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10th Joint Conference on Chemistry

To cite this article: 2016 *IOP Conf. Ser.: Mater. Sci. Eng.* **107** 011001

IOP Conf. Series: Materials Science and Engineering **107** (2016) 011001 doi:10.1088/1757-899X/107/1/011001

Editorial Materials (preface and photograph)

PREFACE

The 10th Joint Conference on Chemistry is an international conference organized by 4 chemistry departments of 4 universities in central java, Indonesia. The universities are Sebelas Maret University, Diponegoro University, Semarang State University and Soedirman University. The venue was at Solo, Indonesia, at September 8-9, 2015. The total conference participants are 133 including the invited speakers. The conference emphasized the multidisciplinary chemical issue and impact of today's sustainable chemistry which covering the following topics:

- Material innovation for sustainable goals
- Development of renewable and sustainable energy based on chemistry
- New drug design, experimental and theoretical methods
- Green synthesis and characterization of material (from molecule to functionalized materials)
- Catalysis as core technology in industry
- Natural product isolation and optimization

The conference speakers are:

Prof. Takuji Ogawa

Single molecular electronic devices and their integration, Physical Organic Chemistry, Osaka University

Osaka University, Japan.

Research

Prof. Dr. Evamarie. Hey-Hawkins

Catalysis- A powerful means for sustainable developments, Institute of Inorganic Chemistry

Leipzig University, Germany.

Research

Prof. Masaaki Nagatsu

Plasma science and technology, Shizuoka University

Shizuoka University, Japan.

Research

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Prof. Santiago Gómez Ruiz

In search of novel sustainable systems based on nanostructured materials for different catalytic applications.",
Rey Juan Carlos University,
Móstoles (Madrid), Spain
Research

Dr. Henning Störz

Current State and Future Prospects Including Monomer Preparation from Biomass
Institute of Agriculture Technology,
Johann Heinrich Von Thuenen Institute.
Braunschweig Germany.

Dr. Leny Yuliati

Photocatalytic hydrogen production,
Ibnu Sina Institute
University Teknologi Malaysia.
Research

Prof. Effendy, PhD

Molecular Structure Analysis by Single X-ray Diffraction Method,

Dr.rer.nat Hery.Haerudin

Catalytic and sustainable Processes in PERTAMINA.

Dr. Hasanudin Abdurakhman

Advanced Material Research in Toray,
Toray Foundation

Dr. Fitria Rahmawati

Materials for Solid Electrolytes,
Sebelas Maret University
. Research1 Research2

Peer review statement

IOP Conf. Series: Materials Science and Engineering **107** (2016) 011002 doi:10.1088/1757-899X/107/1/011002

Peer review statement

All papers published in this volume of *IOP Conference Series: Materials Science and Engineering* have been peer reviewed through processes administered by the proceedings Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

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Accepted papers received: 21 December 2015

Published online: 05 February 2016

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Enviromental Chemistry

PAPER • **OPEN ACCESS**

Alginate cryogel based glucose biosensor

To cite this article: Amin Fatoni *et al* 2016 *IOP Conf. Ser.: Mater. Sci. Eng.* **107** 012010

IOP Conf. Series: Materials Science and Engineering **107** (2016) 012010 doi:10.1088/1757-899X/107/1/012010

Alginate cryogel based glucose biosensor

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Abstract. Cryogel is macroporous structure provides a large surface area for biomolecule immobilization. In this work, an alginate cryogel based biosensor was developed to detect glucose. The cryogel was prepared using alginate cross-linked by calcium chloride under sub-zero temperature. This porous structure was growth in a 100 µL micropipette tip with a glucose oxidase enzyme entrapped inside the cryogel. The glucose detection was based on the colour change of redox indicator, potassium permanganate, by the hydrogen peroxide resulted from the conversion of glucose. The result showed a porous structure of alginate cryogel with pores diameter of 20-50 µm. The developed glucose biosensor was showed a linear response in the glucose detection from 1.0 to 5.0 mM with a regression of y = 0.01x+0.02 and R^2 of 0.994. Furthermore, the glucose biosensor was showed a high operational stability up to 10 times of uninterrupted glucose detections.

1. Introduction

The biosensor is an analytical device consist of the biological sensing element and a transducer which convert the biological recognition into a measurable output signal [1]. The advantages of the biosensor such as high selectivity, low-cost, high sensitivity, miniaturization and real-time measurement ability, stimulates researchers to develop this method continuously. One of the strategies reported to improve the biosensor performances is increase the sensitivity and stability, such as the use of cryogel, porous materials, for supporting material in the biosensor development [2].

Chitosan cryogel based biosensors have been reported in order to improve the biosensor performances mainly their sensitivity and stability in the biosensor development to detect glucose [3], sialic acid [4] and microalbumin [5]. However, those previous cryogel based glucose biosensor developments were used electrochemical detection with some disadvantages such as complex electrode preparation and relatively use an expensive instrument. The use of lower cost detection systems has been reported such as cheap potentiostat [6], pocket camera, mobile phone camera [7] and commercial scanner [8].The use of color change detection system provide some advantages such as simple instrument, easy to prepare a standard solution and the color change sometimes can be easily detected by naked eyes [9].

In this work, the advantages of cryogel were used to prepare glucose biosensor using an alginate as polymer backbone. The biosensor was built by developing cryogel in a micropipette tip, thus, easy to operate by sucking and pumping the sample solution. Glucose oxidase enzyme was used as the biological sensing element and a redox indicator was use to record the resulting hydrogen peroxide, which was finally its color change detected by a spectrophotometer.

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2. Materials and Methods

2.1. Materials
Glucose oxidase (GOD) (EC 1.1.3.4, Type II-S, 15-50 unit mg^{-1}) were procured from Sigma (Steinheim, Germany). Commercial sodium alginate, calcium chloride and D-(+)-glucose anhydrous (≥ 98.0%), potassium permanganate, hydrogen peroxide, sodium dihydrogen o-phosphate and disodium hydrogen o-phosphate were from Merck (Germany).

2.2. Apparatus and measurements
Scanning electron microscopy (SEM) Table Top TM3000 (Hitachi, Japan) was used to characterize the morphology of the alginate cryogel. The sample colour change was measured using a Shimadzu Biospec 1601 UV-Vis spectrophotometer (Shimadzu, Japan). The alginate cryogel biosensor preparation and measurement were used a 10-100 μL micropipette (Eppendorf, Germany).

2.3. Cryogel Preparation
Alginate solution was prepared by dissolving 2.0 g sodium alginate with sodium acetate buffer (50 mM, pH of 4.5) to make 100 mL solutions. The alginate solution of 100 μL was then placed in the microcentrifuge tube to prepare the cryogel. The glucose oxidase (GOD) enzyme of 35 U was then added to the alginate solution, added 12 μL of calcium chloride solution (5% w/v in distilled water) and mixed immediately. A 100 μL micropipette tip was used to support this biosensor system by filling this tip using the alginate-GOD-calcium chloride solution of 100 μL and a 0.5 mm diameter stainless rod as core template. This micropipette tip was then kept in the freezer (-20 ^{0}C) for 6 h to allow cryogelation. The alginate-GOD cryogel was thawed in the refrigerator (4 ^{0}C) for 1 h. The brief of the biosensor fabrication was described in Figure 1. The porous structure of alginate cryogel was then characterized using a scanning electron microscope (SEM). The pore size of the cryogel was measured using a freeware of ImageJ (http://imagej.nih.gov/ij/).

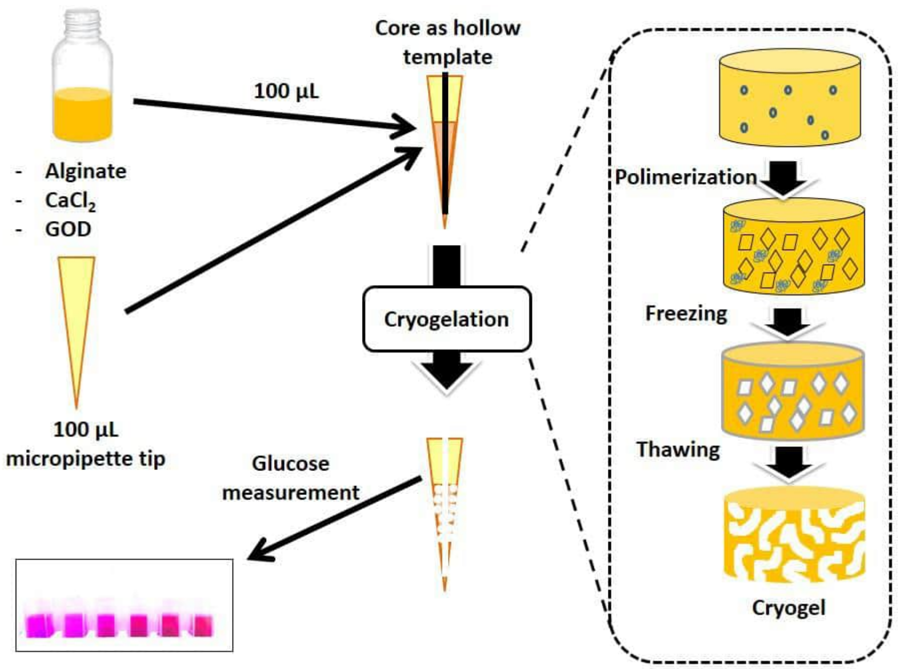

Figure 1. Alginate-GOD cryogel glucose biosensor preparation

2.4. Glucose detection
The glucose detection was based on the redox indicator colour change by the resulted hydrogen peroxide as an enzymatic product of glucose catalysed with glucose oxidase. The redox indicator used was potassium permanganate in appropriate concentration (0.1 mM). The indicator of 2.5 mL was placed in a test tube and then added a 100 µL of sample. The first indicator colour change was studied using a standard solution of 1.0 to 5.0 hydrogen peroxide. Then the biosensor measurement was performed by filling the in-tip cryogel biosensor with 100 µL of glucose sample, allowed the enzymatic reaction for 2 minutes and the reaction product was dropped to the 2.5 ml of redox indicator. The filling and releasing of the sample – product in the biosensor was controlled by micropipette sucking and pumping. The redox indicator colour change by hydrogen peroxide or enzymatic glucose product was recorded using a spectrophotometer UV-Vis. The relation between glucose concentration and the colour change was then plotted in the regression line to get the linear equation of $y = ax + b$.

2.5. Stability study
The stability study was performed by measuring glucose solution at certain concentration uninterrupted, similar to the glucose detection procedure above. The stability was determined by the biosensor response given for more than 90% in a series measurements.

3. Results and Discussion

3.1. Alginate cryogel
Cryogel is one of the interesting porous material, especially due to its large surface area, thus, enhance the biosensor performance when it was used as supporting material in the biosensor development [2-4]. The various polymer can be used to prepare cryogel such as polyvinyl alcohol, chitosan and alginate. In this study, the cryogel as biosensor supporting material was prepared using alginate as polymer backbone. The crosslinking of sodium alginate was by divalent calcium cations of calcium chloride. Native sodium alginate has a functional group of –COONa, which in the aqueous solution became $–COO^-$ and Na^+. The calcium ions of calcium chloride replace the sodium ion in the polymer, each calcium ion can attach two polymer strands. This cross-linking process allows to prepare alginate hydrogel in the room temperature. The cryogel preparation or cryogelation was prepared by crosslinking of the sodium alginate at subzero temperatures, which allowed create hydrogels with large interconnected pores [10]. In this process, the reactants remain in the unfrozen phases and form a cross-linked network upon polymerization, while the ice crystals nucleated from the aqueous phase during freezing, act as pyrogens. The interconnected macroporous networks were formed when the ice crystal melt at a temperature above the freezing temperature of water. Scanning electron microscope image showed a porous structure of the cryogel (Figure 2) with pores size diameter of 20-50 µm.

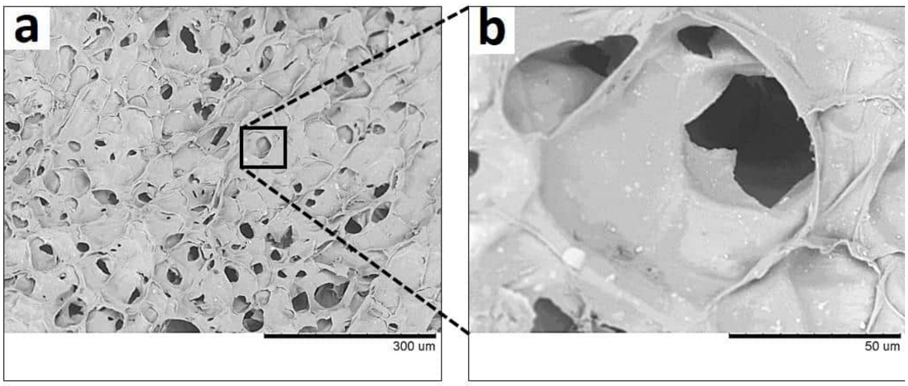

Figure 2. SEM of alginate cryogel surface at x250 (a) and x1500 (b).

3.2. Glucose detection

Glucose detection of the fabricated biosensor was based on potassium permanganate color change by resulted hydrogen peroxide according to the equation (1).

$$H_2O_2 + Mn^{7+}_{(purple)} \rightarrow H_2O + O_2 + Mn^{2+}_{(pale\ yellow)} \quad (1)$$

Furthermore, the color change with the increasing glucose concentration was studied in the range of 1.0 to 5.0 mM. The result showed a good relation (Figure 3) between the color changes of the indicator with the glucose concentration with R^2 of 0.994. The use of potassium permanganate as an indicator was used for the model in this preliminary study to verify the work of the fabricated glucose biosensor since the potassium permanganate was not specific reduce by hydrogen peroxide only. In the real glucose biosensor application, such for glucose detection in the blood, where there are many biological interferences, the specific hydrogen peroxide indicator should be used such as silver nano prism [11], 4-nitrophenyl boronic acid [12] and titanium oxy sulphate [13].

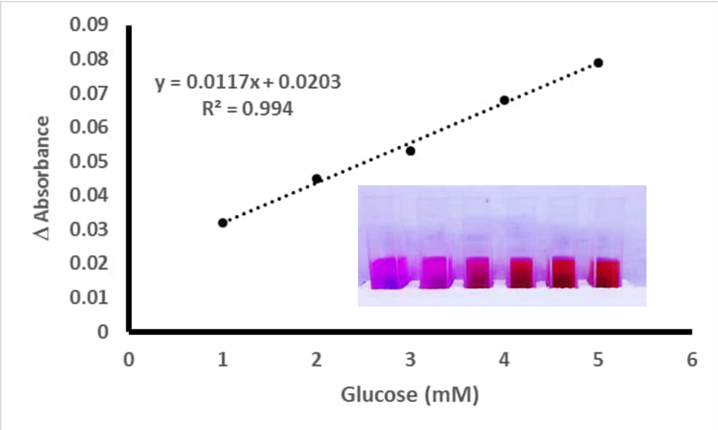

Figure 3. Calibration curve of fabricated alginate cryogel based glucose biosensor at a series glucose concentrations(1.0 – 5.0 mM). Inset, the example of redox indicator colour change by the resulted hydrogen peroxide as glucose conversion by glucose oxidase.

3.3. Operational stability study

The advantages of cryogel in the biosensor development were its ability to hold the enzyme activity, thus, resulted in a high stable biosensor [3, 4]. This was the main purpose of the use of cryogel as biological sensing element supporting material in the biosensor fabrication. However, different polymer material to build the cryogel may result in various stability profile to hold the biological element, due to their nature characteristic, for example, biomaterials usually more biocompatible compare to synthetic material. In this work, the alginate cryogel performance to support the glucose oxidase immobilization was studied by uninterrupted measurement of 3.0 mM glucose solution. The results showed the fabricated biosensor was stable up to 10 times analysis (Figure 4) without significantly lost its activity.

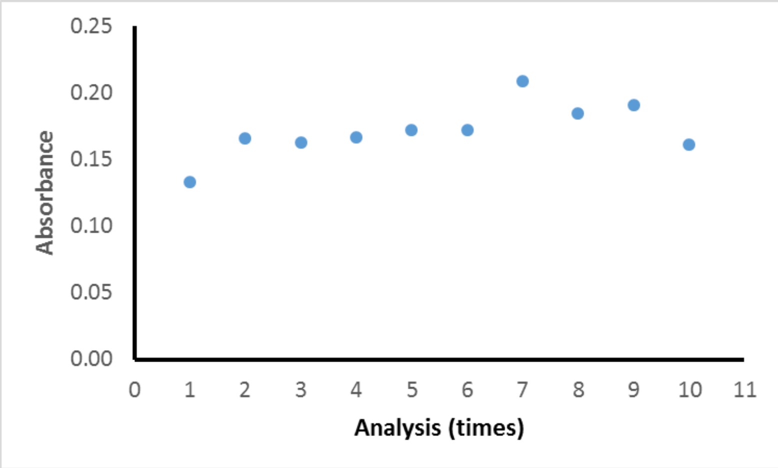

Figure 4. Stability study of fabricated glucose biosensor by measuring uninterrupted analysis of 3.0 mM glucose solution.

4. Conclusion

The alginate cryogel as a supporting material in the developed glucose biosensor showed a porous structure, thus, provide a large surface area for glucose oxidase immobilization. The developed glucose biosensor based on alginate cryogel showed a good linear range in the glucose detection, with an operational stability up to 10 times uninterrupted analysis without significant responses decreasing. This simple alginate cryogel based glucose biosensor with colorimetric detection would be an excellent model for other biosensor application.

Acknowledgement

We would like to thank the Directorate General of Higher Education (DGHE, DIKTI) and the Jenderal Soedirman University for supporting this research through “Riset Unggulan” grant. We also like to thank Fauziyah Sri P and Makhmudita Eka S for their helping in this research.

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United Kingdom

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SUBJECT AREA AND CATEGORY

Engineering
- Engineering (miscellaneous)

Materials Science
- Materials Science (miscellaneous)

PUBLISHER

IOP Publishing Ltd.

H-INDEX

48

PUBLICATION TYPE

Conferences and Proceedings

ISSN

17578981, 1757899X

COVERAGE

2009-2021

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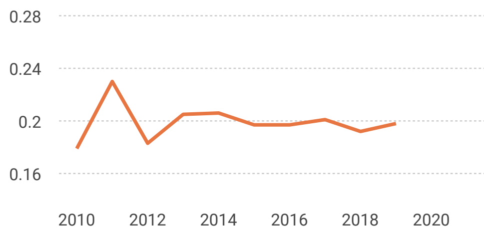

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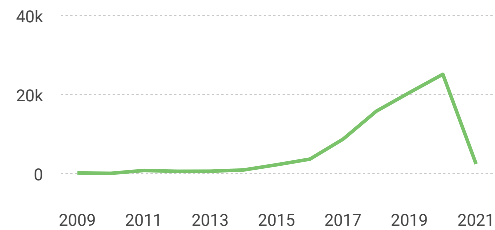

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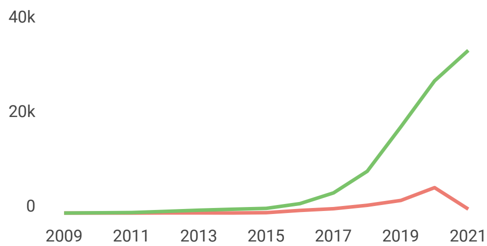

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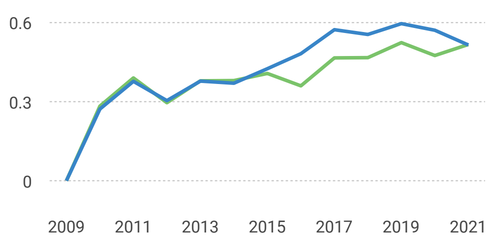

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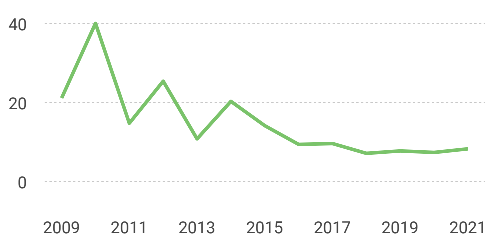

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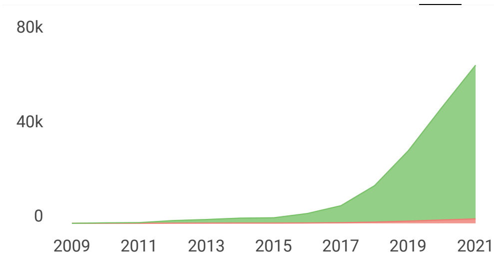

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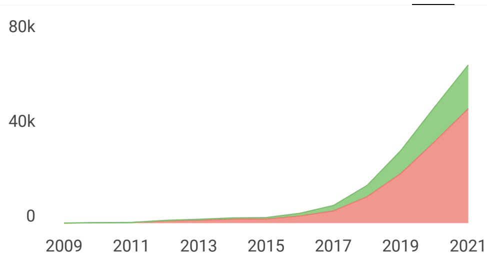

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