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Determination of methyl parathion in liquid phase by nano-composite carbon paste surface biosensor and differential FFT continuous linear sweep voltammetry

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ABSTRACT

A methyl parathion (MP) DNA biosensor was constructed using Au nanoparticles doped in polymeric film on nano-composite carbon paste electrode. The nano-composite carbon paste (NCCPE) was composed of ionic liquid (IL), graphene nanosheets, Pt nanoparticles and graphite. The carbon paste electrode surface was covered by polypyrrole (PPy) film. It was seen that the existence of Pt NPs causes a better electrochemically deposition of PPy on NCCPE surface. The film was then doped with Au nanoparticles, and then, it was modified by a dd-DNA. The characterization of the biosensor surface was studied by scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS) methods. The biosensor response was obtained by differential fast Fourier transformation continuous linear sweep voltammetry (DFFTCLSV) technique, in which the change of the charge under curve (in form of coulomb) in a specific potential range was calculated. The combination of nanoparticles and conducting polymeric layer catalyzed the electron transfer, which amplifies the biosensor sensitivity. The result shows that the biosensor response was proportional to analyte concentration in the range of 10 to 300 nM, with a detection limit of 1.3 nM. Experimental parameters which affect the sensitivity of the biosensors were studied. The biosensor showed good reproducibility, long-term storage stability and accuracy.

1. Introduction

Methyl parathion (MP) (Scheme 1) is one of the typical sensitive and effective organophosphorus pesticides, which is used to control harmful organisms [1]. Thus, it can release into the environment through evaporation, adsorption and scouring. MP is a toxic compound which has some negative effects for the environment. During and after its application in a farm, pesticides are transferred through wind or water. Consequently, increasing the concentrations of pesticides and their metabolites in the surface water and soils causes hazardousness due to their low solubility and bioaccumulation properties [2,3]. Also, the phosphorylation and alkylation of MP can cause neuro-toxicity and even mutation [4]. Therefore, it is vital to develop reliable, highly sensitive and easily operated method for the measurement of its concentration in environment.

Several analytical techniques have been used for the analysis of MP, including gas chromatography (GC) [5], high-performance liquid chromatography (HPLC) [6], gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS–MS) [7,8]. In this work we have tried to develop an electrochemical biosensor for determination of MP.

Carbon paste electrodes have attracted attention as electrodes because of their improved renewability, stable response, and low ohmic resistance compared to other electrodes [9–11]. Most carbon paste usually consists of graphite powder dispersed in a non-conductive mineral oil. Mineral oil has some disadvantages. Its component is not fixed because it is derived from refining of petroleum and processing of crude oil. Thus, matrix components may unpredictably effect on analysis. Ionic liquids (ILs) can be used as a binder instead of mineral oil. It can also improve the electrocatalytic activity. In fact, IL was also used as an electrode modified material in our work due to its high ionic conductivity, good stability and well biocompatibility [12–14].

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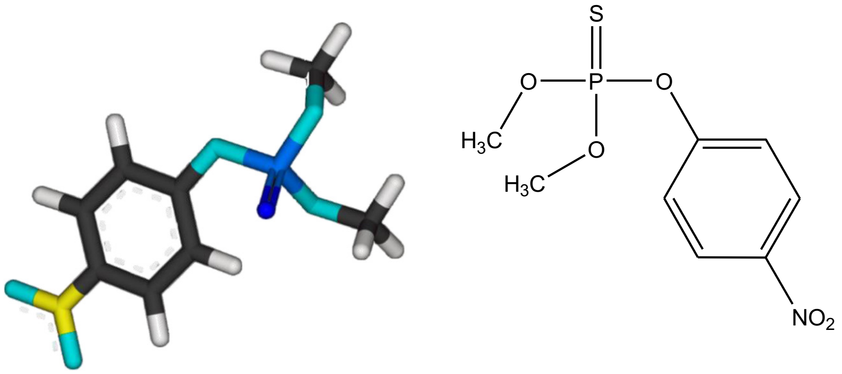

Scheme 1. Structure of methyl parathion.

Further, it is known that graphene is an allotrope of carbon, whose structure is one-atom-thick planar sheets of sp^2-bonded carbon atoms that are densely packed in a honeycomb crystal lattice. Graphene differs from most conventional three-dimensional materials. Likewise, it will be attractive to prepare carbon-based hybrid composites, because such functionalized materials may generate synergy effect and thus enhance their performance in sensing applications. Furthermore, the oxidized rings of functionalized and defective graphene sheets contain abundant C–O–C (epoxide) and C–OH groups, while the sheets are terminated with C–OH and – COOH group. Recently, its combination of IL and GNS, which create a suitable environment for biomolecule electron transfer, has attracted considerable attention in construction of sensors and biosensors [15–17].

Electrochemical polymerization is a simple, relatively inexpensive, and convenient method to prepare conducting polymers film on the electrode surface [18–20]. The use of conducting polymers, however, such as polypyrrole (PPy), as electrode materials has increased in the field of electrochemical biosensor applications [21–23]. PPy can be easily prepared from aqueous and organic solvents by either chemical or electrochemical oxidative polymerization of pyrrole. It has been proposed as suitable substrates for immobilization of biomolecules and can be used to enhance stability, speed, and sensitivity. Studies have shown that electrooxidation of PPy provides a suitable surface for DNA immobilization [24,25]. When the PPy doped with Au nanoparticles (NPs) provides a surface for adsorption of DNA, it can lead to construct a new generation of biosensor. In fact, electrochemical DNA based biosensors become especially attractive because they enable rapid, simple, and selective determination of materials.

In this work, using a mixture of Pt NPs, graphene nanosheets (GNS) and ionic liquid in construction of the carbon paste provides a condition for improving electrocatalytic activities in the electrochemical oxidative polymerization of pyrrole. Then, nano-composite carbon paste electrode (NCCPE) surface is covered by PPy film, and doped with Au NPs. Finally, it is modified with thiol-modified double-stranded DNA (dsDNA), which is adsorbed on the modified surface. In fact, concerning the optimal conditions, the biosensor exhibited a linear response to MP. The fabrication of DNA/Au/PPy/NCCPE involves two sequential stages, which are characterized by SEM and electrochemical impedance spectroscopy (EIS) methods. Application of differential fast Fourier transform continuous linear sweep voltammetry (DFFTCLSV) technique is used to measure the response of the biosensor.

2. Materials and techniques

2.1. Apparatus

The electrochemical DFFTCLSV measurements were carried out using a homemade potentiostat that was connected to a PC. An analog to digital board (PCL-818H, Advantech Co.) was used for controlling the potentiostat and data acquisition. During the experiments the computer was dictated by the condition for the data acquisition requirements electrochemical software was developed using Delphi 6.0. The program was used to generate an analog waveform and acquire current readings. The potential waveform for DFFTLSV is shown in Fig. 1. The preconcentration time was 0.5–2 s and scan rate was 5–2000 mV/s.

The potential waveform was repeatedly applied to the working electrode and then the data was acquired, and stored by the software. Also, the program was able to process and plot the data in real time. Electrochemical studies were performed using a conventional three-electrode system with a platinum wire as an auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The working electrode was a modified nano-composite carbon paste electrode with a diameter of 3.0 mm. EIS measurements were performed in 3 mM $K_3Fe(CN)_6$ in 0.05 M KCl. A stock solution of 2 mM MP was firstly prepared, and then an aliquot was diluted to the appropriate concentration.

2.2. Reagents

Thiol-functionalized double-stranded Herring sperm DNA (dsDNA; HS-$(CH_2)_6$-5′~) was obtained from local company (Nedayefan, Iran). Room temperature IL, 1-butyl-3-methyl imidazolium hexafluorophosphate ([BMIM][PF_6]) was purchased from Merck Company. MP was purchased from Sigma-Aldrich, and stored frozen. Graphene nanosheets (GNS) were purchased from local company in Iran. Graphite powder with a 1–2 μm particle size (Merck) and high purity paraffin oil (Aldrich) were used for preparation of the carbon pastes. All chemicals and solvents used were of analytical grade and were used as received. Double distilled water was used throughout the experiment. Au (50 nm) and Pt (15–20 nm) nanoparticles were purchased from Beijing Chemical Reagent Company (China). The solution 3 mM $Fe(CN)_6^{4-}/^{3-}$ in 0.05 M KCl was used.

2.3. The biosensor fabrication

At first, nano-composite carbon paste was prepared by mixing 20% wt. of 1-butyl-3-methyl imidazolium hexafluorophosphate ([BMIM][PF_6]), 5–7% wt. reduced GNS, 1–3% Pt wt. NPs, 10% wt. of liquid paraffin and 62% of graphite powder. The nano-composite carbon paste (NCCPE) was packed firmly into a glass tube ($D = 4$ mm) and the electrical contact was established via a copper wire to the paste in the inner hole of the tube. The NCCPE surface was gently smoothed on a piece of weighing paper just before use. Indeed, composition of Pt NPs and graphene was proposed to provide a good conductivity and quality of the surface for polymerization.

The electrodes were then prepared by electropolymerization of pyrrole from a 0.1 M PBS containing 0.1 M pyrrole and 0.1 M $LiClO_4$ using pulse voltammetry. A series of potential cycling was imposed from 0 to 750 mV at a scan rate of 0.5 V/s, for 9–72 s. This process was repeated

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