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Construction and Characterization of Conductometric Biosensor for Determination of the Diazinon Concentration

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Abstract

Excessive diazinon residue in vegetables can endanger human health. Therefore, a simple, fast, and accurate method is needed to detect residue. A conductometric biosensor is a good choice because it also offers high selectivity and sensitivity. The principle of detection of the conductometric biosensor is based on enzymatic hydrolysis of diazinon into O,O diethyl phosphorothiate,2-isopropyl-6-methylpyrimidin-4-ol, and H^+ catalyzed by organophosphate hydrolase (OPH). The optimum amount of organophosphate hydrolase added to the screen-printed carbon electrode (SPCE) modified with BSA-glutaraldehyde is 118.5 µg, while the optimum pH is 8.5. This biosensor has a response time of 30 sec, a linear dynamic range of 0 to 1 ppm, sensitivity of 42.21 µS/ppm, and limit of detection of 0.19 ppm.

Abstrak

Pembuatan dan Karakterisasi Biosensor Konduktometri untuk Penentuan Konsentrasi Diazinon. Residu diazinon yang berlebihan pada sayur-sayuran dapat membahayakan kesehatan manusia. Oleh sebab itu, diperlukan suatu metode yang sederhana, cepat, dan akurat untuk menentukan kadar residu diazinon tersebut. Salah satu pilihan yang tepat adalah biosoensor konduktometri karena biosensor ini juga menawarkan kepekaan dan selektifitas yang tinggi. Prinsip deteksi dari biosensor konduktometri didasarkan pada pengukuran daya hantar larutan hasil hidrolisis diazinon menjadi O,O dietil fosforotioat, 2-isopropil-6-metilpirimidin-4-ol dan H^+ yang dikatalisis oleh enzim organofosfat hidrolase (OPH). Jumlah enzim optimum yang ditambahkan pada SPCE yang dimodifikasi oleh BSA-glutaraldehida adalah 118,5 µg, sementara pH kerja optimum adalah 8,5. Biosensor ini memiliki waktu respon 30 detik, kisaran konsentrasi linier 0 hingga 1 ppm, kepekaan 42,21 µS/ppm dan batas deteksi 0,19 ppm.

Keywords: BSA-glutaraldehyde, conductometric biosensor, diazinon, organophosphate hydrolase, SPCE

1. Introduction

Diazinon is an organophosphorus compound widely used as a pesticide. Excessive use of diazinon can leave residues that endanger human health. It can inhibit acetylcholine esterase activity in the nervous system, which leads to serious symptoms and death [1]. Therefore, determining the diazinon residual concentration in agricultural products is important.

General methods for determining the concentration of diazinon and other organophosphorus compounds are gas chromatography (GC) and high-performance liquid chromatography (HPLC). However, these methods require high operation cost and complicated optimization. A biosensing method using a biosensor overcomes the disadvantages of GC and HPLC. A biosensor offers many advantages: It is highly selective and sensitive, easy to use, fast, and portable [2-3].

A biosensor is a device incorporated with biological sensing elements (enzymes, antibodies, microbes, etc.) that are connected to a transducer. Depending on the transducer, biosensors are electrochemical, optical, piezoelectric, or thermal. The most common biosensor used for determining organophosphorus compound concentrations is the electrochemical biosensor. Electrochemical biosensors are potentiometric, amperometric, field effect transistor (FET), or conductometric. We used a conductometric biosensor because it has a small electrode, has good sensitivity, does not require an alternating current (AC) power supply or a standardized

reference electrode, and has the potential to be produced in large scale due to its low cost [3-6].

Diazinon concentrations are determined based on conductance measurements when diazinon hydrolyzes into O,O diethyl phosphorothioil, 2-isopropyl-6-methyl-pyrimidin-4-ol [5], and H+ catalyzed by organo-phosphate hydrolase (OPH). OPH is immobilized on the surface of the electrode so it can be used multiple times.

In this research, the OPH immobilization method is adsorption. This method is simple and does not significantly change the activity of OPH [6]. The immobilization medium for this method is a screen-printed carbon electrode (SPCE) modified by bovine serum albumin (BSA) and glutaraldehyde (GA) [7-11]. OPH is immobilized at the negative pole of the SPCEs because the target molecule of measurement is H^+ (because H^+ provides the highest conductivity compared to other ions).

Temperature, pH, and the amount of the enzyme influence enzyme activity. Therefore, they also affect biosensor performance. The optimum temperature and pH for free OPH is 50–55 °C and 9, respectively [12]. However, immobilized OPH has different optimum conditions [13]. In this study, the temperature remained constant at room temperature to avoid excessive evaporative losses during the course of the experiment and ease of operations [13]. The main objectives of this research are to study the effect of pH and the amount of enzyme on the biosensor performance as well as analyze the biosensor at optimum conditions. The amount of enzyme was studied at the biosensor constructing stage while the pH was studied during the characterization of biosensor. The second objective of this research was to see whether this device is applicable in vegetable samples.

2. Experiment

Reagents and materials. Diazinon, glutaraldehyde, NaOH, tris (hydroxyl methyl amino methane), concentrated HCl, $CuSO_4.5H_2O$, $(NH_4)_2SO_4$, KH_2PO_4, sucrose, distilled water, Na-K-Tartrate, $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, Na_2EDTA, $ZnSO_4$, yeast extract, bovine serum albumin, and organophosphate hydrolase isolated from *Pseudomonas aeruginosa* based on reported protocols were used [14].

Construction of biosensor. About 10 µL of BSA (5% w/v) was added on the SPCE surface and then 10 µL 0.1% (v/v) glutaraldehyde was added. The SPCEs were dried in the oven at 40 ^oC for 1 h, and then cooled at ambient temperature for 10 min. Afterward, 39.5 µg of OPH was added on the membrane surfaces (BSA+ glutaraldehyde) and stored in the refrigerator for 24 h. The same procedure was repeated for adding 79.0, 118.5, 158.0, and 197.5 µg OPH. The unmodified SPCE was connected to the positive pole of the multimeter. Meanwhile, the modified SPCE (BSA + glutaraldehyde + enzyme) was connected to the negative pole of the multimeter.

Measurement of solution's conductance. The biosensor electrodes were immersed in standard diazinon solutions (which ranged from 0 to 1 ppm) or sample solutions. Then, the resistance was measured every 15 sec for 1 min. The resistance was then converted into conductance. The optimum experimental conditions and characterization of the biosensors were determined from the measurement results.

3. Results and Discussion

Construction of enzyme biosensor. Several factors that influence biosensor performance can be studied at the biosensor manufacturing stage. One factor is the amount of OPH added to the surface of the BSA-glutaraldehyde membrane. The effect of the amount of OPH on the biosensor performance is determined by evaluating the sensitivity of the biosensor with different amounts of OPH. Sensitivity shows the change in signal per unit change in the amount of analyte. A biosensor with higher sensitivity can distinguish the signal between two different samples better than a biosensor with lower sensitivity. The sensitivity calculations are summarized in Table 1.

Biosensor A (without enzyme) has the lowest sensitivity, which indicates that the conductance is relatively constant although there are increases in diazinon concentration. This phenomenon proves that OPH plays an important role in the enzyme biosensor.

The increased sensitivity from biosensor B to D is attributed to the increase in the amount of OPH. The increase in the amount of enzyme by two times (from B to C) should be followed by an increase in sensitivity by

Table 1. The Effect of the Amount of Enzymes on Biosensor Sensitivity *

Biosensor Code**	Amount of OPH (µg)	Sensitivity (µS/ppm)	S_D of Sensitivity (µS/ppm)
A	0.0	1.23	1.74
B	39.5	13.71	5.70
C	79.0	19.04	3.05
D	118.5	22.89	1.86
E	158.0	14.72	2.64
F	197.5	20.16	2.44

* pH = 9, adjusted using Tris-HCl buffer

** These are just a label to distinguish biosensors with different amounts of OPH

two times. This logic is based on the following equation [6]:

$$v = \frac{d[P]}{dt} = \frac{-d[S]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_M + [S]} \quad (1)$$

K_M is the Michaelis-Menten constant, $[E_0]$ is the initial enzyme concentration, [S] is the substrate concentration, [P] is the product concentration, and [ES] is the enzyme-substrate complex concentration. The K_M of OPH is 0.45 mM, or equal to 136935 ppm. This value is very large compared to the maximum concentration of diazinon used in this study (the maximum concentration is 1 ppm). Therefore, the substrate concentration can be ignored:

$$v = \frac{k_2[E_0][S]}{K_m} \quad (2)$$

$$v = K_{total}[E_0][S] \quad (3)$$

Equation (3) shows that increasing the amount of enzyme increases the hydrolysis rate of diazinon. In this study, the conductance measurements were performed in the same time frame. Thus, at the same diazinon concentration, the signal produced by a larger amount of enzyme will be greater than a smaller amount. Consequently, the sensitivity would have been increased by two times. However, the results show that the increase in the amount of enzyme is inconsistent with the increase in sensitivity. The cause of this problem is probably the inhomogeneous pore size of BSA-glutaraldehyde (Fig. 1A). Even though almost all of the surface of BSA-glutaraldehyde is covered by OPH (Fig. 1B), it does not guarantee that all the OPH molecules added at the surface are immobilized. This phenomenon weakens the signal produced, leading to a decrease in performance.

Increasing amounts of OPH should increase the sensitivity (even though the increment is inconsistent). However, that logic is not necessarily true. The performances of biosensor E are lower than biosensor D although biosensor E has more enzyme. This phenomenon shows that an excessive amount of enzyme can inhibit the diffusion of the diazinon hydrolysis product to the transducer. The signal produced weakens, thus leading to a decrease in performance.

The biosensor with the best performances was biosensor D, with sensitivity of 22.89 µS/ppm. Therefore, the optimum amount of OPH is 118.5 µg. This amount was used as the dependent variable to characterize the biosensor.

Characterization of biosensor. The acidity level (or pH) causes a change in OPH activity [12]. Therefore, pH also affects biosensor performance. The biosensor's sensitivity at pH 7.5 to 9.5 is shown in Table 2. The lowest performance occurred at pH 7.5. The optimum pH was 8.5 although it had lower sensitivity than pH 9.0. pH 8.5 has a better standard deviation than pH 9.0.

Changes in pH affect biosensor performance in two ways. The first is the nature of diazinon itself. The hydrolysis rate constant of diazinon can change due to the change in pH. The hydrolysis rate constant increases abruptly at pH > 10.0 and pH < 4.0 [5]. Therefore, the effect of the nature of diazinon at range 7.5 to 9.5 contributes only a little to the change in performance. The second is the nature of OPH. A change in pH leads

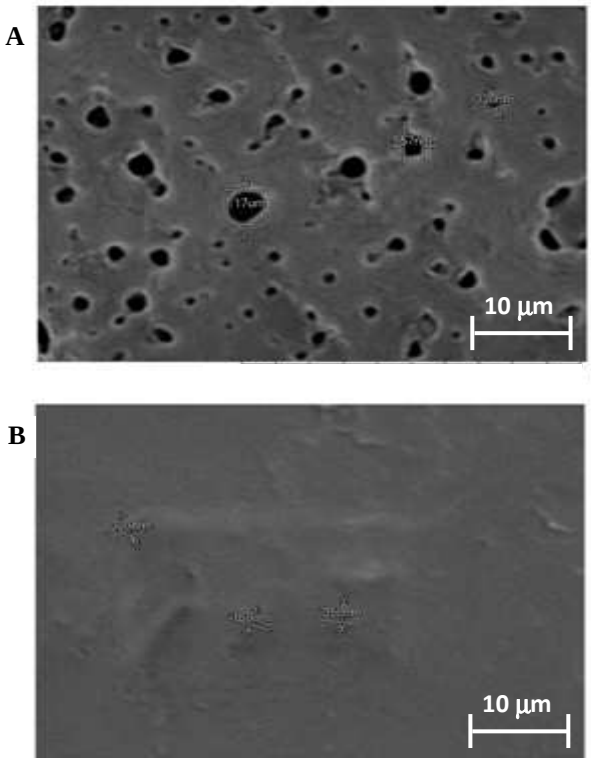

Fig. 1. Scanning Electron Microscope Image of BSA-glutaraldehyde Surface without OPH (A) and with OPH (B)

Tabel 2. Effect of pH on the Sensitivity of the Conductometric Biosensor*

pH	sensitivity (µS/ppm)	S_D of sensitivity (µS/ppm)
7.5	17.22	4.08
8.0	18.55	3.98
8.5	29.35	2.02
9.0	31.83	6.42
9.5	26.49	4.27

* Amount of OPH = 118.5 µg

to a change in charge distribution [15] because there are many amino acid residues with different pKa. The change in charge distribution can affect the geometric structure of the enzyme [16], especially the structure of an active site. Altered active sites affect OPH activity, and thus the biosensor performance.

The optimum pH for analysis is 8.5 instead of 9.0. It has a sensitivity of 29.35 µS/ppm with a standard deviation of 2.02 µS/ppm. The optimum pH shifted from 9.0 (free OPH [12]) to 8.5 shows that immobilization can affect structural changes in OPH. Therefore, pH 8.5 was used to analyze organophosphate concentrations in vegetables.

Application of biosensor. Biosensors must be characterized at optimum conditions before being applied in vegetable samples. One purpose is to construct a calibration curve for determining the concentration of diazinon in vegetables. The enzyme biosensor was characterized using a biosensor with 118.5 µg of OPH at pH 8.5. The performance parameters (at optimum conditions) were response time, linear dynamic range, sensitivity, and limit of detection [13].

The response time was 30 s. Therefore, the measurement must be performed at least 30 s before the signal is recorded. Linear dynamic range, sensitivity, and limit of detection were determined by signals that showed at least 30 s. Sensitivity of biosensor was 42.21 µS/ppm, so it can distinguish a 0.1 ppm difference by about 4 µS conductance change. The degree of confidence used in this study was 99.87%, which is 3 times the standard deviation of the blank [17]. Therefore, the limit of detection of this biosensor was 0.19 ppm. The diazinon concentrations in vegetables are shown in Table 3.

The vegetable with the most residue was spinach. However, this result was obtained from the solution used to immerse the vegetables, not from its leaves. The amount of residue (from the leaves) that come into human body should be less than the amount shown in Table 3. Thus, it is very important to wash vegetables before eating or cooking them. The amount of residue in cabbage was below the limit of detection. Therefore, the amount of residue in cabbage is unknown.

Tabel 3. Concentration of Diazinon Residue in Vegetable Samples

Vegetables	Concentration of residue in 5 gram samples (ppm)
mustard	0.9
spinach	1.5
cabbage	< LOD
kale	0.5
lettuce	0.5

4. Conclusions

The construction of a biosensor is affected by the amount of OPH added on the SPCE-BSA-glutaraldehyde, while the biosensor is affected by the pH of the buffer. The optimum amount of enzyme and optimum pH are 118.5 µg and 8.5, respectively. This conductometric biosensor has a response time of 30 sec, a linear dynamic range of 0 to 1 ppm, sensitivity of 42.21 µS/ppm, and limit of detection of 0.19 ppm. The biosensor can detect diazinon residues in all vegetables except cabbage.

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