# Design of Pesticide Biosensor Using Glutaraldehyde Crosslinked-Cellulose Acetate Membrane in Gold Electrode

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***Abstract*** **-** This paper reports on the development of pesticide biosensor using membrane of cellulose acetate and glutaraldehyde in gold electrode analyzed potentiometrically has been committed. The aim of research is to get composition of cellulose acetate that gives optimal work as supporting media of immobilization of acetylcholinesterase (AChE) enzyme. This biosensor design is used for analysis of pesticide chlorpyrifos and getting optimization level of biosensor by doing work test that includes: Nernst factor (sensitivity), measurement range, and detection limit. The biosensor electrode is designed by AChE enzyme immobilized in gold electrode (Au) layered with cellulose acetate membrane with composition variation of 5%, 15% and 25% of glutaraldehyde. Potentiometrical analysis result of research shows that biosensor electrode with composition of cellulose acetate 15% and glutaraldehyde 25% gives most optimal work in analysis of chlorpyrifos pesticide with highest Nernst factor (sensitivity) value of 23.18 mV/decade. Measurement range for all compositions of biosensor electrode membrane exhibit same value, i.e. 1ppm - $10^{-6}$ ppm. The design result of biosensor consisted of glutaraldehyde crosslinked-cellulose acetate membrane in gold electrode can be recommended for detecting residue of chlorpyrifos pesticide in detection limit of $10^{-6}$ ppm or $10^{-3}$ ppb.

***Keywords*****:** Acetylcholinesterase, biosensor, cellulose acetate, pesticide.

## I. Introduction

Indonesia is one of the countries that undergoes rapid development in agriculture. Agriculture development cannot be separated from control of plant pests. Usage of pesticide in agriculture is intended to control plant pests. Moreover, the usage can cause negative effect if the residue remains in foodstuff, it will be dangerous if consumed by human. Chlorpyrifos is one of insecticide from group of organophosphate pesticide. Mean while compounds of organophosphate and carbamate pesticide are characterized to inhibit *cholinesterase* enzyme, i.e. enzyme that plays a role in continuing nerve stimulus. Poisoning can happen due to disturbance of nerve function that will cause death [1,2,3,4].

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Thus, since the mentioned above determination of pesticide residue owing to its potential toxicity has become an important needed to performance. Here, one of equipment can be developed to analysis of chlorpyrifos pesticide residue is enzyme-based biosensor.

According to several reported paper, recently enzyme-based biosensor in membrane electrode is designed to detect pesticide is based on measurement of enzyme inhibition [5]. The biosensor based on enzyme inhibition principal has been used widely to detect an analyte, such as components of pesticide and heavy metals [6]. This system selection is also based on fact that the toxic analyte inhibits normal function of enzyme, and give an analytical response. Generally, development of this biosensing system features quantitative measurement in enzyme activity before and after being reacted or being contacted with a target analyte [7,8,9,10].

Nowadays acetylcholinesterase (AChE) is an enzyme that is used in biosensor to provide a simple, fast analysis procedure [11]. Development of pesticide biosensor based on acetylcholinesterase and its enzyme can be immobilized in a matrix with various techniques, such as adsorption, entrapment, covalent bond, *cross linking* and encapsulation. Ability of biosensor made with method of enzyme immobilization is very influenced by technique of immobilization and types of supporting material or selected matrix. Vikas and Pundir fabricated cholesterol biosensor by using cellulose acetate membrane as supporting material in electrode of platinum (Pt) and obtained value of response time in 30 seconds. It was also shown that cellulose acetate membrane has good performance as supporting material in design of a biosensor [12].

Therefore, in fabricated of biosensor, cellulose acetate and glutaraldehyde are used as supporting material in enzyme immobilization with *cross linking* technique. On the other hand, membrane of cellulose acetate has good stability to various chemical substances, good mechanical strength so that it can resist to selective, high pressure and can inhibit very small materials, while glutaraldehyde is selected because it has an action capability as *cross linking agent* and a bond regulator between covalent molecules with polymer chain. Based on literature study, then in this research enzymatic biosensor was designed with variation of cellulose acetate

membrane as supporting material to analyze pesticide of chlorpyrifos.

## II. Experimental Section

### A. Materials and Equipment

Materials used are gold wire, bronze wire, silver wire, tin wire, acetylcholinesterase (AChE) enzyme (EC. 3.1.1.7.) purchased from Sigma Aldrich , cellulose acetate (CA) Mr=61,000 g/mol, glutaraldehyde (GA), acetylthiocholine chloride (ATCh) Mr=197.73 g/mol, chlorpyrifos pesticide, $NaH_2PO_4.H_2O$, $Na_2HPO_4.12H_2O$, aceton p.a, ethanol p.a, potassium chloride (KCl) p.a, aquades, parafilm.

Equipments used are a pH meter Orion Model 710A/potentiometer, digital multimeter Model : UX-837TR, a magnetic stirrer, platinum wire, battery, an oven, an analytical scale, a stopwatch, a solder, a freezer, blue tape, and laboratory glasses.

### B. Methods

**1. Preparation of Buffer Solution of Phosphate pH 8.0**

Phosphate buffer solution with pH of 8.0 was prepared by mixing 140 mL of solution of $NaH_2PO_4.H_2O$ 0.2 M (solution A) and 20 mL of solution of $Na_2HPO_4.12H_2O$ 0.2 M (solution B) into volumetric flask of 1000 mL and aquades was added till the mark.

**2. Preparation Solution of KCl 1 M and KCl 0.1 M**

7.445 gram of KCl was weighed, and then dissolved with aquades in volumetric flask of 100 mL, and then aquades was added till reaching the mark. To this solution, then dilution was committed in concentration of 0.1 M by diluting 10 mL of KCl 1 M with aquades.

**3. Preparation Inhibitor Solution of Chlorpyrifos Pesticide**

Standard solution of chlorpyrifos pesticide 10 mg/L was made by diluting main solution of chlorpyrifos 100 mg/L as much as 10 mL with ethanol into volumetric flask of 100 mL until the mark, then dilution was committed in concentration of 1 ppm – $1\times10^{-6}$ ppm.

**4. Preparation Standard Solution Substrate of Acetylthiocholine chloride (ATCh)**

Solution of acetylthiocholine chlorida $1\times10^{-1}$ M was made by solving substrate of acetylthiocholine chloride with buffer phosphate pH 8.0 then it was inserted into volumetric flask of 100 ml and added with phosphate solution . To this solution, then dilution was committed in concentration of $1\times10^{-3}$ M.

**5. Preparation Cellulose Acetate (CA) Dope Solution**

Dope solution cellulose acetate 5% and 15% was made by weighing each 0.5 g and 1.5 g of cellulose acetate then each of them was solved into 10 mL of acetone.

**6. Preparation Solution of Acetylcholinesterase (AChE) Enzyme**

Solution of acetylcholinesterase enzyme was made by solving its enzyme with 9 mL of buffer solution of phosphate pH 8.0 and 1 mL of acetone.

**7. Preparation Standard Electrode of Ag/AgCl**

First step, design of comparator electrode of Ag/AgCl consists of making wire of Ag/AgCl and installation of comparator electrode of Ag/AgCl. Making wire of Ag/AgCl was committed by electrolyzing wire of silver (Ag) with solution of KCl 0.1 M for ± 30 minutes. Wire of platinum (Pt) and silver (Ag), each has length of 5 cm and a battery was prepared. Wire of silver was electrolyzed until the wire was layered by AgCl, which was marked with color change of the wire to be black greyish.

Next, wire of Ag/AgCl that had been formed was inserted into electrode body (tip) to make comparator electrode of Ag/AgCl. In the electrode body, comparative solution of KCl 0.1 M was inserted sufficiently until wire of Ag/AgCl was really dowsed and ensured that there was no air bubble and in edge of the electrode, a parafilm was lifted. The edge of electrode body had been clogged using silica gel to prevent solution penetration out when being used.

Then, wire of Ag/AgCl was inserted into electrode body (tip) which, in it, solution of KCl 0.1 M was inserted suficiently until wire of Ag/AgCl was really dowsed and ensured that there was no air bubble and in edge of the electrode, a parafilm was lifted. The edge of previous electrode body had been clogged by using silica gel.

**8. Design of Biosensor Electrode in Type of Layered Wire**

Body of biosensor electrode was made from layered copper wire with size of 7 cm and diameter of 1 cm which was connected with wire of gold (Au) with size of 2.5 cm and diameter of 0.4 mm then it was connected using wire of tin. Then it was inserted into tip with position of gold wire was protruded out 1.5 cm.

After that, body of electrode was made by connecting gold wire with copper wire by being connected using tin wire. Copper wire functions as current conductor. Then, the wire was inserted into tip and parafilm was lifted as adhesive of tip with wire of Cu and Au. In each electrode, parafilm plastic was lifted as adhesive of blue tip with wire of Cu and Au.

**9. Preparation Biosensor Membrane**

Body of electrode that had been made, in its edge, it was dowsed into membrane material. Edge of electrode body, i.e. gold wire, was dowsed in homogen solution of membrane material cellulose acetate (5% and 15%). After layering of cellulose acetate was made, electrode was rinsed with aquades and in part of Au wire that had been layered with membrane of cellulose acetate was soaked in solution of glutaraldehyde 25% for 6 hours. There after layer of cellulose acetate that contained glutaraldehyde was formed, electrode was rinsed with aquades and buffer phosphate then electrode membrane (Em) was formed, and then Em was soaked in enzyme Acetylcholinesterase (AChE) for 48 hours. Before used,

electrode (Em) was kept being dowsed into buffer phospate pH 8.0 in temperature of 4°C.

**10. Working Test of Biosensor**

Potential measurement of enzymatic electrode biosensor with inhibitor of pesticide chlorpyrifos was committed by dowsing electrode biosensor that had been conducted into buffer solution of phosphate pH 8.0 for 10 minutes before used, the electrode biosensor was used to measure potential substrate of acetyltiocholine chloride with concentration of $10^{-3}$ M. After a constant response was obtained, then the electrode was lifted and rinse with aquades, after that the electrode was soaked into solution of pesticide $10^{-6}$ ppm for 30 minutes, next the electrode was again inserted into substrate solution of acetylthiocholine chloride $10^{-3}$ M.

After a constant response was obtained, the electroda was lifted and dowsed in buffer of phosphate pH 8.0 for then the same treatment was carried out in concentration variation of pesticide chlorpyrifos $10^{-6}$ ppm, $10^{-5}$ ppm, $10^{-4}$ ppm, $10^{-3}$ ppm, $10^{-2}$ ppm, $10^{-1}$ ppm and 1 ppm. After a constant response was obtained, it was noted as value of potential biosensor.

The designed biosensor performance was determined through: range of working concentration, Nernst factor (sensitivity), and detection limit.

Working test of pesticide biosensor was determined with composition difference of membrane of SA and GA as supporting media in potentiometrical analysis of chlorpyrifos pesticide with various concentrations, beginning from $10^{-6}$ ppm–1 ppm (Figure 1).

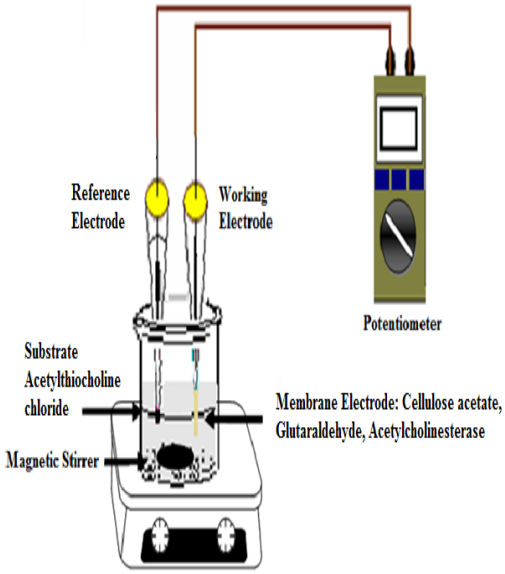


Fig. 1 Analysis design of biosensor electrode

## III. Result And Discussion

First let us demonstrate working test of biosensor was based on inhibition reaction of enzyme AChE by inhibitor of chlorpyrifos pesticide in substrate hidrolysis of acetylthiocholine to thiocholine and acetic acid [13,14]. The existence of chlorpyrifos pesticide as inhibitor caused reaction of phosphorylation, i.e. addition of phosphate group from chlorpyriphos into active side of enzyme AChE (for example, serine amino acid) so that the catalytic activity of enzyme in substrate hydrolysis of acetylthiocholine (ATCh) was decrease. The enzymatic hydrolysis of these substrates produces electroactive thiocholine.

**11. Measurement Range, Nernst Factor and Detection Limit**

The basic element of this chlorpyrifos pesticide biosensor was a gold electrode modified using membrane of glutaraldehyde and cellulose acetate. The immobilized acetylcholinesterase enzyme layer was formed by glutaraldehyde cross linked with cellulose acetate. The response characteristics of the biosensor were measured and discussed. Measurement range is concentration range of analysis solution. Determining value of Nernst factor is needed to know sensitivity of biosensor, detection limit is the smallest concentration of an analyte that can be detected in analysis process. Measurement range was obtained from linearity by looking at the relation of -log [chlorpyrifos] with potential value and this measurement range data will be used next for test work of biosensor pesticide chlorpyrifos. Value of sensitivity can be seen from Nernst factor value of 29.6 mV/decade in temperature of 24°C ± 1°C. It happened because oxidation of acetylthiocholine involved two electrons.

Table I and Figure 2 showed measurement range, sensitivity value and detection limit of biosensor with membrane composition of CA 5% and GA 25% (A).

TABLE I
Range of working concentration and Nernst factor biosensor for the composition of membrane CA 5%, GA 25% (A)

| No | Substrate concentration [acetylthiocholine chloride] M | Inhibitor concentration [chlorpyrifos] ppm | Potential (mV) |
|---|---|---|---|
| 1 | $10^{-3}$ | 1 | 80.5 |
| 2 | $10^{-3}$ | $10^{-1}$ | 85.9 |
| 3 | $10^{-3}$ | $10^{-2}$ | 96.4 |
| 4 | $10^{-3}$ | $10^{-3}$ | 119.5 |
| 5 | $10^{-3}$ | $10^{-4}$ | 125.8 |
| 6 | $10^{-3}$ | $10^{-5}$ | 136.2 |
| 7 | $10^{-3}$ | $10^{-6}$ | 144.1 |
| Nernst factor (Slope) | | 11.45 mV | |
| R (coefficient of correlation) | | 0.973 | |
| $E^0$ (K) | | 78.25 | |
| Range of working concentration | | 1 ppm – $10^{-6}$ ppm | |

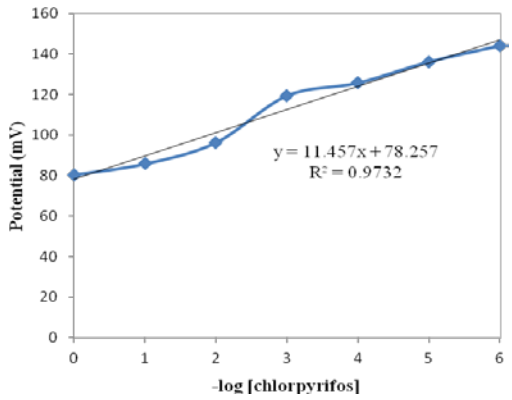


Fig. 2 Curve of potential towards – log [chlorpyrifos] for membrane composition of CA 5%, GA 25% (A)

Figure 2 shows measurement range of biosensor with composition of SA 5% and GA 25%, i.e. 1ppm – $10^{-6}$ ppm, value of Nernst factor (sensitivity) for electrode biosensor is 11.45 mV/decade with correlation coefficient value of 0.973. This value is still far from ideal value, i.e. 29.6 mV/decade, the low concentration of CA causes not really much enzyme trapped in, resulting small potential value and Nernst factor.

TABLE II
RANGE OF WORKING CONCENTRATION AND NERNST FACTOR BIOSENSOR FOR THE COMPOSITION OF MEMBRANE CA 15%, GA 25% (B)

| No | Substrate concentration [acetylthiocholine chloride] M | Inhibitor concentration [chlorpyrifos] ppm | Potential (mV) |
|---|---|---|---|
| 1 | $10^{-3}$ | 1 | 37 |
| 2 | $10^{-3}$ | $10^{-1}$ | 40.2 |
| 3 | $10^{-3}$ | $10^{-2}$ | 50 |
| 4 | $10^{-3}$ | $10^{-3}$ | 78.4 |
| 5 | $10^{-3}$ | $10^{-4}$ | 110.3 |
| 6 | $10^{-3}$ | $10^{-5}$ | 150.1 |
| 7 | $10^{-3}$ | $10^{-6}$ | 160 |
| Nernst factor (Slope) | | 23.18 mV | |
| R(coefficient of correlation) | | 0.943 | |
| $E^0$ (K) | | 19.88 | |
| Range of working concentration | | 1 ppm – $10^{-6}$ ppm | |

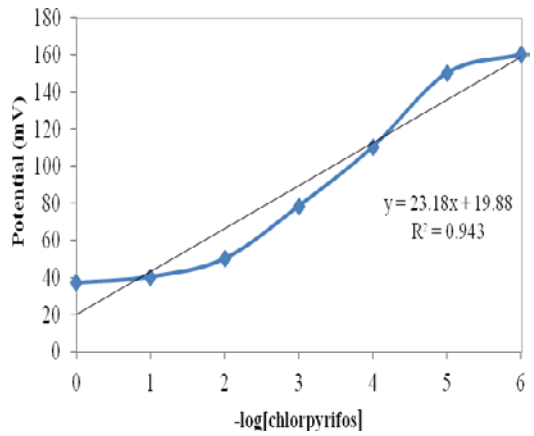


Fig. 3 Curve of potential towards - log [chlorpyrifos] for membrane composition of CA 15%, GA 25% (B)

On the other hand, Table II and Figure 3 shows bigger sensitivity value of electrode with membrane composition of CA 15% and GA 25% , compared with the previous electrode, i.e. 23.18 mV/decade with correlation coefficient value of 0.943, with the same measurement value, i.e. from 1ppm – $10^{-6}$ ppm. Difference of membrane composition cause difference of potential value in every electrode that influence the sensitivity value.

Based on research result, for all various concentrations of pesticide that is analyzed shows linear line as shown on Figure 2 and Figure 3, therefore, detection limit for both electrode biosensor in analyzing pesticide chlorpyrifos has range of $10^{-6}$ ppm.

## IV. CONCLUSION

Enzymatic biosensor using electrode of gold / cellulose acetate / glutaraldehyde / acetylcholinesterase (Au / CA / GA / AChE) for analysis of pesticide chlorpyrifos potentiometrically has been successfully designed.

Further, potentiometrically analysis result shows that bahwa electrode biosensor with composition of CA 15% and GA 25% gives most optimal working in analysis of chlorpyrifos pesticide with the highest Nernst factor (sensitivity) value of 23.18 mV/decade. Measurement range for all composition of electrode biosensor membrane shows same value, i.e. 1ppm - $10^{-6}$ ppm. Thus, it is pointed out that design result of biosensor consisting of membrane of cellulose acetate (CA) and glutaraldehyde (GA) in gold electrode analyzed potentiometrically can be recommended to detect residue of pesticide of chlorpyrifos in detection limit of $10^{-6}$ ppm or $10^{-3}$ ppb.

## REFERENCES

[1] Squibb, K., 2002, Pesticides: Program in Toxicology NURS Applied Toxicology

[2] Klein, G.M., Rao, R. B.,Flomenbaum, N. E., Nelson, L. S., and Farmer, B. M., 2008, Disarter Preparedness: Emergency Response to Organophosphorus Poisoning, A Supplement to Emergency Mediciene

[3] Tuovinen, K., Kaliste-Korhonen, E., Raushel, F.M., and Hanninen, O., 1994, Phosphotriesterase-A Promising Candidate for Use in

detoxification of Organophosphates, *Fundam. Appl. Toxico,*. 23, 578-584.

[4] Sassolas, A., Prieto-Simón, B., Marty, J. , 2012, Biosensors for Pesticide Detection: New Trends, *American Journal of Analytical Chemistry*, 2012, 3, 210-232

[5] Rodriguez-Mozaz, S., Marco, M., Lopez de Alda, M., Barcelo, D., 2004, Biosensors for Envirometal Monitoring of Endocrine Disruptors: a review Article. *Anal Bional Chem*. 378 : 588 – 598.

[6] Mostafa, G. A. E.,2010, Electrochemical Biosensors for the Detection of Pesticides, *The Open Electrochemistry Journal, 2,* 22-42.

[7] Amine, A., Mohammadi, H., Bourais, I. and Palleschi, G., 2006, Enzyme Inhibition-Based Biosensors for Food Safety and Environmental Monitoring. *Biosensors and Bioelectronics.* 21 : 1405 – 1423.

[8] Dan Du, Shizhen, C., Jie, C., Aidong, Z., 2008, Electrochemical Pesticide Sensitivity Test Using Acetylcholinesterase Biosensor Based on Colloidal Gold Nanoparticle Modified Sol-gel Interface. *Talanta*. 74: 766-772.

[9] Renedo, O.D., Alonso-Limillo, M.A. and Martinez, M.J., 2007, Recent Developments in the Field of Screen-Printed Electrodes and their Related Applications. *Talanta.* 73 : 202 – 219.

[10] Tudorache, M. and Bala, C., 2007, Biosensors Based on Screen-Printing Technology, and their Applications in Environmental and Food Analysis. *Anal Bioanal Chem*. 388 : 565 – 578.

[11] Mashuni, Syahrul, M., Ahyar, A. and Wahab, A. W., 2010, Pesticides Using A Biosensor-Based Enzyme Acetylcholinesterase Determination of Carbamate and Cholin Oxidase on Platinum Electrode, *Indonesian Journal of Chemistry*, 10 (3), 290-294.

[12] Vikas and Pundir, C.S., 2008, Farication of Pt based Amperometric Cholesterol Biosenosr Using Cellulose Acetate Membrane, *Journal of Scientific and Industrial Research*, 67, 299-306.

[13] Prieto-Simon, B., Campas, M., Andreescu, S. and Marty, J., 2006, Trends in Flow-based Biosensing Syatems for Pesticide Assessment. *Sensors*. 6 : 1161 – 1186.

[14] Ciucu, A.A., Negulescu,C., Baldwin, R.P., 2003, Detection of Pesticides Using an Amperometric Biosensors Based on Ferophthalocyanine Chemically Modified Carbon Paste Elekcrode and Immobilized Bienzymatic System. *Biosensors and Bioelctronics.* 18 : 303 – 310.