the scattered light will be less, so that the turbidity decreased and the water will look clear as more and more light is transmitted

.

3.2.4 Fe metal content. The presence of iron ions give the water a reddish color in the peat. The iron content before adsorption of 1.192 mg / L. The results of the analysis of the iron content in the peat water after adsorption can be seen in Figure 4. In Figure 4, adsorption at the contact time of 30 minutes at a dose of 2 grams (A2,0-30) with residual Fe content in the sample is 0.148 mg / L. The longer the contact time, the more opportunity for bagasse charcoal particles in contact with ferrous metals that are bound in the pores of the charcoal. Long contact time between the adsorbate with the adsorbent allows more bonds are formed between the particles of charcoal with iron metal. Selection of contact time and dosage of the best views of the effectiveness of bagasse charcoal in reducing the iron content in water samples of peat [4].

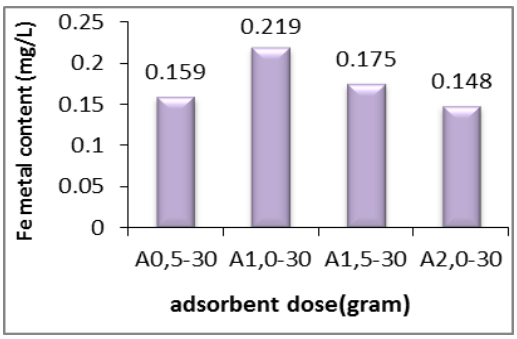

Figure 4. Analysis of iron in water samples of peat (initial iron content; 1.192 mg / L); water standard of 1.0 mg / L

The results of the analysis indicate the color of each sample not meet clean water requirements, just decrease the color intensity of each sample. Charcoal is considered able to reduce the intensity of the color of the water early 2028 Pt-Co. is A2,0-30 (Pt-Co 1114). In the analysis of parameters of turbidity, and the metal content of Fe, indicating overall bagasse charcoal adsorbent dose variation (A0,5-30; A1,0-30; A1,5-30; A2,0-30) qualified water below the threshold which have been set while the pH of the sample as a whole there is no eligible. Increasingly low pH value is influenced by the pH of acidic peat water, but it is affected by the acid sites on the solid surface of bagasse charcoal

4. Conclusion

Charcoal bagasse has been created through the process of carbonization. After the adsorption process, the variation of the dose at 30 minutes contact time were affecting each parameter were observed. Overall results of the analysis of turbidity and iron content of peat water after adsorption meet the standards prescribed by PERMENKES 416 / Menkes / Per / IX / 1990. For color intensity and pH parameters not meet the standards.

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