TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Optimization Assay of Enzymatic Biosensors for Determination of Carbaryl Pesticides

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Abstract

Pesticides are chemicals used worldwide to destroy or control insects, fungi, and other pests. In agriculture, farmers use numerous pesticides to protect seeds and crops. Application of pesticides compounds has indeed significantly increased the yield of agricultural products such as vegetables and fruits. The excessive use of pesticides somehow negatively affects both human and environment. The bioaccumulation characteristic has allowed them to accumulate and remain persistent in the environment for a long period. The presence of pesticides in the environment is particularly hazardous, and prolonged exposure may leads to several health problems like asthma attacks, skin rashes and neurological diseases. Carbaryl is one of the most widely used pesticides due to its powerful effect and low cost. At present, pesticides are detected through conventional analytical techniques. However, such techniques requires high skills personnel, expensive instruments and time-consuming. A demand for simple, fast and effective method is necessary for pesticide detection. This lead to the development of enzymatic biosensor which the objective is to immobilize butyrycholinesterase enzyme based on chitosan onto the glassy carbon electrode via cross-linking with glutaraldehyde. Optimization of the experimental parameters for the biosensor performance was conducted using cyclic voltammetry which includes pH, time, scan rate and the effect of methylene blue. Upon the optimizations, it found that pH7 of electrolyte solution, 40s of response time and 50mVs^{-1} was identified to provide the optimum conditions for the proposed biosensor that potentially can be used as a tool for pesticide detection. The optimized parameters will be employed for further experiments for designation of sensitive enzymatic biosensor for detection of pesticides from the vegetables.

Keywords:
Biosensor; Butyrylcholinesterase; Carbaryl; Chitosan; Pesticide

Introduction

The wide uses of carbamate pesticide in modern agriculture are due to its low persistence in environment and high insecticidal action (Amine *et al.*, 2006; Rekha *et al.*, 2000). Despite their less persistence in environment, carbamate pesticide possessed with toxicity which can cause serious problem to the environment and food safety. The residue of this pesticide accumulated in soils, foods and other form of matrices have commonly detected using several method including gas chromatography with mass spectrometry (Hu *et al.*, 2013; Su *et al.*, 2011), flame photometry detection

(Hu *et al.*, 2013) and liquid chromatography with fluorescence/UV (Lawrence *et al.*, 1976), diode-array (Valencia & de Llasera, 2011) and mass spectrometry detection (Alves *et al.* 2012). Such mentioned methods offer high sensitivity, reliability and precision. Nevertheless, these conventional methods requires highly skilled personnel, expensive instruments and time-consuming. In order to solve the problems, enzyme based biosensor technique is introduced which able to overcome the limitations of the conventional methods.

The toxicity of carbamate pesticide is shown by their ability to inhibit the activity of acetylcholinesterase (AChE), a key enzyme in human and animal nervous system. This AChE enzyme catalyze the hydrolysis of neurotransmitter acetylcholine (ACh) into choline and acetic acid. In the presence of carbamate pesticide, it will cause the hydrolysis of neurotransmitter acetylcholine (ACh) failed. Thus, high accumulations of acetylcholine lead to abdominal cramps, muscular tremor, hypotension, breathing hardship, slow hearbeat and ultimately death [3].

Although the physiological role of BChE is still unknown, this enzyme can hydrolyzes butyrylcholine (BCh) and other esters such as ACh. These native substrates can be replaced by the acetylthiocholine (ATCh) (Su *et al.* 2011; Alves *et al.* 2012) and butyrylthiocholine (BTCh) (Hu *et al.* 2013; Lawrence *et al.* 1976; Valencia & de Llasera, 2011) producing the electroactive species of thiocholine and corresponding carboxylated acid after hydrolysis in a similar proportion as the original substrates. The quantification of pesticide is determined based on the enzymatic inhibition of cholinesterases (ChE) with the presence of pesticide. In this study, an optimization assay for carbaryl pesticide determination was described and an enzyme based biosensor was constructed with the crosslinking strategy based on glutaraldehyde (GA) for immobilization of BChE on a chitosan (CS) modified electrode. Cyclic voltammetry (CV) method was applied to characterize the sensor electrochemically.

Methodology

Materials

BChE (246 units/mg solid) from equine serum and glutaraldehyde were purchased from Sigma. BTCh chloride and chitosan obtained from Aldrich. Bovine serum albumin, carbaryl were obtained from Sigma-Aldrich. The mediator, methylene blue and PBS solution (di-Sodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) were from Systerm. GCE (disk diameter 3 mm) obtained from Metrohm–India (Delhi, India) were used. All other chemicals were of analytical reagent grade. Double distilled water (DW) was used throughout the experiments.

Apparatus

Electrochemical measurements were carried out on an electrochemical analyzer Potentiostat Galvanostat Autolab machine. Three electrode system with a glassy carbon electrode as working electrode (3mm), a platinum wire counter electrode and a silver/silver chloride reference electrode are employed. All experiments were performed in an electrochemical cell filled with 20ml of 0.1M PBS with 0.5mM BTCh chloride at room temperature.

Preparation of the electrode

The bare GCE was polished with alumina slurry (diameter 0.05 mm) and then cleaned ultrasonically in water, followed by thorough rinsing with DW. The cleaned electrode was dried in air. To prepare the enzyme electrode, 5 µl of Chit solution (CS, 2% w/v) was placed on the surface of unmodified GCE, followed by 5 µl of a mixture of BChE (0. 1 mg/ml) and BSA. Then, 3 µl of glutaraldehyde solution (GA, 0.25%) was placed to cross-link with the immobilized enzyme. The prepared electrode was dried in air for 3 to 4 hours prior to measurement.

Measurement procedure and optimization of parameters

A cyclic voltammogram of the prepared electrode was recorded in the potential range of -1.0 to +1.0 V at a scan rate of 50 mV s^{-1} versus Ag/AgCl as reference electrode and Pt as counter electrode in 20 ml of 0.1 M phosphate buffer (pH 7.0) containing 0.5mM of BTCh chloride. Before the measurement, the pretreated BChE/CS/GA/GCE was incubated with carbaryl solution using dropping method for 10 min incubation time. Then, the BChE/CS/GA/GCE was transferred into the electrochemical cell of 20 ml 0.1M PBS (pH 7.0, containing 0.5mM BTCh chloride) to study the electrochemical response of the inhibition on BChE by carbaryl.

Result and discussion

Effect of methylene blue

Methylene blue (MB) is a chemical used as a redox indicator. MB solution will increase the electron transfer rate in electrochemical biosensor. Figure 1 shows the CV of BChE biosensor with MB (blue) and without MB (red) in 0.1M PBS (pH 7) containing 0.5mM BTCh chloride. BChE biosensor was applied with MB for further experiments conducted.

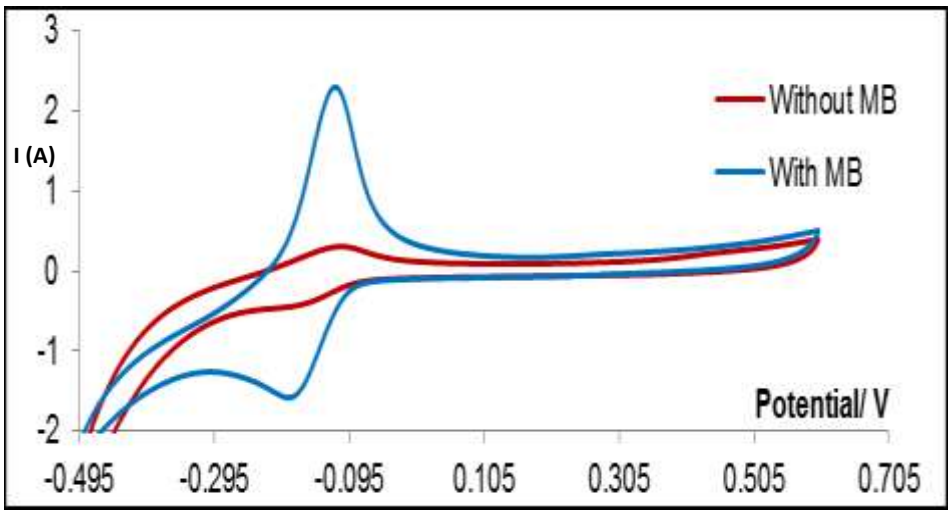

Figure 1. CV of GCE with (blue) and without (red) MB in 0.1M PBS (pH 7) containing 0.5mM BTCh chloride.

Effect of pH

The pH plays a significant role in obtaining good analytical performance. The response currents of the enzyme electrode were investigated in the ranges of PBS (0.1M, pH from 6 to 8) including 0.5 mM BTCh chloride. Figure 2 presents the cyclic voltammetry response of the enzyme electrode at different pH buffer solution. The highest peak value was at pH 7. Thus, a pH 7 was selected as the optimum pH for the experiments.

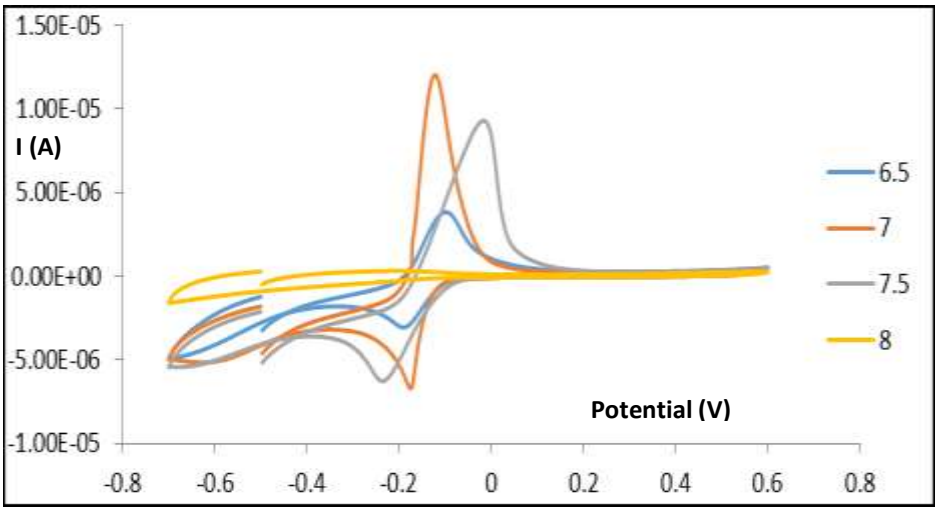

Figure 2. CVs of BChE/CS/GA/GCE in 0.1 M PBS containing 0.5 mM BTCh chloride at different pH values from pH 6.5 to pH 8.0.

c. Effect of response time

The effect of response time on the performance of biosensor was studied and the result was shown in Figure 3. The maximum value of the response current was at 40s and was used in the subsequent experiment.

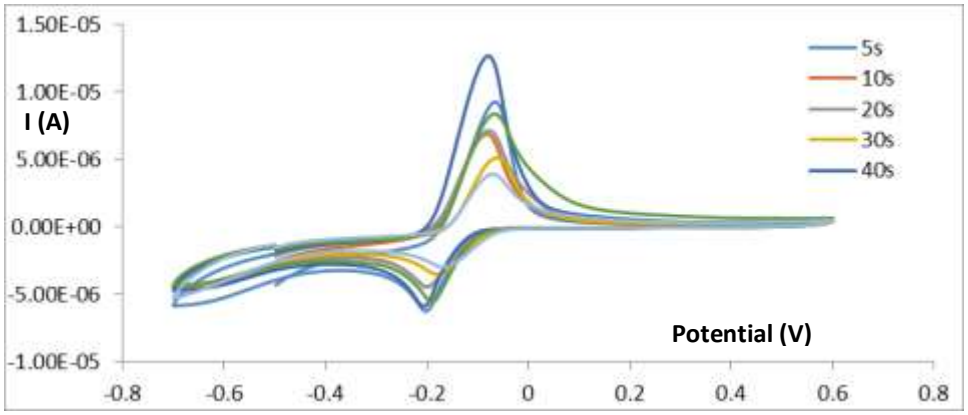

Figure 3. CVs of BChE/CS/GA/GCE in 0.1 M PBS containing 0.5 mM BTCh chloride at different response times from 5 to 60 s.

d. Effect of scan rate

The effect of scan rate on the voltammetric response of BTCh chloride at BChE/CS/GA/GCE has also been investigated in the series applied scan rate (from 20 to 70 mVs^{-1}). The maximum peak obtained at the scan rate of 50 mV s^{-1} just slightly above the 40 mV s^{-1} as seen in **Fig. 4** and was chosen for further experiments.

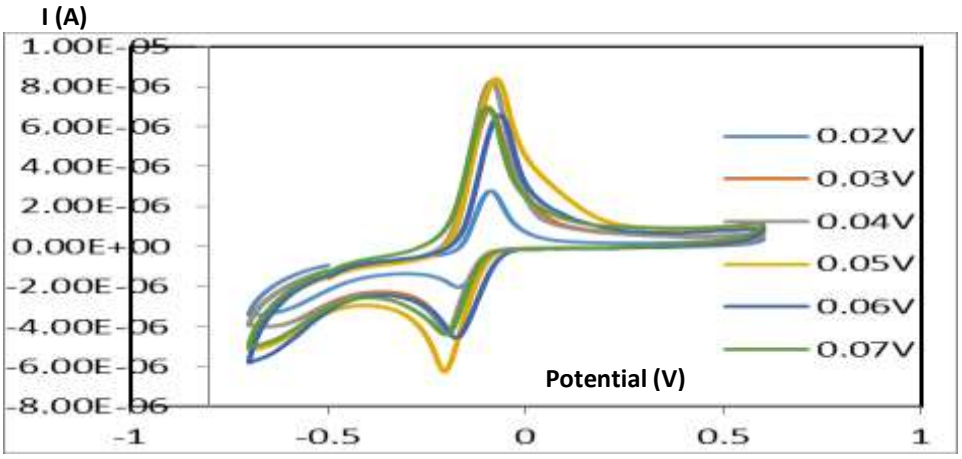

Figure 4. CVs of BChE/CS/GA/GCE in 0.1 M PBS containing 0.5 mM BTCh chloride at different scan rates from 20 to 70 mV s^{-1}.

Conclusion

In summary, an optimization assay for carbaryl detection was carried out successfully based on the crosslinking of BChE on a chitosan modified electrode through glutaraldehyde reagent. Several experimental parameters such as pH electrolyte, response time and scan rate was optimized. Additionally, the effect of methylene blue was also tested. The optimized parameters will be employed for further development of sensitive biosensor for carbaryl determination. The developed biosensor will be applied in the real vegetable samples. This will be very useful in order to ensure the consumed vegetables are safely used by the consumers.

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