Talanta 82 (2010) 957–961

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Screen-printed poly(3,4-ethylenedioxythiophene) (PEDOT): A new electrochemical mediator for acetylcholinesterase-based biosensors

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ARTICLE INFO

Article history:
Received 2 April 2010
Received in revised form 25 May 2010
Accepted 30 May 2010
Available online 8 June 2010

Keywords:
PEDOT:PSS
Acetycholinesterase
Mediator
Organophosphates

ABSTRACT

This work describes the use of a PEDOT:PSS-based conductive polymer for designing AChE-based biosensors. The transducers were obtained directly by screen-printing a PEDOT:PSS suspension on the surface of thick film carbon electrodes. The obtained working electrodes showed a high conductivity when compared with electrodes modified with conventional mediators like cobalt phthalocyanine or tetracyanoquinodimethane. The PEDOT:PSS polymer was shown to be suitable for thiocholine oxidation, allowing the measurement of AChE activity at 100 mV vs Ag/AgCl. The high conductivity of PEDOT:PSS allowed the accurate detection of the organophosphate insecticide chlorpyrifos-oxon at concentrations as low as 4×10^{-9} M, corresponding to an inhibition ratio of 5%.

1. Introduction

In the last 10 years, conducting polymers have attracted much interest in the development of biosensors, and more particularly amperometric enzyme-based sensors [1]. Such materials exhibit interesting electrical properties that were previously encountered only in inorganic materials like silicon [2]. For this reason, they are often qualified as synthetic metals. Since their discovery in 1977 [3], conducting polymers have been investigated for many technological applications, such as organic lightweight batteries, microelectronics, optical displays, antistatic coatings and electromagnetic shielding [4,5]. In the field of biosensors, the main conducting polymers investigated have been polyaniline (PANI), polypyrrole (PPy) and polythiophene (PTh). These polymers are generally synthesized in situ by electrochemical polymerization, either at constant potential or using scanning or sweeping techniques [1]. In some reports, such conducting polymers have also been used as immobilization matrix [6–8], but they are generally used to dramatically enhance the conductivity of the transducer.

Poly(3,4-ethylenedioxythiophene) (PEDOT) is becoming one of the most successful conducting polymers for commercial applications, due to the interesting optical transparency in its conducting state, high stability and moderate band gap and low redox potential [9]. These extremely promising materials have traditionally been synthesized by monomer oxidation in the presence of a strong oxidant showing a high insolubility and intractability once synthesized [10], but this inconvenience was overcome using a polymer electrolyte such as poly(styrenesulfonate) (PSS) in the reaction media [11]. PEDOT:PSS dispersions consist on a conducting poly(3,4-ethylenedioxythiophene) polycation (PEDOT) and a poly(styrenesulfonate) polyanion (PSS), that having a higher molecular weight than the previous one acts as counter ion and stabilizer keeping the PEDOT chain dispersed in the aqueous medium. PEDOT:PSS polymers are generally deposited as thin films directly on the surface of the transducer by solvent casting, dip-coating, spin-coating [12] or inkjet microdeposition [13].

In this work, a chemically synthesized PEDOT:PSS is presented as a new electroactive material to be incorporated in acetylcholinesterase (AChE)-based biosensors. Several AChE-based inhibition systems have been already described for the detection of organophosphate neurotoxins by monitoring the anodic oxidation of enzymatic product thiocholine [14,15]. However, thiocholine oxidation occurs at relatively high potential that cause high background current and interference from other electroactive compounds. To overcome this problem, mediators, such as tetracyanoquinodimethane (TCNQ) [16–18], Prussian blue [19] or cobalt-phthalocyanine (CoPC) [20–22] have been successfully used to lower the detection potential. These electronic mediators are generally incorporated in a carbon paste, which is deposited on the surface of the transducer by screen-printing technology [23]. The

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doi:10.1016/j.talanta.2010.05.070

aim of this work is to demonstrate the ability of a water-soluble PEDOT:PSS to act both as a highly conductive polymer and as a mediator for thiocholine oxidation.

2. Experimental

2.1. Chemicals and stock solutions

Ethylenedioxythiophene (EDOT) monomer (99%), poly(sodium-4-styrenesulfonate) (PSS) and ammonium peroxydisulphate (APS) were purchased from Sigma–Aldrich Chemicals S.A. Acetylcholinesterase (EE-AChE) extracted from electric eel was provided by Sigma (Saint Quentin Fallavier, France). Before immobilization, enzymatic activities were measured spectrophotometrically using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB-Ellman's reagent) provided by Sigma [24]. The substrate acetylthiocholine chloride (ATCh) was purchased from Sigma. A 0.1 M ATCh stock solution was daily prepared in water and stored at 4 °C.

The organophosphorus insecticide chlorpyrifos-oxon (phosphoric acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester) (CPO) was purchased from Dr Ehrenstorfer (Augsburg, Germany). Stock solution of pesticide (10^{-3} M) was prepared in acetonitrile and stored at 4 °C.

Azide-unit pendent Water-soluble Photopolymer (AWP) was provided by Toyo Gosei Kogyo Co (Chiba, Japan).

The pastes used for screen-printing, Electrodag PE-410, 423SS and 6037SS were obtained from Acheson (Plymouth, UK), cobalt-phthalocyanine-modified carbon paste (CoPC) was purchased from Gwent Electronic Materials, Ltd (Gwent, UK). A glycerophthalic paint (Astral, France) was used as insulating layer. Transparent PVC sheets (200 mm × 100 mm × 0.5 mm) (SKK, Germany) were used as screen-printing substrates.

2.2. Methods

2.2.1. Synthesis of PEDOT:PSS dispersion

The PEDOT:PSS aqueous dispersion was prepared using an Ultrasonic Processor (model UP 400 S from Dr. Hielscher GmbH) during the synthesis. 1 mL ethylenedioxythiophene (9.4 mmol) and 3.5 g of poly(styrene sulfonate, sodium salt) were dissolved in 100 mL of distilled water. To this mixture, an equimolar amount of ammonium peroxydisulphate (6.58 g, 28.8 mmol) dissolved in 50 mL of water was added dropwise over a period of 4 min. After 1 h of reaction under ultrasonic irradiation a dark blue PEDOT:PSS aqueous dispersion was obtained.

2.2.2. Fabrication of PEDOT:PSS-modified electrodes

Screen-printed electrodes were produced using a semi-automatic DEK 248 screen-printing system (DEK, UK). The working electrode was a 4 mm diameter circle, the auxiliary electrode was a 16 mm × 1.5 mm curved line and the Ag/AgCl pseudo-reference electrode was a 5 mm × 1.5 mm straight line. The PEDOT:PSS dispersion was directly screen-printed on the working electrode or mixed with an Electrodag 423SS graphite paste (GP) in three different ratios, 4:1, 1:1 and 1:4 (polymer dispersion:GP). The final PEDOT percentage was thus respectively 100%, 80%, 50% and 20%. The electrodes were left to dry during 30 min at 60 °C.

2.2.3. Determination of sensor topography and conductivity

The topography of the working electrode was studied using a PicoPlus Atomic Force Scanning Probe Microscope (Molecular Imaging, USA) operating in tapping mode.

The electrical conductivity of the biosensor different layers (or combination of layers) was measured using 4 cm × 4.3 cm pieces of PVC substrates previously printed using the corresponding pastes. The conductivity was measured using a standard four-probe technique at room temperature using a homemade instrument (CIDETEC, Spain). All the layers, corresponding to each fabrication step, were measured at five different spots. Average data of the five measurements will be presented.

2.2.4. Immobilization of enzymes

The immobilization of enzymes was performed by physical entrapment in a polyvinylalcohol-based photopolymer (AWP), as described in previous works [20]. AWP polymer was mixed with EE-AChE solution in a ratio 1:1 (v/v). The mixture was vortex-mixed and briefly centrifuged to eliminate the foam. A volume of 3 μL was then spread onto the working electrode using a micropipette. The concentration of the initial enzymatic solution was adjusted in order to obtain a final enzyme loading of 1 mU/biosensor. The electrodes were exposed to neon lights (2 × 8 W) for 3 h at 4 °C to allow the photo-polymerization between azide groups. After drying for 48 h at 4 °C, the biosensors were ready to use.

2.2.5. Cyclic voltammetry measurements

Cyclic voltammetry measurements were performed using an AUTOLAB PGSTAT12 electrochemical station (Eco Chemie B.V., The Netherlands). The sensors based on PEDOT:PSS were tested in 10 mL of 0.1 M phosphate buffer pH 7, under soft and constant magnetic stirring (100 rpm). The oxidation peak generated upon injection of thiocholine at a final concentration 1 mM was determined by scanning the potential from −400 to +500 mV vs Ag/AgCl at a scan rate of 5 mV s^{-1}.

2.2.6. Amperometric measurements

Amperometric measurements were carried out with a 641VA potentiostat (Metrohm, Switzerland), connected to a BD40 flatbed recorder (Kipp & Zonen, The Netherlands). The measurements were performed in a 10 mL glass cell thermostated at 30 °C. The electrodes were tested in 0.1 M phosphate buffer pH 7 at four different working potentials: 50 mV, 100 mV, 150 mV and 410 mV vs Ag/AgCl. The current intensity was recorded and, after current stabilization, ATCh or thiocholine was injected to a final concentration of 1 mM. The time necessary to reach the plateau was 2–3 min. The measured signal corresponded to the difference of current intensity between the baseline and the plateau. The cell was washed with distilled water between measurements.

The pesticide detection was made in a three-step procedure as follows: first, the initial response of the electrode to 1 mM ATCh was recorded three times, then the electrode was incubated in a solution containing a known concentration of insecticide, and finally the residual response of the electrode was recorded again. The percentage of inhibition was then correlated with the insecticide concentration.

3. Results and discussion

3.1. Characteristics of PEDOT:PSS-modified transducers

3.1.1. Conductivity of PEDOT:PSS-modified electrodes

The conductivity of electrodes was shown to increase with the amount of PEDOT:PSS used. Surprisingly, the conductivity of CoPC-modified electrodes was comparable with the one of bare electrodes. The use of 100% PEDOT:PSS allowed to reach conductivities twofold higher than the one of non-modified electrodes (Table 1). As confirmed by atomic force microscopy (Fig. 1), the use of PEDOT:PSS did not lead to a significant increase of electrode specific surface. The huge conductivity of PEDOT:PSS-modified electrodes was thus directly correlated to the intrinsic properties of PEDOT:PSS polymer.

Table 1
Effect of the percentage of PEDOT:PSS on the conductivity of the developed transducers. Comparison with electrodes made of CoPC-modified carbon paste.

Electronic mediator	Conductivity (S cm^{-1})
No mediator	899 ± 114
20% PEDOT:PSS	1120 ± 122
50% PEDOT:PSS	1473 ± 270
80% PEDOT:PSS	1514 ± 243
100% PEDOT:PSS	2111 ± 310
CoPC	907 ± 34

3.1.2. Cyclic voltammetry experiments

The ability of PEDOT:PSS-modified electrodes to detect thiocholine was studied by cyclic voltammetry, by scanning the potential between −400 mV and +500 mV vs Ag/AgCl. Fig. 2 shows the voltammograms obtained using screen-printed electrodes modified by PEDOT:PSS or CoPC, in the presence of 1 mM thiocholine. The sensors modified by CoPC present an oxidation peak starting at 0 mV vs Ag/AgCl, characteristic of the mediated detection of thiocholine [22], while PEDOT:PSS-modified electrodes showed an oxidation peak at 150 mV vs Ag/AgCl. As thiocholine oxidation is generally performed at potentials around 400 mV vs Ag/AgCl [20], PEDOT:PSS can be considered as a new effective mediator for the amperometric detection of thiocholine. This observation was confirmed by studying the response of sensors modified by PEDOT:PSS towards thiocholine at concentrations varying from concentration 2 to 8 mM (Fig. 3).

3.2. Amperometric measurement of AChE-based biosensors response

AChE-based biosensors were obtained by entrapment 1 mU of EE-AChE in an AWP polymer deposited on the surface of working electrodes. The electrochemical response of the biosensors was tested by measuring the oxidation current obtained in response to 1 mM ATCh at different oxidation potentials, i.e. 50 mV, 100 mV, 150 mV and 410 mV vs Ag/AgCl. Table 2 presents the results obtained for the different types of sensors designed.

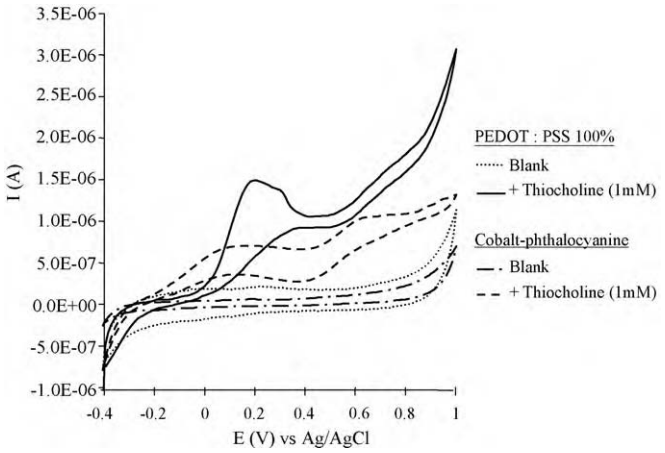

Fig. 2. Cyclic voltamograms obtained in 0.1 M phosphate buffer pH7 in absence or the presence of thiocholine 1 mM. Comparison between electrodes modified with PEDOT:PSS (100%) or CoPC (scan rate: 5 mV s^{-1}).

All the biosensors modified with PEDOT:PSS presented a maximum response at potentials higher than 100 mV vs Ag/AgCl. As expected, the highest responses were achieved using biosensors made of 100% PEDOT:PSS, the average signal being 2.5 times higher that the response of the classically used CoPC-modified electrodes.

The stability of the biosensors needs to be evaluated in order to ensure that the decrease in the signal during inhibition measure-

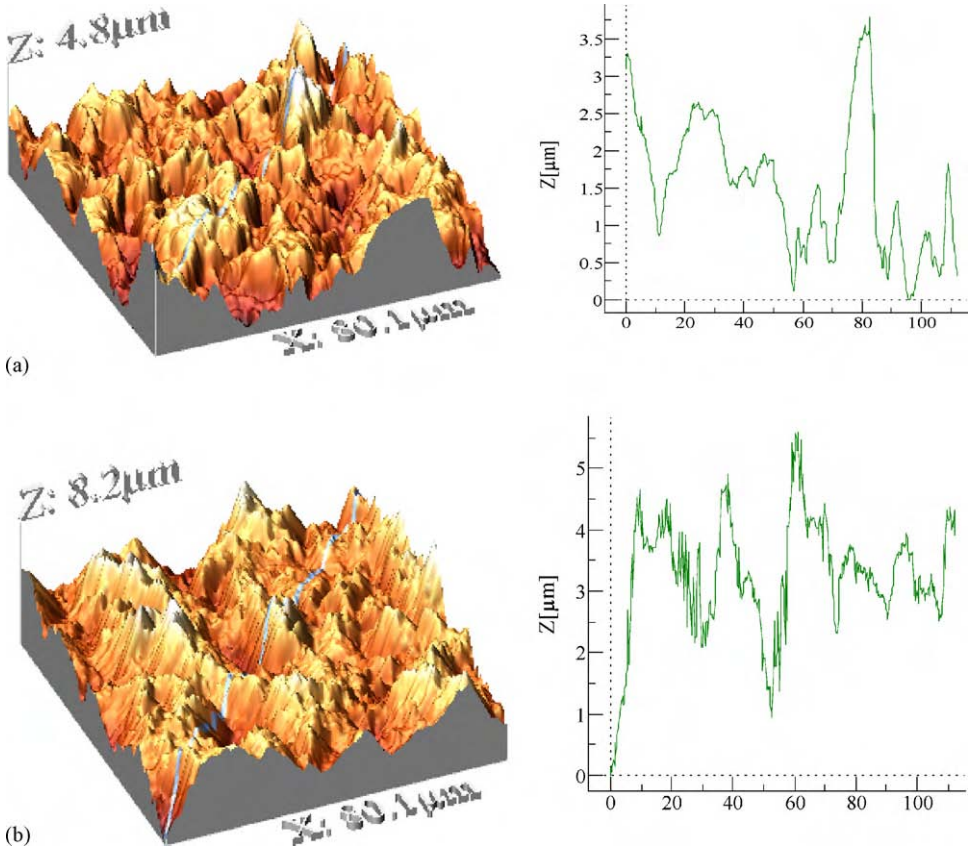

Fig. 1. Atomic-force microscopy images of the surface of electrodes modified with PEDOT:PSS (100%) (a) or CoPC (b).

Table 2
Amperometric response to ATCh 1 mM of AChE-based sensors modified with CoPC or PEDOT at different oxidation potentials (vs Ag/AgCl).

	50 mV	100 mV	150 mV	410 mV
No mediator	0	0	0	50 ± 5 nA
20% PEDOT:PSS	50 ± 10 nA	160 ± 15 nA	175 ± 20 nA	175 ± 25 nA
50% PEDOT:PSS	90 ± 10 nA	240 ± 20 nA	260 ± 20 nA	260 ± 35 nA
80% PEDOT:PSS	180 ± 15 nA	450 ± 20 nA	480 ± 20 nA	500 ± 35 nA
100% PEDOT:PSS	200 ± 20 nA	600 ± 25 nA	650 ± 30 nA	650 ± 45 nA
CoPC	250 ± 10 nA	250 ± 20 nA	250 ± 20 nA	250 ± 25 nA

Table 3
Biosensors responses and inhibition ratios obtained with various concentrations of CPO. Comparison between biosensors based on CoPC and PEDOT:PSS (100%).

CPO (M)	CoPC		PEDOT:PSS	
	Initial current (nA)	Final current (nA)/inhibition %	Initial current (nA)	Final current (nA)/inhibition %
3.3×10^{-9}	255	0	605	0%
4×10^{-9}	245	<10%	610	<5%
4.4×10^{-9}	250	<10%	600	570/5%
6×10^{-9}	265	25/10%	590	530/10%
5.0×10^{-8}	235	120/49%	585	285/51%
7.6×10^{-7}	260	0/100%	620	0/100%

ments is due to enzyme inhibition and not to enzyme leaching. The operational stability of immobilized EE-AChE was therefore estimated by successively measuring the response of the same enzyme electrode to 1 mM ATCh. It was shown that all the designed sensors displayed a maximum response for at least 10 consecutive assays, allowing inhibition measurements to be performed.

3.3. Detection of chlorpyrifos-oxon by PEDOT:PSS-modified biosensors

The incubation time of sensors with various concentrations of CPO was optimized in order to achieve the highest sensitivity of detection. It was observed that an incubation time of 10 min was sufficient to achieve the maximum inhibition percentage whatever the pesticide concentration used. The inhibition effect of CPO on EE-AChE-based sensor was thus studied using an incubation time of 10 min. The high sensitivity of the sensors modified by PEDOT:PSS allowed accurate measurements of inhibition rates lower than 10%, which is generally used to define the detection limit of sensors based on other mediators like CoPC or TCNQ [21]. The LOD was therefore considered as the pesticide concentration inducing a loss of signal of 5%, corresponding to a decrease in intensity of approximately 30 nA. Table 3 shows a comparison between the inhibition rates obtained using the classical CoPC based sensor and the biosensor with 100% PEDOT:PSS. The main difference with the classical CoPC-based sensor concerns the detection limit, the high sensitivity of PEDOT:PSS allows lowering the LOD to concentrations as low as 4.4×10^{-9} M (1.3 μg L^{-1}).

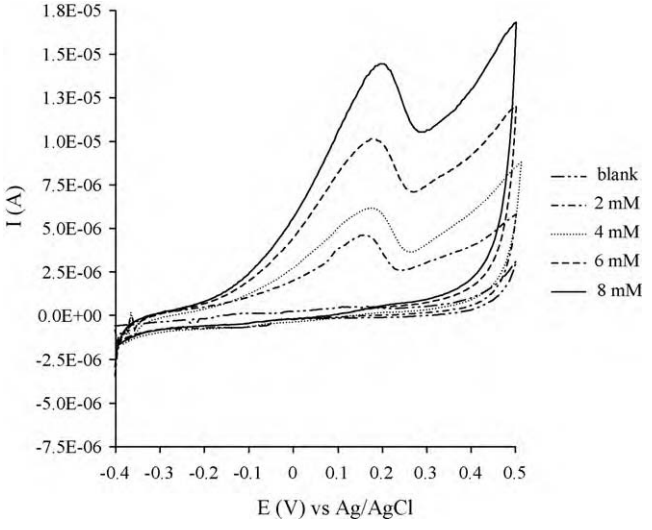

Fig. 3. Cyclic voltamograms obtained with electrodes modified by PEDOT:PSS (100%) in the presence of thiocholine at concentrations ranging from 2 to 8 mM, in phosphate buffer 0.1 M pH7 (scan rate: 5 mV s^{-1}).

4. Conclusions

This paper describes the possibility of screen-printing a conductive suspension of PEDOT:PSS on the surface of electrodes for designing AChE-based biosensors. The obtained sensors were shown to display conductivities two times higher than conventional transducers based on CoPC or TCNQ-modified carbon pastes. In addition, PEDOT:PSS was shown to be an efficient mediator for thiocholine oxidation, allowing the measurement of AChE activity at potentials as low as 100 mV vs Ag/AgCl. The high conductivity of this polymer led to high amperometric signals, allowing the accurate detection of concentrations of CPO inducing inhibition rates as low as 5%.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.05.070.

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