



# An effective surface design based on a conjugated polymer and silver nanowires for the detection of paraoxon in tap water and milk



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## ABSTRACT

In this study, a novel approach for the fabrication of a biosensor utilizing a conducting polymer and silver nanowires is reported. To obtain immobilization platform for butyrylcholinesterase (BChE), a graphite electrode was modified with the poly(5,6-bis(octyloxy)-4,7-di(thieno[3][3,2-b]thiophen-2-yl)benzo[c][1,2,5]oxadiazole) (PTTBO) which has a hydrophobic alkyl chain as the pendant group providing hydrophobic nature to the matrix. Since biomolecules contain both hydrophobic and hydrophilic parts in their structure, alkyl chains interact with the proteins which provide an enhanced stability. Biosensor performance was improved through the deposition of silver nanowires on the polymer coated surfaces which enhances the charge transfer rate. This enabled the development of rapid, highly sensitive and stable amperometric sensors for the quantitative determination of organophosphorus pesticide; paraoxon. Fabricated biosensor showed two linear ranges between 0.5–8  $\mu\text{M}$  and 10–120  $\mu\text{M}$  with a low detection limit of 0.212  $\mu\text{M}$  when butyryl thiocholine iodide is used as the substrate. Surface modifications were monitored by scanning electron microscope (SEM) and cyclic voltammetry (CV) techniques. Under optimal operational parameters, fabricated sensors were tested for paraoxon detection in milk and tap water based on the inhibition of the enzyme molecules, where recovery tests proved the applicability of the designed system.

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## 1. Introduction

Environmental monitoring and food control are the two main concerns in many research fields due to the excessive use of pesticides in agriculture, industry and chemical warfare [1]. Among the pesticides, organophosphates and carbamic pesticides are the most commonly used ones in agriculture as insecticides in order to fight pests and to increase the yield. Unfortunately, toxicity of these compounds causes serious health defects on mammals due to their inhibitory effect on cholinesterase enzyme (ChE) [2].

Blockage of the active sites of ChE by pesticides can result in disturbance of normal neuronal function, which leads to immunological diseases, nerve disorders and eventually death [3]. Since contamination of agricultural products and aquatic systems cause these lethal effects, the need for pesticide detection gains high priority. Chromatographic methods, such as high performance liquid chromatography (HPLC), gas chromatography (GC), mass-spectrometry (MS) among others have been employed for monitoring pesticides in contaminated water and food products [4–6]. Such analytical methods provide very sensitive and selective detection. Nevertheless, they have also several drawbacks as they require long analysis time, complicated pretreatment methods, qualified operators and intricate equipments [7]. To minimize these complications, cholinesterase enzymes based bioanalytical systems integrated with amperometric, potentiometric and optical methods have been employed over the last few years [8–13]. These analytical systems not only provide fast and highly sensitive detection but also are potentially applicable for use in field. Recently, enzyme inhibi-

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tion based biosensors have been employed for the detection of pesticides in many matrices [14–16]. These systems are generally constructed to determine the decrease in the enzyme activity caused by the pesticide exposure. Cholinesterase biosensors are strong candidates for pesticide monitoring.

There are two types of cholinesterases used in biosensor construction; namely acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). AChE is present primarily in the blood and neutral system whereas BChE is found primarily in liver. They are characterized by different substrate specificity. Substrate binding and catalytic mechanism of the enzymes is another route to distinguish AChE and BChE [17]. Although they have a similar structure, the enzymes have unequal sensitivity to inhibitors [18]. Arduini et al. reported prussian blue-modified screen printed electrodes to investigate inhibition effect of several carbamates and organophosphates using AChE and BChE [19]. Wei et al. developed an AChE biosensor based on ionic liquid gold nanoparticles-porous carbon composite matrix to detect dichlorvos in lettuce leaves [20]. In addition, carbon black nanoparticle modified screen-printed biosensors were developed for the determination of paraoxon utilizing inhibition of BChE [21]. Shamagsumova et al. fabricated an AChE biosensor based on glassy carbon electrodes modified with carbon black and pillar[5]arene [22]. It was tested in the analysis of aldicarb, carbofuran and malathion residuals in spiked samples of peanut and beetroot. Moreover, in our previous work, a combination of multiwalled carbon nanotubes (MWCNTs) and a functional conducting polymer were used as the substrate matrix and AChE was covalently attached to the graphite surface. The biosensor was utilized for the analysis of paraoxon, parathion and clofenvinphos in tap water samples [23].

The amperometric measurements with BChE biosensors are mainly based on the oxidation of the electroactive product thiocholine which is the hydrolyzed form of *S*-butyrylthiocholine iodide. During the reaction, one electron is transferred from thiocholine resulting in dimerization to disulphide [24]. The conversion of thiocholine to disulphide strongly depends on the surface properties of the electrode. For that reason, many mediators and various nanomaterials have been introduced in biosensor construction [25–27]. Nanomaterials enhances the efficiency of inhibitive biosensors. Sensitivity can be improved by increasing conductivity of the electrodes improving the electron transfer reaction [28]. In addition, their antifouling capabilities lead to enhanced repeatability of the biosensors. Nanomaterials also provide a decent enzyme microenvironment, thereby improving working and storage stabilities [29]. Such modifications improve the characteristic of the transducer matrix providing high electrical conductivity as well as a biocompatible environment and high surface area for enzyme loading. The key point for the wide spread application is the ease of processing of the device. In order to fabricate nanostructure based enzyme sensor, drop casting is the most popular route due to its simplicity and rapidity. However, deposited film may be inhomogeneous [29].

Silver nanowires (AgNWs) have been used for enhancing the sensitivity and reliability of the determination of target molecules in biosensor systems. As silver exhibits high conductivity, improved electron transfer rate can be obtained using AgNWs [30]. AgNWs also provide large specific surface area, effective biomolecule absorption and biocompatibility [31]. Hence, AgNWs offer improved surface modification for the biosensor fabrication.

Enzyme based biosensors constructed with immobilization techniques require an appropriate platform for enzyme deposition to maintain their biological activity measurements with high sensitivity. Conducting polymers are well suited for that purpose since they offer appropriate surface area, good mechanical and chemical stability as well as biocompatibility [32]. Moreover, they can be easily deposited onto surfaces via electropolymerization which

provides control over the morphology, thickness and homogeneity [33]. The combination of 1-D metallic arrays with conducting polymers led to a synergistic effect improving the electronic and mechanical properties of each component. The designed system combines the advantages of each component. To the best of our knowledge, there is no report on the use of conducting polymers in conjunction with the AgNWs for BChE biosensors.

Herein, we describe a BChE amperometric biosensor incorporating silver nanowires with a conducting polymer for the determination of paraoxon content in different matrices. With this aim, 5,6-bis(octyloxy)-4,7-di(thieno[3][3,2-b]thiophen-2-yl)benzo[c][1,2,5]oxadiazole (TTBO) was electrochemically polymerized on the surface of graphite electrode. While polymer film serves as an excellent immobilization platform, further improvement of the characteristics of support material was achieved by introducing AgNWs to the sensing system. Afterwards, BChE was immobilized on the modified surface using glutaraldehyde (GA) as the crosslinking agent. Surface characteristics of the enzymatic biosensor were investigated using cyclic voltammetry (CV) and scanning electron microscopy (SEM). Following the optimization of operating and experimental conditions, the system was tested for the analysis of an organophosphate; paraoxon in spiked samples of tap water and milk.

## 2. Experimental

### 2.1. Materials and methods

Butyrylcholinesterase (BChE, EC 3.1.1.8, 10 Umg<sup>-1</sup> from equine serum), butyryl thiocholine iodide, paraoxon and chemicals used for the synthesis of the monomer and electropolymerization were purchased from Sigma-Aldrich Co., LCC. (St. Louis, USA). Dichloromethane (DCM), acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany).

AgNWs were characterized using scanning (SEM) and transmission (TEM) electron microscopes. SEM studies were performed on a FEI NOVA NANO SEM 430 microscope operated at 10 kV. For the preparation of SEM samples, ethanol solution of nanowires was simply drop casted onto SEM stubs and allowed to dry. No coating was utilized for the analysis. The TEM was performed on a JEOL TEM 2100F microscope, operated at 200 kV. For the preparation of TEM samples, ethanol solution of nanowires was drop casted onto lacey carbon coated copper grids and allowed to dry.

All amperometric measurements were performed with a Compact-Stat potentiostat (Ivium Technologies B.V., Eindhoven, Netherlands) in a three electrode cell consisting of a graphite electrode (RingsdorffWerke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) as the working electrode, a platinum electrode (Metrohm, Switzerland) as the counter electrode and a Ag wire as the reference electrode. In amperometric analyzes, the data were given as the average of three measurements and standard deviations were recorded as  $\pm$ SD. All measurements were carried out at ambient conditions (25 °C). For the determination of surface characteristics, scanning electron microscope (SEM) (JEOL JSM-6400 model, Japan) was used.

### 2.2. Synthesis of the monomer

Syntheses of 1,2-bis(octyloxy) benzene, 1,2-dinitro-4,5-bis(octyloxy) benzene, 5,6-bis(octyloxy) benzo[c][1,2,5]oxadiazole, 4,7-dibromo-5,6-bis(octyloxy)benzo[c][1,2,5]oxadiazole and tributyl(thieno[3][3,2-b]thiophen-2-yl) stannane are given in supporting information. Mono-stannylated thienothiophene was obtained in high yield by lithiation with *n*-butyl lithium and

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