

DETERMINATION OF CARBAMATE PESTICIDES USING A BIOSENSOR BASED ON ENZYME ACETYLCHOLINESTERASE AND CHOLINE OXIDASE ON PLATINUM ELECTRODE

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ABSTRACT

In recent years, instead of their potential hazard to human health, carbamic pesticides are widely used in agriculture. Therefore, there is a growing interest in rapid and accurate determination for food safety and environmental monitoring. The aim of this research is to design a biosensor for analyzing carbamate pesticide residue in sample with composite variables of glutaraldehyde (GA) concentration in electrode membrane. Enzyme acetylcholinesterase (AChE) was co-immobilised with choline oxidase (ChO) onto a platinum (Pt) surface using a solution of 5%, 10%, 15% cellulose acetate and 25% glutaraldehyde. The result of this research shows that detection limit of the sensor using cellulose acetate 5%, 10% and 15% are $10^{-7.7}$ M, $10^{-8.7}$ M and $10^{-7.6}$ M respectively. The results are approximately equal to 2.2 ppb–0.2 ppb, which means that this biosensor is very sensitive for determining carbamate pesticide residue. Detection limit of biosensors are comparable to that of the conventional instrument such as Gas Chromatography (GC) and High Pressure Liquid Chromatography (HPLC), i.e. 1.5 ppb and 2.0 ppb respectively. The proposed electrochemical pesticide sensitivity test exhibited high sensitivity, desirable accuracy, low cost and simplified procedure.

Keywords: Biosensors, glutaraldehyde, cellulose acetate, immobilised, carbamate pesticides

INTRODUCTION

Among all environmentally hazardous chemical substances, pesticides are the most common, especially in soil, water, atmosphere, and in agricultural products. Their existence in our environment is so common that they have become problems to human health. One of the efforts to improve food safety is to have enough stock of nutritious and healthy food. Food safety from pesticide residues can be controlled quickly and accurately through the pesticide biosensors.

Some pesticides are very toxic, while accumulated pesticides in living organisms may cause serious illness. The existence of small but stable amount of pesticide may cause acute toxicity that quick detecting system is required to protect human health and to control of food products and environmental pollution [1].

In the last several decades, carbamate and organophosphate compounds, the two most commonly found pesticide groups, have been widely used in many crops. The two compounds are applied in the agriculture due to their low environmental stability compared to organochlorine [2-3]. The efficiency of carbamate and organophosphate as pesticides and their toxicity to human and animals are caused by their ability to inhibit

the enzyme of hydrolyses called esterase. Acetylcholinesterase enzyme (AChE) is very important for the central nerve system of human and insects [4].

Acetylcholinesterase enzyme hydrolyses acetylcholine of the electrode membrane to prevent accumulation. Obstruction of AChE causes the accumulation of acetylcholine which leads to the dysfunction of several nerve systems and caused the damage in breathing system and might lead to death [5-6].

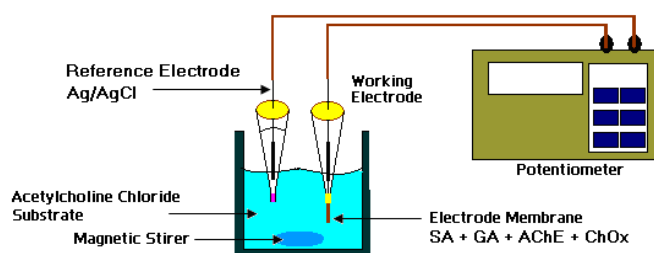
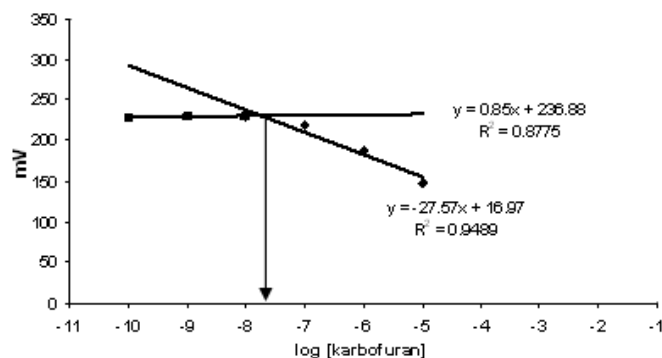
Some international organization such as the Food and Agricultural Organization (FAO), the World Health Organization (WHO), the European Community (EU) regulate the limit of maximum pesticide residue in water and food consumption by human and animals. One of the policies they made was to monitor the limit of a pesticide for one product being 0.1 µg/L and the total pesticide concentration should not be higher than 0.5 µg/L [4].

Pesticide analyses frequently had done using Gas Chromatography (GC) and High Pressure Liquid Chromatography (HPLC). The weakness of this method is needed a long time in requirement of extraction and purity treatments in the laboratory. To overcome this weakness, an analysis using biosensor

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Table 1. The composition of carbamate pesticide biosensor

Composition of Membrane			
Cellulose Acetate (SA)	Glutaraldehyde (GA) (%)	AChE (IU/mL)	ChO (IU/mL)
5%			
10%	25	49.8303	4.95
15%			

**Fig 1.** The design of the measurement of biosensor response**Fig 2.** Detection limit of the biosensor for the composition of membrane of SA 5%, GA 25% at the concentration substrate 10^{-3} M (A)

Biosensor Membrane

Membrane was made from enzyme Acetylcholinesterase (AChE) and choline oxidase (ChO_x) which was immobilised in supporting materials Cellulose Acetate (SA) and Glutaraldehyde (GA) with the composition shown in Table 1.

Design of Electrode Biosensor of Layered Wire

Electrode bodies were made from a 7 cm bronze wire of 1 cm in diameter which was connected with a 2.0 cm platinum wire of 0.4 mm in diameter, which was then soldered with tin wire. It was then put into a blue tape with the platinum wire (Pt) sticking out at 1.5 cm which was use as the electrode bodies. At the bodies of all electrodes were the parafilms coiled around as Cu and Pt wires. The edge of electrode, which was Pt wire, was immersed in homogenous Cellulose Acetate. After the Cellulose Acetate was formed, the electrodes were washed using aquades 3 times. Next, the part of Pt wire layered with membrane Cellulose Acetate was immersed

in Glutaraldehyde for 6 h. After that, the electrodes were washed with buffer phosphate pH 8.0. This formed membrane electrode (Em), which was then immersed in buffer phosphate pH 8.0 containing enzyme Acetylcholinesterase and enzyme choline oxidase for 48 h. Membrane electrode (Em) which had not been used remained immersed in the buffer phosphate pH 8.0 at the temperature of 4 °C.

Measurement of Detection Limit of the Carbamate Pesticide Biosensor

The potential of Acetylcholine Chloride substrate, with carbofuran pesticide inhibitor, was measured by way of immersing the electrode biosensor made in buffer phosphate pH 8.0 solution before being used, and then the electrode biosensor was used to measure the substrate potential of acetylcholine chloride with the concentration of 10^{-3} M which had been added with carbofuran solution with varying concentration between 10^{-3} – 10^{-9} M. The measurement of biosensor response is shown in Fig. 1.

RESULT AND DISCUSSION

Specific analysis method to determine the quantity of a substance or molecule in a very small number of samples always faces limited detection which is indicated as the lowest concentration of a substance determined. The detection limit of an electrode can be determined by putting tangents at the linear function which is Nernstian and non Nernstian. The intersection of the two lines is extrapolated to X to obtain the detection limit concentration. The result of the determination of electrode detection limit of the biosensor designed can be seen in Fig. 2 to Fig. 4.

Fig. 2 the extrapolation to X – log [carbofuran] it was found that the detection limit of the biosensor comprising membrane SA 5%, GA 25% was $10^{-7.7}$ M (\approx 0.00221 ppm or 2.2 ppb). Fig. 3, the detection limit of the biosensor comprising membrane 10%, GA 25% was $10^{-8.7}$ M (\approx 0.000221 ppm or 0.2 ppb). The Biosensor comprising this membrane was the best of all. Fig. 4 the result of extrapolation to X – log [carbofuran] it was found that the detection limit of the biosensor $10^{-7.6}$ M (\approx 0.00221 ppm). This is relatively smaller compared with the detection limit of the other biosensors designed. The reason was that the membrane's

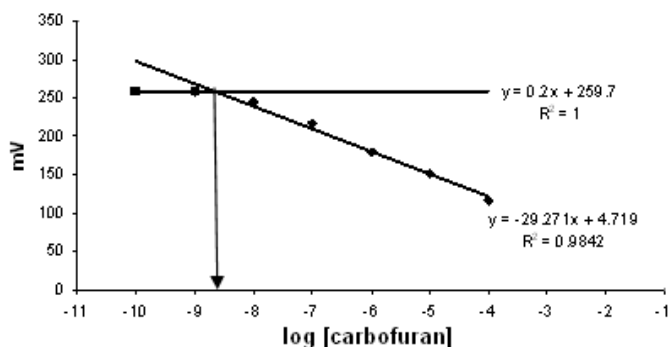


Fig 3. Detection limit of the biosensor for the composition of membrane of SA 10%, GA 25% at the concentration substrate 10^{-3} M (B)

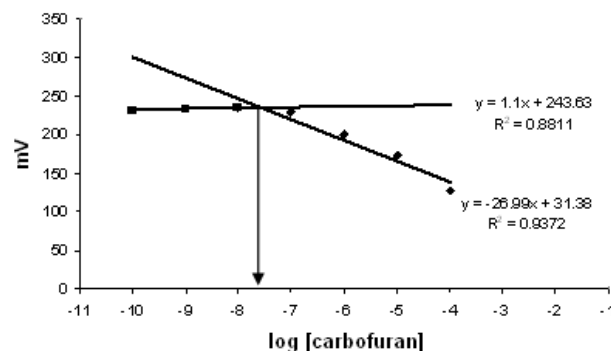


Fig 4. Detection limit of the biosensor for the composition of membrane of SA 15%, GA 25% at the concentration substrate 10^{-3} M (C)

Table 2. Carbamate biosensor performance

Type Membrane	Membrane composition		Biosensor performance
	SA	GA	Detection Limit (LD)
A	5%		$10^{-7.7}$ M
B	10%	25%	$10^{-8.7}$ M
C	15%		$10^{-7.6}$ M

supporting substance i.e. concentration SA was higher than the composition of other membranes, which was 15%. Immobilization of enzyme AChE and ChO_x at the supporting material cellulose acetate and glutaraldehyde can be done in making the membrane of pesticide carbamate biosensor. Cellulose acetate membrane is very stable against various kinds of chemical substances, has good mechanical strength making it enduring in high and selective pressure so that it can hold very tiny materials. Biosensor based on the enzyme inhibition principle can be widely used to detect an analyte such as pesticide component and heavy metals. The selection of the system of enzyme/analyte was based on the fact that this toxic analyte obstructed the function of the normal enzyme. In general, the development of this biosensing system relies on the quantitative measurement of the enzyme's activities prior to and after being reacted or contacted with a target analyte. The complete detection limits of every biosensor membrane composition are presented in Table 2.

CONCLUSION

The result of the study indicates that as for the membrane composition SA 5% and GA 25% the detection limit is $10^{-7.7}$ M, for SA 10% and GA 25% is $10^{-8.7}$ M, for SA 15% and GA 25% is $10^{-7.6}$ M. This result is equal to 0.0022 ppm or 2.2 ppb). The work performance of the biosensor includes high specificity and sensitivity, quick response, relative inexpensive cost, relatively compact instrument, and easy operation of the instrument and safety. Therefore, the biosensor is an important instrument to detect biological and

chemical components for health, food, environment monitoring. In addition the combination of electrochemical transducer and an enzyme as a biological component make it to be used widely.

Detection limit of carbamate biosensor ranges between 10^{-7} – 10^{-9} M. The maximum detection limit is at the composition of membrane cellulose acetate (SA) 10% and glutaraldehyde (GA) 25%. This indicates that Acetylcholinesterase-and-Choline oxidase-enzyme-based biosensor can be used to detect carbamate pesticides up to the detection limit of 10^{-9} M equal to 0.2 ppb. As in the case of other measuring instruments like GC and HPLC which have carbamate detection limit of 0.2 ppb, the biosensor designed in this study can be applied to detect carbamate pesticides more efficiently because it is portable to the field.

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