Environmental Biosensor Development for Organochlorine Pesticides Detection based on Carbon Paste Electrode Modified by Nanoparticle

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INTRODUCTION

Increasing of activity in the agricultural and industrial sectors become undivided part of life. Besides positive impact for humans, these activities also had negative impact in the form of environmental damage caused by contamination. In the agricultural sector, farmer used various types of pesticides to increase quality and productivity of agricultural products. However, the use of pesticides also give bad effect to the environment and human life. Based on Stockholm Convention (2001), pesticides included into persistent organic pollutants (POPs), namely that is organic compounds that are resistant to environmental degradation through chemical, biological, and photocatalytical. Pesticide compound group of organochlorines was the most toxic of organophosphates or carbamates.

Various methods have been developed and used as a analysis method in environmental monitoring. Generally the methods currently used for the identification of pesticide are the method of gas chromatography and high performance liquid chromatography. However, the methods that have been used is relatively expensive methods. These methods also cannot be used for direct identification of pesticide compounds in the environment. To solve that problem, biosensor technology become alternatively main selection. Important step in the development of biosensors is the process of modifying the electrode and immobilization of biological component. In this research was used nanoparticle zeolite as electrode modifier and immobilization matrix of biological component. Acetylcholinesterase enzyme (Ache) was used as biological component with acetylcholine chloride as substrate. Electrochemical measurement use cyclic voltammetry. Optimization of independently variables factors to obtain optimum immobilized Ache activity response that includes pH, Ache concentration, and the concentration of acetylcholine chloride.

MATERIALS AND METHODS

The materials used in this study was HCl (pH 2, 3 M, 0.2 M), K3 [Fe (CN) 6] 1 mM, FeCl3 • 6H2O 0:01 M, AgNO3 0.1 M, the enzyme Acetylcholinesterase (Ache) (Electrophorus electricus Sigma) substrate solution Acetylcholine Chloride 2.1 mM solution of Tetra-chloroetylene organochlorines (TCE), a phosphate buffer solution of pH 6-8, Bayah nano-sized zeolite, graphite, and liquid paraffin. The tools used in the

study is an electrode compartment, mortar, nylon, dialysis membranes, yarns paranylon, microliter pipette, waxed paper, glass cup, potentiostat DY2300 and computers that have been installed as well as the data processing program Design Expert v.10.

Zeolites Modified by Iron (Balal et al. 2009)
Modifications made by mixing zeolite FeCl3 concentration, 0:01,. Zeolites are already activated as much as 1 g soaked in 250 mL FeCl3, then stirred with a magnetic stirrer for 48 hours. Zeolite is then filtered and washed with distilled water and HCl pH 2 to 3 times restating manghilangkan chloride ions. Then the zeolite was dried in a desiccator to dry.

Pasta Carbon Electrodes (Balal et al. 2009)
The electrode was prepared by grinding 55 mg of graphite powder with 35 mL of liquid paraffin in a mortar until smooth mixture is obtained. Then the zeolite which has been modified Fe mixed with graphite and then added 2 mL of diethyl ether. The mixture is stirred until the solvent evaporates. Copper wire having a diameter of 3 mm incorporated into the glass tube until the remaining space along the 5 mm at the end of the tube as a carbon paste. A modified carbon paste electrodes to put in a full and dense. Then leveled with scratching on waxed paper.

Characterization of Carbon electrodes Pasta (modification Taufik 2013)
The electrode that has been made is characterized by measuring the response of the electrode with a solution of K3 [Fe (CN) 6] 1 mM using cyclic voltammetry technique, speed Microscopy 50 MVS-1 at an interval of a potential 0.0 to 3.5 V.

Enzyme Immobilization AChE (Ikeda et al. 1998).
AChE enzyme solution with a certain concentration is dripped onto the surface of the carbon paste electrode, the electrode then dried to evaporate the solvent. The tip electrode covered with a dialysis membrane, nylon net, and tied with paranylon. Electrode immersed in a phosphate buffer solution and can be used in the measurement of the activity of AChE with electrochemical method.

Electrochemical measurement
Electrochemical measurements performed using cyclic voltammetry using a potentiostat DY2300. The electrode used is an electrode Ag / AgCl, platinum, and CPZ / AChE successively as a comparison electrode, auxiliary electrode (counter), and the working electrode. Parameter measurements are made with cyclic mode, initial E-700 mV, the final E 3500 mV, rate of 50 mV / s, low E-700mV. Phosphate buffer solution as much as 1.9 mL and 100 mL of solution AChE added into the measurement cell. The observed peak anode current is set as blank. Then add 1 ml of 2.1 mM substrate acetylcholine chloride and measured changes in peak current anode. Furthermore tertrachloroetylene solution (TCE), written and seen a decrease in the flow of the anode due to the addition of TCE with a certain concentration.

Optimization of Activity of AChE Enzyme
Optimization is done is pH 6.5 ~ 8, and the concentration of AChE (0.0249 ~ 0.0499 U / mL). The method used to optimization of AChE activity is Response Surface Method. This method is done by inserting a combination of factors independent variables on a statistical software Design Expert. After that, experiments were conducted in accordance with the resulting combinations to get the optimum value of the activity.

Characterization Electrode
Biosensor electrode CPZ / cache in optimum condition characterized by parameters Linearity and limit of detection, stability, and precision test.

Linearity, limit of detection (LOD) and Limit quantization (LOQ)

Linearity measurement is measured by measuring the current response to the concentration of tetrachlorethylene. Each concentration used was 0.8, 1.6, 3.5, 7, 15, 25, 35, and 60 ppb. The data is then plotted in the curve with the x-axis is the concentration of tetrachlorethylene and response current (mA) on the y-axis. Extrapolation line provides linearity, detection limit, and the limit of quantitation.
Limit of detection and quantitation were determined by the following formula:

$$LOD = \frac{3 \times \sigma \text{ analit}}{b} \qquad LOQ = \frac{10 \times \sigma \text{ analit}}{b}$$

Information:

σ = standard deviation of the response analyte
b = the slope of the linear equation

Stability
The stability of the biosensor CPZ / AChE was determined by measuring the resulting current in the measurement. The entire value of the activity obtained in the initial measurement is considered at 100%. Activities were measured again for 3 days with 1 day intervals. Percent biosensor activity was measured using the formula:

$$\% \text{ Activity} = \frac{\text{I day -n } (\mu A)}{\text{I begin } (\mu A)} \times 100\%$$

Precision test
Precision is measured as the standard deviation or relative standard deviation. Precision can be expressed as repeatability (repeatability). Precision is determined by calculating the standard deviation (SD) of the test 3 pieces electrode CPZ / AChE and standard deviation (% RSD) is calculated as follows:

$$\%RSD = \frac{SD}{X} \times 100\%$$

Information:

SD: Standard deviation measurement
X: The average measurement

Activation of Nano-zeolite

Activation nanozeolite aims to increase the porosity and adsorption capacity of the zeolite. In addition, activation can also modify the ratio of Si / Al zeolites. Activation can be done in physics and chemistry, in physics that is by heating while chemical activation is by mixing with acids or strong bases. In this study, two treatment chemical activation, the activation using HCl and NaOH 3 3 M. Molarity and the same volume used for the activation process either acidic or alkaline. It is intended that the number of H + or OH- ions in each of equal treatment. Remaining liquid in the activation process by acid yellow, in contrast to the rest of the liquid in the activation of colored translucent base equal to the initial color its base premix (Figure 1).

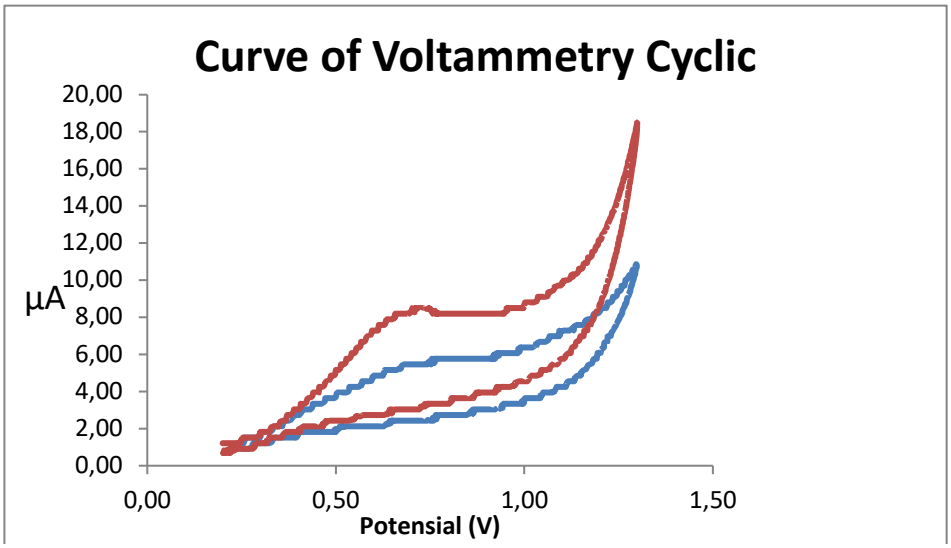
Nano-zeolite Composited by Fe

Modification of zeolites by adding FeCl3 0:01 M. The zeolite that has been activated as much as 1 g soaked in 250 mL FeCl3, then stirred with a magnetic stirrer for 48 hours. Modifications done either already activated zeolite using acidic or alkaline. Zeolite is then filtered and washed with distilled water and HCl pH 2 to 3 times restating remove chloride ions. Then the zeolite was dried in a desiccator to dry. Some of the Si atoms in zeolites is replaced by Al atoms.

The difference in charge between the tetrahedron (AlO4) 5- and (SiO 4) 4- will produce zeolite structures are negatively charged. In addition to the difference in charge have resulted in zeolites have a low power absorption against anions, immobilization of enzymes required for zeolite surface character more positive. Therefore, modification of the zeolite with the addition of cations (Fe 3+) of FeCl3 solution. To improve the character of the zeolite surface becomes more positive as the immobilization of enzymes, it needs to be modified zeolite. This is done with the principle of exchange of the metal cations in the zeolite pores. According to Hanafi (2005) onsite metal cations in the zeolite will not change the crystal structure of the zeolite tetrahedron, but will change the affinity of the zeolite absorption power. Results modifications nano-zeolite with yellow colored iron powder form (Figure 2). After nano-zeolite modified by the addition of Fe3 + ion is expected to be more positive nano-zeolite characters so as to facilitate interactions with negatively charged or electronegative groups of the enzyme and will strengthen the interaction between the enzyme with the electrode surface, so that the enzyme is not easily detached from the surface of the electrode.

Characterization of Carbon-electrode Pasta Nanozeolit-Fe

Cyclic voltammetry is a technique used to obtain qualitative information about an electrochemical reaction with the principle of measuring changes in current as a result of oxidation-reduction reactions. The emergence of currents caused by the oxidation-reduction reaction at the electrode surface and is proportional to the concentration of the analyte in solution. Carbon paste electrodes made by mixing 55 mg of graphite with 35 mL of liquid paraffin. EPK then be modified by adding zeolite-Fe. The electrode that has been created is characterized by using K3Fe (CN) 6 1 mM. Characterization using K3Fe (CN) 6 produces a peak oxidation and reduction. Oxidation peak occurs due to the oxidation solution of [Fe (CN) 6] 4- to [Fe (CN) 6] 3- and peak reduction occurs because the solution of [Fe (CN) 6] 3- is reduced to [Fe (CN) 6] 4 -. Carbon paste electrode-nano-zeolite-fe for nano-activated zeolite acidic or alkaline than the peak current performance. The results obtained showed that the oxidation peak current electrode with nano-zeolite activation base provides greater flow than the nano-zeolite acid activation (Figure 3).



Optimization of PDH Activity

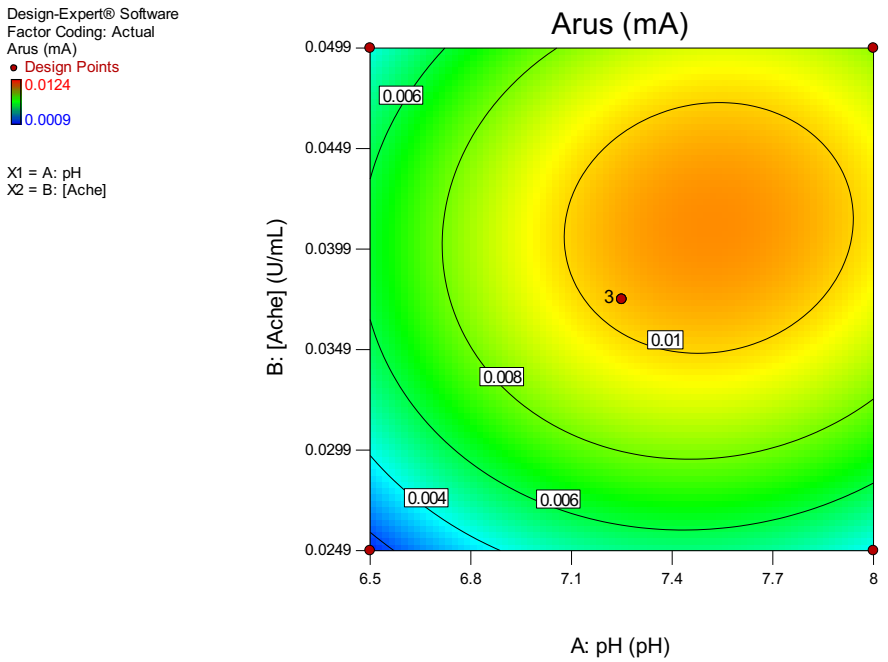
To produce optimum performance of the enzyme, then conducted searches optimum condition. There are two pieces of the parameters used to find the optimum conditions, ie pH (6.5-8), and [AChE] (0.0249-0.0499 U / mL). Both parameters were analyzed using Response Surface Method on software Design Expert and produced as many as 11 combinations of measurement.

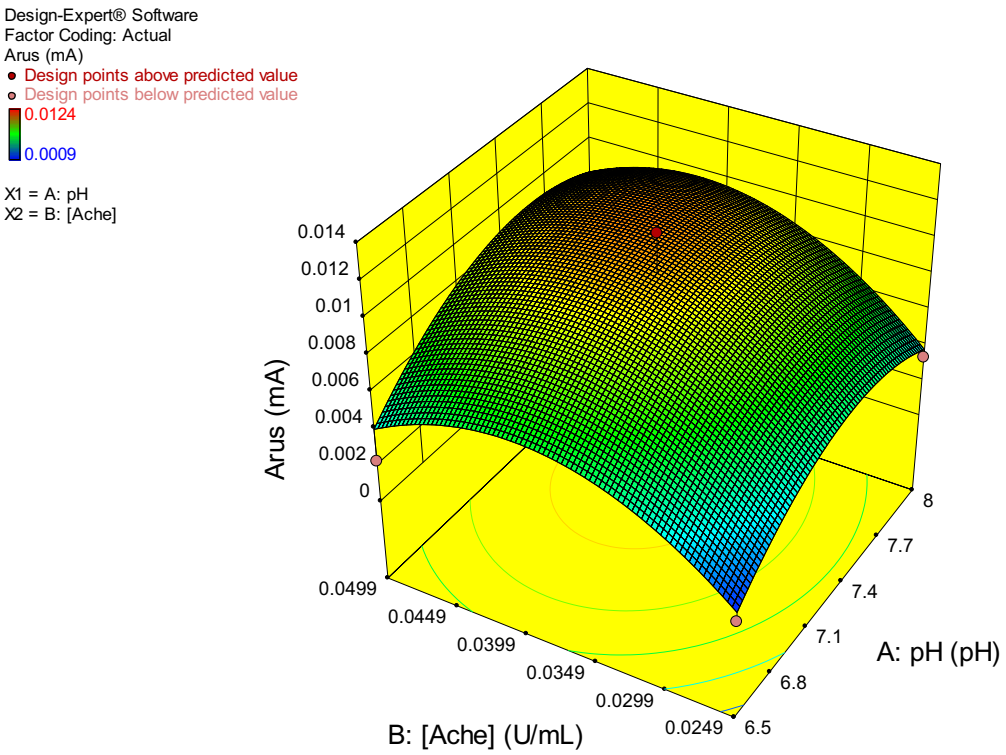
AChE enzyme activity is determined using cyclic voltammetry technique. Measurements were performed by measuring the phosphate buffer solution and a solution of AChE with the smallest concentration defined as a blank, then added substrate acetylcholine chloride in accordance with a combination of measurements. The optimum conditions of activity of the enzymes AChE is [AChE] 0.0374 U / mL, as seen in Figure which shows a plot contours two dimensional between (a) [AChE] and pH on peak current of oxidation, or plot contours and 3 dimensional (b) [AChE] and the pH of the oxidation peak current.

The second plot shows the optimum activity of the enzyme shown in contour area that is dark yellow.

Tabel 1 Optimization variable pH and [ACE] and the resulting current

	pH	[Ache]	Arus
1	7.3	0.0197	0.0013
2	6.5	0.0499	0.0023
3	8.0	0.0249	0.0034
4	6.5	0.0249	0.0009
5	8.3	0.0374	0.0086
6	7.3	0.0374	0.0124
7	7.3	0.0551	0.0081
8	7.3	0.0374	0.0087
9	6.2	0.0374	0.0041
10	8.0	0.0499	0.0065
11	7.3	0.0374	0.0098

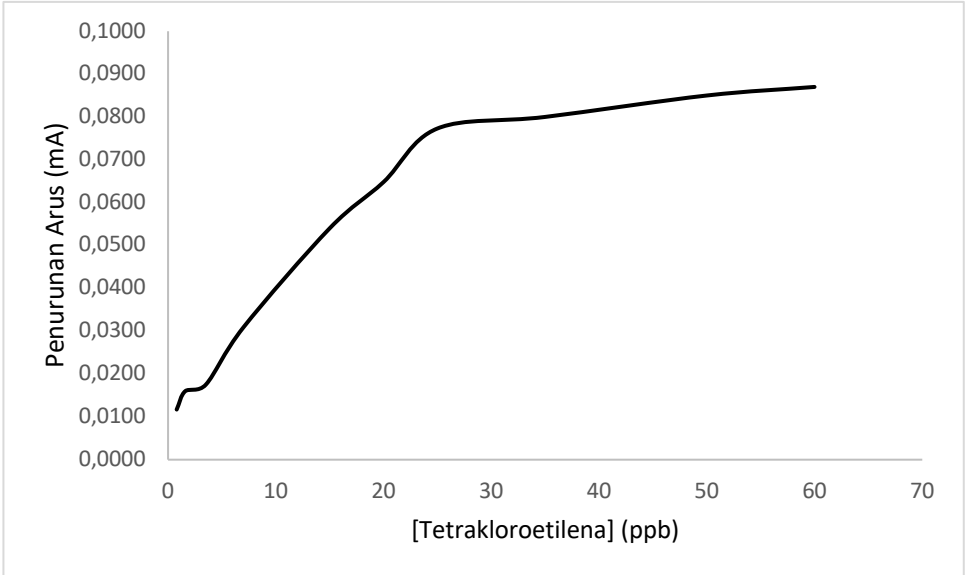




Characterization Electrode

Linearity, limit of detection (LOD) and Quantitative Limit (LOQ)

Linearity, LOD and LOQ determined by measuring the activity of the enzymes AChE against organochlorine tetrachlorethylene in optimum conditions of pH 7.3, [PDH] 0.0374 U / mL, using one electrode and 3 repetitions. Determination of linearity was obtained from the graph circuiting and concentration of tetrachlorethylene.
Tetrachlorethylene measurements performed in the range of 0.8-60 ppb (Figure 6). Measurements in this range aims to determine linearity, limit of detection and quantitation limit biosensor CPZ / AChE made. The measurement results on the electrode showed linearity of the AChE enzyme activity against tetrachlorethylene concentration range that has been set. Range of linearity produced long enough to obtain accurate and high value linear regression, ie R2 = 0.9954. From the line equation y = 0.0027x + 0.0104 prescribed limit value detection and quantitation limit of measurement (Figure 7).



Limit of detection is the lowest concentration of an analyte that can be detected by a process of analysis and the limit of quantitation is the amount of analyte smallest in the sample can be determined quantitatively at the level of accuracy and precision were good. Based on the calculation, the value of LOD and LOQ respectively 2:15 and 7:17 ppb.

Stability

To obtain a high stability in the biosensor is carried to enzyme immobilization process. In a study conducted immobilization of enzymes on nanozeolit particles. According Hamsah (2007), the nano-sized composites can improve the properties, stability, structure, and strength of materials. Weniarti (2011) also states that the immobilization of enzymes on the zeolite will create increased enzyme stability and can be reused.
Measurement of the enzyme activity of AChE stability against tetrachlorethylene performed at the optimum condition with the measurement time for 3 days. It is seen that the stability of the biosensor electrode CPZ / AChE decreased every day. On the first day of the stability of the electrode decreased activity that is not too big, so even on the second day, but on the third day decreased significantly electrode activity. On the fourth day percentage drops sharply biosensor performance reached 34.53%. From the results of this electrode stability, it can be said that the measurement or analysis using arsenic biosensor to produce a fairly good stability on the first, second, and third, while on the fourth day of the electrode has a low stability. So the stability of the electrodes need to be improved.

Tabel 2 The percentage reduction in biosensor current versus time

Day	Persentase (%)	Current (mA)
0	100.00	0.1341
1	95.30	0.1278
2	88.67	0.1189
3	74.20	0.0995
4	34.53	0.0463

Precision Test

Precision test is the test used to determine the repeatability of an electrode. Repeatability is determined by looking at the activity of three pieces of electrodes are measured in optimum condition and the tetrachlorethylene 3.5 ppb. Repeatability is used to determine the accuracy of a method used by standard deviation (SD) and the relative standard deviation (% RSD). In Table generated standard deviation of the AChE enzyme activity measurements tetrachlorethylene at 0.00075 and the% RSD for 4:06%. These results can be said that the repeatability of 3 pieces electrode activity is quite good because it was produced% RSD less than 5% (Yashin, 2011).

Tabel 3 Precision test biosensor electrode organochlorines

Elektrode	Aktivitas
1	0.01790
2	0.01850
3	0.01940
Rerata	0.01860
SD	0.00075
%RSD	4.06

CONCLUSION

Biosensor electrode organochlorine nanozeolit-Fe-modified particles using AChE enzyme has been successfully created. Biosensor performance optimum pH of the solution, and the AChE enzyme concentrations of 7.3 and 0.0374 U / mL. Biosensor measurement linearity tetrachlorethylene with CPZ / AChE were in the range 0.8-25 ppb. LOD and LOQ value resulting from the measurement of 2:15 and 7:17 ppb. This value is the same than the LOD measurements using GC / HPLC which is 2 ppb. Stability measurement electrode tends to fall every day, so the measurement is better done with the condition that fresh enzymes and generated repeatability of a good electrode activity with value% RSD at 4:06%.

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