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The Performance of Organophosphate Pesticides Determination Using Biosensor based a Small Device Potentiometer as a Transducer †

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Abstract: The need to control pesticide residues in foodstuffs in a fast and straightforward analysis for the field scale is required. Therefore, this research develops a transducer-based biosensor with a small device potentiometer (SDP) to produce a fast and accurate pesticide detection tool. The biosensor based on Pt/Au electrodes by immobilizing the acetylcholinesterase (AChE) enzyme coated membrane cellulose acetate (CA) 15% (w/v) cross-linked glutaraldehyde (GA) 25% (v/v) and SDP as a transducer that produces a potential value. The biosensor testing results on the organophosphate pesticide class, namely diazinon and profenofos, in which they showed the sensitivity of 21.204 and 21.035 mV.decade^{-1}, Limit of Detection (LoD) 10^{-8} mg.L^{-1}, selectivity coefficient $K_{i,j} < 1$ and accuracy of 99.497 and 94.765 %, respectively. The results showed that the biosensor connected to an SDP transducer had an excellent performance in determining the presence of organophosphate pesticides.

Keywords: small device potentiometer; biosensor; organophosphate; pesticide; sensitivity; limit of detection; selectivity; accuracy

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1. Introduction

In recent decades, biosensors have become a popular research area capable of identifying pesticide residues and other chemicals. Biosensors are "self-standing devices", devices that record physical, chemical, or biological changes and convert them into measurable signals from the sample and monitor the analyte of interest [1,2]. The sensor contains a recognition element that allows a selective response to a specific analyte or group of analytes, minimizing interference from other sample components. Another significant component of a sensor is a transducer or detection device that produces a signal [3].

Electrochemical biosensors are a subclass of chemical sensors that combine sensitivity, such as low detection limit, electrochemical transducers with the high specificity of biological recognition processes. These devices contain biological recognition elements (enzymes, proteins, antibodies, nucleic acids, cells, tissues or receptors) that selectively react with the target analyte and generate an electrical signal related to the measured analyte concentration [1,4]. Enzyme-based electrochemical biosensors have advantages over conventional methods due to their excellent sensitivity, selectivity, mini size and fast response [5].

Maintaining the catalytic activity on the efficient immobilization of the acetylcholinesterase (AChE) enzyme is an essential consideration when developing electrochemical biosensors that can be used for practical applications. The highlight of the electrochemical biosensor is its unique ability to generate digital signals that can measure by converting the catalytic signal with the help of microfabricated electronics [6]. The electrochemical method using measurement tools is based on potentiometric [6], amperometric [7] or conductometric [8]. Potentiometric biosensors are suitable for measuring the response value of pesticide detection measurements [9].

Potentiometric are efficient when used in field analysis because they are more straightforward and ideal for real-time analysis. The detection system using potentiometric developed by Timur, S., & Telefoncu, A., 2004 [10], has the underlying principle of inhibition of AChE activity due to its properties in identifying organophosphate compounds. The enzyme was immobilized on the surface of the electrode with the help of a chitosan membrane [11]. Without a pre-concentration step, in both aqueous and organic media, detection of organophosphates without the requirement of trained personnel proved to be advantageous for the proposed portable biosensor. Pesticides were effectively detected in the range of 0.1-100 mM for parathion-methyl and methamidophos and 0.6-600 mM for Malathion. However, in the presence of higher pesticide concentrations, only partial regeneration of the enzymatic activity was regenerated [9,10].

The combination of potentiometric-based AChE enzyme biosensors as transducers with analytical techniques has been widely reported in the literature as a suitable method. In this work, we report the development of a small device potentiometric (SDP)-based biosensor as a transducer for the determination of pesticide organophosphates, based on the AChE enzyme immobilized on cellulose acetate (CA) and glutaraldehyde (GA) membrane-coated Au electrodes.

2. Materials and Methods

2.1. Meterials

Acetylcholinesterase (AChE, from *electrophorus* sigma aldrid 1.17 mg ith activity 425.94 units per mg (EC. 3.1.1.7)), cellulose acetate (CA, 15% v/v in water added 10 acetone), glutaraldehyde (GA, 25% v/v in water), potassium chloride (KCl, 10^{-1} M) and acetone (C_3H_6O), phosphate buffer solutions (PB) with various pH values were prepared by mixing standard stock solutions of 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4. Standard solution of acetylthiocholine chloride (ATCl) substrate with concentrations of 10^{-1}, 10^{-2} and 10^{-3} M in PB solution. The pesticides used in the OP group are diazinon and profenofos as inhibitors, each made in concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} and 10^{-1} mg.L^{-1} using ethanol as solvent.

2.2. Apparatus

The potentiometer is used as an experimental tool for measuring the potential value of analyte detection [12]. The working electrodes used are Au and a platinum (Pt) as a catalyst to accelerate the electrolysis process and an Ag/AgCl as a standard electrode.

2.3. Electrolysis of Ag/AgCl

Standard Ag/AgCl electrodes were carried out by electrolyzing Ag wire with 0.1 M KCl solution for ± 20 minutes. The length of time electrolysis will affect the thickness of AgCl on Ag wire, where the more extended the electrolysis process, the thicker it will be to a certain extent, and vice versa. Next, the Ag/AgCl wire that has been formed is then dried in the open air. Finally, Ag/AgCl wire that has been dried at room temperature is inserted into the electrode body as a comparison electrode for Ag/AgCl [13].

2.4. Preparation of Au electrode biosensor

The ends of the Au electrode bodies were immersed in a 15% CA membrane solution. The CA membrane layer formed was rinsed with distilled water and then dipped in 25% GA solution for 12 hours. Furthermore, the electrode was rinsed with distilled water and

PB solutions pH 8, then a membrane electrode (ME) was formed. Then, ME was immersed in the AChE enzyme for 2 × 24 hours at room temperature. Before measuring the response to the biosensor, the components in the measurement such as standard electrodes, coated wire type working electrodes, ATCl substrate and inhibitor solution need to be left at room temperature for about 2 hours components is stable and produces a good response [13].

2.5. Measurement of the potential value biosensor

Measurement of the potential value of the enzymatic biosensor electrodes with the pesticide inhibitors diazinon and profenofos in concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} and 10^{-1} mg.L^{-1} using a potentiometer. Em was immersed in PB pH 8.0 for 10 minutes and then used ME to measuring the potential value of 10^{-3} M ATCl substrate to obtain a constant value. Next, ME was removed and rinsed with distilled water, and then ME was immersed in a pesticide solution for 30 minutes and then dipped again into the ATCl substrate solution. Furthermore, it made observations to obtain a constant potential value.

2.6. The performance test of biosensor

2.6.1. Sensitivity

The sensitivity value (Nernst factor) is determined using a graph of the relationship between the potential value and –log of inhibitor concentration. Then, we can see the linear equation from the chart to obtain the sensitivity range of the diazinon and profenofos pesticide electrode.

2.6.2. Limit of detection (LoD)

LoD is measured by the linear equation of the calibration curve, y = ax + b, and the equation for the value of y at the detection limit is based on equation of Miller's (1991) [14].

2.6.3. Selectivity

Selectivity is expressed as the degree of bias by adding contaminants or foreign compounds to the sample and comparing it to the analysis of samples that do not contain other added ingredients. Sample analysis used a concentration of 10^{-5}; 10^{-4}, and 10^{-3} mg.L^{-1}. The potential value was measured using a potentiometer, and the selectivity coefficient (Ki, j) was calculated based on the equation of Harmita (2004) [15].

2.6.4. Accuracy

Determination of the accuracy value is obtained by calculating the % recovery, where the sample used is mustard greens with the addition of inhibitor concentrations of 10^{-4}, 10^{-3} and 10^{-2} mg.L^{-1}. Measurement of potential value using a potentiometer and calculated % recovery based on the equation of Harmita (2004) [15].

3. Results and Discussions

As shown in Figure 1, an analysis of the performance of the biosensor on the pesticide diazinon and profenofos using a small device potentiometric (SDP)-based biosensor as a transducer was carried out. SDP-based biosensor performance tests including sensitivity, LoD, selectivity and accuracy are essential parameters in biosensors.

The sensitivity or Nernst factor is one of the general parameters of biosensor performance testing using SDP as a transducer indicated by the slope resulting from the calibration of the electrode potential response to the analyte's activity. Analysis using potentiometric is based on the potential change in each variation of ion concentration [16]. The feasibility of a tool used in detecting an analyte is seen from how much sensitivity is in the measurement process, so in this study, the Nernst factor value was determined to see how well the sensitivity of the tool and the measurement range of an electrode was suitable for use as a pesticide detection tool. The results of the measurement of the potential value of the biosensor performance are presented in Table 1.

Figure 1. Shows the sensitivity of the performance of SDP-based biosensors to the detection of pesticide diazinon and profenofos of 21.204 and 21.035 mV.decade^{-1}, respectively. The sensitivity is the slope value of the linear regression equation from the graph of the relationship -log [inhibitor] with the potential value measured using a potentiometer. The value of the Nernst factor is more ideal if it is close to the value of 29.6 mV.dekade^{-1} [16].

Table 1. Measurement of potential value, sensitivity value and LoD of biosensor

Substrate Concentration (M)	Inhibitor Concentration (mg.L^{-1})	Potential Value	
		Diazinon	Profenofos
10^{-3}	10^{-1}	50	50.7
	10^{-2}	64.8	69.3
	10^{-3}	89.5	86.1
	10^{-4}	121.1	104.2
	10^{-5}	135.7	126.5
	10^{-6}	159.9	148.7
	10^{-7}	175.8	169.4
	10^{-8}	192.9	189.6
Potential value of substrate (mV)		199.8	195.7
The reference solution (mV)		199.1	196,3
Inhibitor Concentration, 10^{-8} mg.L^{-1} (mV)		195.5	194.6
Sensitivity (mV.decade^{-1})		21.204	21.035
Linear regression equation (R^2)		0.992	0.998
LoD (mg.L^{-1})		10^{-8}	10^{-8}

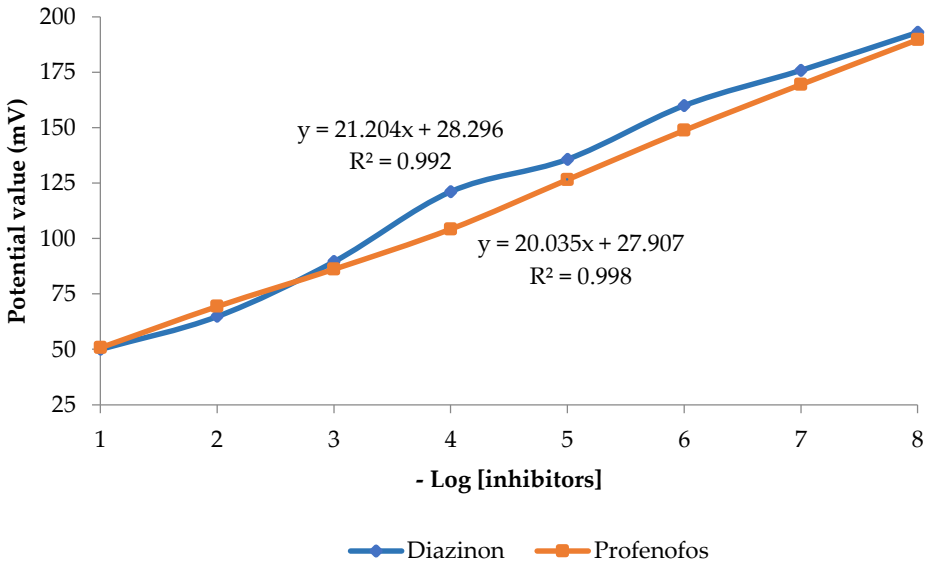

Figure 1. Graph of the relationship of –Log [inhibitors] with the potential value of biosensor based SDP

The characteristics of a biosensor are also determined by its ability to detect the concentration of an analyte. The smaller the attention that can see, the better the features of

the biosensor. Limit of Detection (LoD) is the minor analyte concentration that gives a sufficiently large signal and can be distinguished from the signal obtained from the blank with a 99% confidence level [15]. The optimum performance of a biosensor can be determined by its ability to detect the concentration of an analyte. The LoD was determined using the standard deviation of the intercept and the slope of the calibration line. Table 1 shows the LoD value of the SDP-based biosensor as a transducer, which is 10^{-8} mg.L^{-1}. So, the smaller the concentration that can detect, the better the characteristics of the biosensor [17].

Table 2. Selectivity of biosensor based SDP

a_i (mg.L^{-1})	a_j (mg.L^{-1})	Potential Value (mV)				$K_{i,j}$, Selectivity	
		a_i		a_j			
		[diazinon]	[profenofos]	[profenofos]	[diazinon]	[diazinon]	[profenofos]
10^{-5}	0	160	126.5	0	0	0	0
	10^{-9}			159.5	127.9	-0.24	0.64
	10^{-8}			158.9	126.1	-0.53	-0.19
	10^{-7}			158.6	125.9	-0.67	-0.29
	10^{-6}			158.1	124.7	-0.91	-0.87
	10^{-5}			157.4	124.5	-1.26	-0.96
10^{-4}	0	131.1	104.2	0	0	0	0
	10^{-9}			130.5	106.3	-0.29	0.96
	10^{-8}			130.1	105.7	-0.48	0.68
	10^{-7}			129.8	104.8	-0.63	0.27
	10^{-6}			129.5	103.3	-0.77	-0.43
	10^{-5}			129.1	102.2	-0.97	-0.97
10^{-3}	0	90	80.1	0	0	0	0
	10^{-9}			89.8	82.2	-0.1	0.95
	10^{-8}			89	81.9	-0.49	0.81
	10^{-7}			88.7	80.5	-0.63	0.17
	10^{-6}			88.2	79.6	-0.88	-0.25
	10^{-5}			88	78.1	-0.98	-0.98

*a_i is the concentration of the analyte/main compound, a_j is the concentration of the analyte/interference compound, $K_{i,j}$ is the selectivity coefficient

Table 3. Accuracy of biosensor based SDP

$[C'_A]$	$[C_A]$	$[C_F]$	Potential Value (mV)						Accuracy, % Recovery	
			Diazinon			Profenofos			Diazinon	Profenofos
			$[C'_A]$	$[C_A]$	$[C_F]$	$[C'_A]$	$[C_A]$	$[C_F]$		
10^{-2}	10^{-3}	10^{-2}	64.8	131.1	79.1	80.4	118.6	76.9	79.123	76.899
10^{-3}		10^{-3}	90.0		84.8	118.6		99.2	84.790	99.232
10^{-4}		10^{-4}	131.1		107.7	130.8		108.2	107.690	108.165
Mean of % Recovery									99.497	94.765

*C'_A is the concentration of analyte/compound added, CA is the concentration of the sample, CF is the total concentration of the sample obtained from the measurement

The determination of selectivity is carried out to determine the method's ability to measure the presence of pesticides carefully and thoroughly in interfering components.

The ideal biosensor is expected to only respond to the primary analyte to be detected. If the electrode is highly selective towards i rather than ion j, then $K_{i,j} < 1$. Conversely, if the electrode is highly selective towards j than ion i, then $K_{i,j} > 1$. The value of $K_{i,j}$ is never constant for the activity of component i and component j. Variations in the value of $K_{i,j}$ depend on the electrode's response and the environment of the elements in the solution. The selectivity coefficient value obtained is smaller than +1 (Table 2). The overall values obtained for the range of concentrations of the low nuisance components are still within tolerance. The average selectivity coefficient value received still meets the specified selectivity value standard, more excellent than -1 and more minor than +1, so $K_{i,j} < 1$, is a very selective electrode for pesticide detection compared to interfering compounds [18].

Accuracy is a measure that shows the degree of closeness of the analysis results to the actual analyte content. Accuracy is expressed as the % recovery of the added analyte. In general, the acceptance criteria for accuracy (% recovery) are 80-110% [15]. The data in table 3 shows that the average % recovery of the SDP-based biosensor as a transducer has an accuracy rate of 99.497 and 94.765% for diazinon and profenofos pesticide detection, respectively.

4. Conclusions

Based on the results and data obtained from the study of SDP-based biosensors as transducers in the detection of organophosphate pesticides, the sensitivity was 21.204 and 21.035 mV.decade^{-1}, LoD 10^{-8} mg.L^{-1}, selectivity coefficient Ki,j < 1 and accuracy of 99.497 and 94.765%. Thus, potentiometric biosensors with CA and GA membranes immobilized by AChE enzymes have good sensitivity, selectivity and accuracy in detecting the presence of organophosphate pesticides in a sample and LoD from tiny biosensors is effective for detecting at low scale and concentration.

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