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Electrosynthesis of a poly(1,5-diaminonaphthalene) – polypyrrole nanowire bilayer for trichlorfon insecticide biosensing

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Abstract

In this study, an acetylcholinesterase enzyme biosensor was developed based on a bilayer of poly(1,5-diaminonaphthalene) and polypyrrole nanowire structures modifying carbon screen-printed electrodes (SPEs). A polypyrrole nanowire inner layer was electrodeposited on the surface of SPEs to enhance conductivity and specific areas. A poly(1,5-diaminonaphthalene) outer layer was used for immobilizing acetylcholinesterase through glutaraldehyde agent. On the basis of the inhibition of organophosphate pesticides on the enzymatic activity of acetylcholinesterase enzyme, the acetylcholinesterase-immobilized bilayer of the conductive polymer electrode was designed for electrochemical determination of trichlorfon insecticide, one of the popular organophosphate pesticides.

Keywords. Acetylcholinesterase, biosensors, poly(1,5-diaminonaphthalene), polypyrrole nanowire, trichlorfon.

1. INTRODUCTION

Trichlorfon is a moderately toxic organophosphate pesticide. It is used to control insects on numerous crops. However, the contamination of trichlorfon insecticide present in the eco-systems creates a lot of pollution problems. Current analytical methods such as gas or liquid chromatography and mass spectroscopy have been widely used for the determination of organophosphate pesticides in common [1]. Nowadays, the food safety control problems and environmental pollution require the development of such techniques that are simpler, faster and more convenient in analysis.

Meanwhile, electrochemical biosensors in general and enzyme sensors in particular, have received considerable attention regarding the detection of pesticide residues owing to the advantages of low cost, simplicity and high sensitivity, thus making them excellent candidates for portable detection devices. In living beings organophosphate pesticides bind irreversibly to the active site of the acetylcholinesterase (AChE) enzyme [2]. Thus acetylcholinesterase (AChE) biosensors based on the inhibition on AChE have shown satisfactory results for pesticides analysis, where the enzyme activity was employed as an indicator of a quantitative measurement for such insecticides. When AChE is immobilized on the working electrode surface, its interaction with the substrate of acetylthiocholine produces the electroactive product of thiocholine. The inhibition on the enzyme system can be monitored by measuring the oxidation current of thiocholine [3].

$$\text{Acetylthiocholine} + H_2O \xrightarrow{\text{AChE}} \text{Thiocholine} + \text{Acetic acid}$$

$$2\text{Thiocholine} \xrightarrow{\text{anodic oxidation}} \text{dithio-bischoline} + 2H^+ + 2e^-$$

The immobilization of enzyme to solid electrode surface still remains a great challenge for the fabrication of biosensors. The use of conducting polymepolypyrrole (PPy) matrix for entrapment enzyme has received great attention in the past few years. PPy exhibits a high conductivity, easy preparation, and excellent environmental stability. However, it cannot attach enzyme directly due to the absence of functional groups such as carboxyl or amino-groups. To resolve this problem, a poly(1,5-diaminonaphthalene) [P(1,5DAN)] outer layer with functional amino groups was electrosynthesized onto polypyrrole nanowire (PPy NWs) which was

previously template-free electrodeposited. The proposed bilayer configuration (labeled as P(1,5DAN)/PPy NWs) performed for covalent immobilization AChE in the design of electrochemical biosensors in rapid determination of trichlorfon.

2. EXPERIMENTAL

2.1. Reagents and Apparatus

Pyrrole monomer (Fluka) was purified by distillation under nitrogen atmosphere before use. Acetylthiocholine chloride (ATCl), AChE (VI-S, 1000 IU/mg from electric eel), 1,5 diaminonaphthalene monomer, Trichlorfon ([2,2,2-trichloro-1-hydroxyethyl]-phosphonic acid dimethyl ester), glutaraldehyde (25%), Bovine Serum Albumin (BSA), $HClO_4$ and $LiClO_4$ were purchased from Sigma–Aldrich.

The 0.1 M PBS solution (pH 7.4) was prepared by mixing the solutions of 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, 0.137 M NaCl and 2.7 mM KCl. The de-ionized water was used throughout the experiments.

The electrochemical experiments were carried out with an Autolab PGSTAT302N potentiostat interfaced to a GPES 4.9 software (EcoChemie, The Netherlands) using the screen-printed electrodes (SPEs) at room temperature. SPEs were fabricated by thick-film technology with DEK Albany 247 printing machine (Weymouth, UK). SPEs with a standard three-electrode cell were printed onto polyethylene terephthalate film. The working and counter carbon-based screen-printed electrodes were prepared from the carbon ink Electrodag PF-407A. The diameter of the working carbon electrode was 3 mm. The reference electrode was printed from the silver ink Electrodag 418 S with $E_{ref} \approx +0.3$ V *vs*. standard hydrogen electrode (SHE) [4]. Finally, a last layer of a thick film curable dielectric was covered with PVC insulator, leaving a defined rectangular shaped (circular well of 5 mm diameter) working area (Fig. 1).

The surface morphology was analysed by Field Emission Scanning Electron Microscope (FE-SEM) with Hitachi S-4800.

2.2. Electropolymerization of P(1,5DAN)/PPy NWs bilayer

Firstly, the electropolymerization of PPy NWs on SPEs was carried out by using 0.15 M pyrrole monomer in 0.2 M Na_2HPO_4 in the presence of various concentration of $LiClO_4$ (from 1 mM to 15 mM) [5] under potentiostatic conditions at E = +0.75 V in 500 s. Then, P(1,5DAN) layer was covered onto PPy NWs surface in the mixture 0.01 M $HClO_4$ and 0.1 M $LiClO_4$ solution containing 5.10^{-3} M of 1,5 diaminonaphthalene monomer using cyclic voltammetry (CV). The parameters for CV: scan rate 50 mV/s; the potential range for electropolymerization is between –0.02 and +0.75 V. Afterwards, the produced electrodes (P(1,5DAN)/PPy NWs/SPEs) were washed with deionized water and incubated in PBS solution.

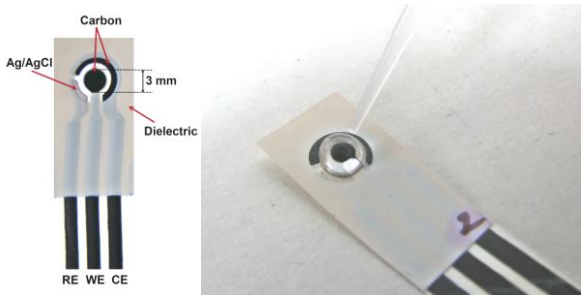

Fig. 1: The picture of the integrated screen-printed electrode

2.3. Fabrication of AChE sensors

A volume of five microliters of 2.5 % glutaraldehyde was coated on P(1,5DAN)/PPy NWs/SPEs to form the covalent bond between amino groups on P(1,5DAN) surface with aldehyde groups of glutaraldehyde agent. The modified electrode obtained was treated with 10 μL AChE solution (300 mU, containing 5 g/L BSA to maintain the stability of AChE) and kept at room temperature (25^{o}C) for 2 h for covalent linkage of the AChE to the electrode surface [6-8]. Then, the obtained sensors (labeled as AChE-P(1,5DAN)/PPy NWs/SPEs) were washed with PBS (pH 7.4) to remove the excess of AChE. The sensor was stored in the refrigerator at 4^{o}C for further use.

2.4. Electrochemical measurement

As can be seen in Fig. 1, 50 μL droplet deposited over the electrochemical microcell delimited by the circular dielectric layer. The AChE-P(1,5DAN)/PPy NWs/SPEs were tested by cyclic voltammetry (CV) in PBS solution, and the anodic current was recorded as I_0. Then, 1.0 mM ATCl solution (optimized value was determined from [3]) was injected into the microcell, the anodic current was recorded as $I_{control}$. For the measurements of trichlorfon insecticide, the PBS solution containing the different standard concentrations of trichlorfon was injected into the microcell. The anodic current of ATCl on AChE-

P(1,5DAN)/PPy NWs/SPEs with trichlorfon inhibition is I_i. The inhibition of trichlorfon (I%) was calculated as [1]:

$$I\% = [(I_{control} - I_i)/(I_{control} - I_0)] \times 100\%$$

where I_0 is the anodic current in blank solution (PBS); $I_{control}$ is the anodic current in PBS solution containing 1.0 mM ATCl substrate specificity; and I_i is the anodic current after the injection of i concentration of trichlorfon insecticide into PBS containing 1.0 mM ATCl. The anodic currents were recorded at +0.65 V

3. RESULTS AND DISCUSSION

3.1 Electropolymerization and characterization of the P(1,5DAN)/PPy NWs bilayer

The P(1,5DAN)/PPy NWs bilayer-modified electrodes were obtained as described in Section 2.2. First, the synthesis voltammograms and surface morphology of PPy NWs were given in Fig. 2.

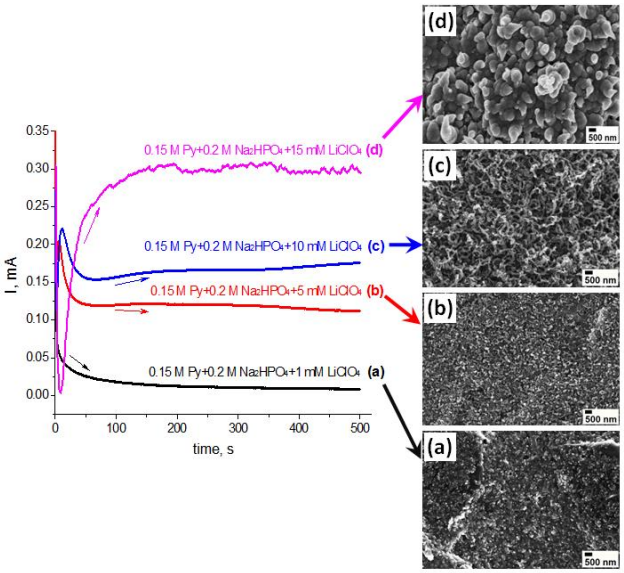

Fig. 2: Current density versus time of polarization at 0.75 V in 0.15 M pyrrole and 0.2 M Na_2HPO_4 containing (a) 1 mM, (b) 5 mM, (c) 10 mM, and (d) 15 mM $LiClO_4$; and SEM images of PPy deposits obtained, respectively

The rate of polymerization increases on further increases of the $LiClO_4$ concentration. At 1 mM $LiClO_4$, the anodic current decreases versus time to a very low value (curve a). The PPy film on the electrode is so thin and incomplete (Fig. 2a). When - $LiClO_4$ concentration (5 mM and 10 mM) increases in monomer solution, first the anodic current response increases highly then it decreases rapidly. In observations on the voltammograms, the continuous current (curve b) is slowly decreased or saturated, but the continuous current (curve c) is higher and it slightly increases with time. The morphology in Fig. 2b and Fig. 2c shows that PPy NWs generated in 10 mM $LiClO_4$ is the best. Besides, in the Fig. 2, curve d corresponding to 15 mM $LiClO_4$, the PPy cauliflower-like was observed. Thus, 10 mM $LiClO_4$ concentration was selected for polymerization of PPy NWs. This PPy NWs structure has uniform pattern, high effective surface area, and low diffusion resistance so it is a suitable candidate for the purpose of electrode surface modification [9]. A P(1,5DAN) layer was covered on PPy NWs in order to improve the ability to immobilize enzyme AChE onto the electrode surface via covalent binding. Fig. 3 shows the cyclic voltammograms (CVs) taken during the course of electropolymerization of P(1,5DAN) on PPy NWs/SPEs. The anodic current increases at the first cycle due to the oxidation of 1,5DAN monomer. The appearance of two typical redox systems (from the second cycle, at 0.23 V/0.07 V and 0.45 V/0.40 V) and the current continuously increased during scans reflecting the growth of P1,5DAN film on the PPy NWs/SPEs surface. The SEM image (inset) showed the diameter of the nanowires ranges from 80 to 100 nm.

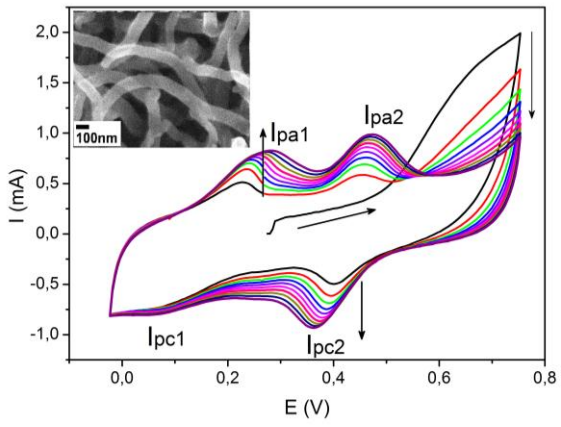

Fig. 3: Cyclic voltammograms (CVs) registered during electropolymerization of P(1,5DAN) on PPy NWs and SEM image (inset)

Fig. 4 showed CVs behavior of PPy/SPEs, PPy NWs/SPEs and P(1,5DAN)/PPy NWs/SPEs in 0.1 M PBS (pH 7.4) solution. As can be seen, the electrochemical property of cauliflower-like PPy is lower than the PPy NWs. It indicated PPy NWs have higher electrochemical activity and/or the specific surface area. Two observed redox couples are corresponding to the transformation of oxidation

state of P(1,5DAN) [10] clearly seen when compared with CV of pure PPy NWs under the same conditions. The bilayer was formed by an inner layer of PPy NWs (high specific surface and good conductivity) and an outer layer of P(1,5DAN) (where immobilization AChE via amino group), thereby promising enhancement sensitivity for biosensing.

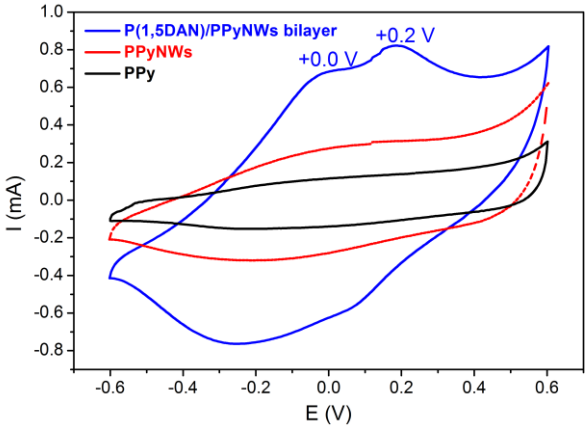

Fig. 4: CVs responses of cauliflower-like PPy, PPy NWs and P(1,5DAN)/PPy NWs film-coated SPEs in 0.1 M PBS (pH 7.4), scan rate: 50 mV/s

3.2. Trichlorfon biosensing based on AChE-P(1,5DAN)/PPy NWs

AChE-immobilized electrodes were studied by CV technique between –0.1 V and 0.75 V, scan rate 50 mV/s (Fig. 5). The cyclic voltammograms of the AChE-P(1,5DAN)/PPy NWs/SPEs in PBS solution (curve 1) and in PBS containing 1.0 mM ATCl (curve 2). The AChE-P(1,5DAN)/PPy NWs/SPEs exhibited significant electrocatalysis to the oxidation of ATCl from *ca.* +0.2 V to +0.75 V. When AChE-P(1,5DAN)/PPy NWs/SPEs were injected with standard solutions of Trichlorfon at given concentrations, the currents decreased drastically (curve 3-7) compared with the case of no inhibitor (curve 2). The current values at +0.65 V were decreased to 24.12% when the concentration was 50 ng/L (curve 3). It is due to Trichlorfon acting as one of the trichlorfon involved in the irreversible inhibition action on AChE, thus reduced the enzymatic activity to its substrate. At exposure to higher Trichlorfon concentration from 100 ng/L (curve 4) to 300 ng/L (curve 7), the anodic current decreased to 32.79% and 78.78%, respectively. The AChE activity inhibition of trichlorfon (I %) and trichlorfon concentration (ng/L) have a certain linear relationship. The regression equation was I(%) = 0.2284×C(ng/L) + 13.6 with the coefficient of determination equals 0.9715 (Fig. 5, inset).

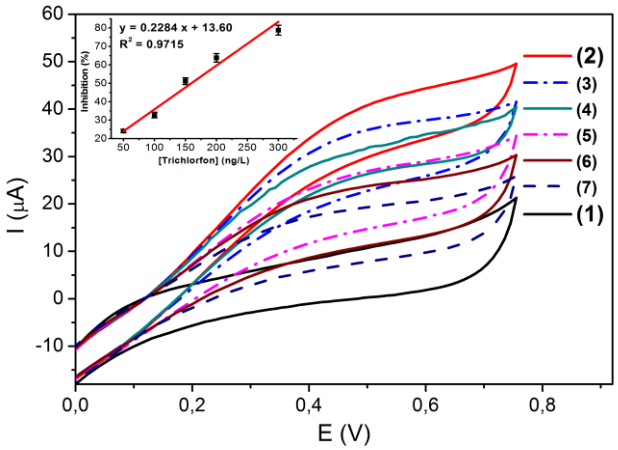

Fig. 5: CVs of AChE-P1,5DAN/PPy NWs/SPEs in PBS (1) containing 1.0 mM ATCl (the control experiment) (2) with Trichlorfon concentrations of 50 ng/L (3), 100 ng/L (4), 150 ng/L (5), 200 ng/L (6), and 300 ng/L (7). Inset: The calibration curve shows the relation between inhibitions and concentrations of trichlorfon insecticide

4. CONCLUSION

This work successfully demonstrated two-step electrodeposition of P(1,5DAN)/PPy NWs bilayer film on the integrated screen-printed electrode. The positive combination of the most outstanding aspects of P(1,5DAN) and PPy NWs exhibits the film with high specific surface area, relatively high conductivity, easy preparation and ability of covalent immobilization of biomolecules via amino groups. The disposable AChE biosensor base P(1,5DAN)/PPy NWs bilayer has been developed for the determination of Trichlorfon. These features provide scope for utilizing the methodology proposed in the present study to immobilize other biomolecules in the process of fabricating biosensors.

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