Journal of Electroanalytical Chemistry 735 (2014) 43–50

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Development of an amperometric biosensor based on a novel conducting copolymer for detection of anti-dementia drugs

Janset Turan [a,1], Melis Kesik [a,1], Saniye Soylemez [a,1], Seza Goker [a,1], Marit Kolb [c,*], Müfit Bahadir [c,2], Levent Toppare [a,b,d,e,*]

[a] Department of Chemistry, Middle East Technical University, Ankara 06800, Turkey
[b] Department of Biotechnology, Middle East Technical University, Ankara 06800, Turkey
[c] Institute of Environmental and Sustainable Chemistry, Technische Universität Braunschweig, Hagenring 30, 38106 Braunschweig, Germany
[d] Department of Polymer Science and Technology, Middle East Technical University, Ankara 06800, Turkey
[e] The Center for Solar Energy Research and Application (GUNAM), Middle East Technical University, Ankara 06800, Turkey

ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form 1 October 2014
Accepted 6 October 2014
Available online 17 October 2014

Keywords:
Conducting copolymer
Biosensor
Acetylcholinesterase
Anti-dementia drugs

ABSTRACT

In this study, a new amperometric biosensor was developed for the detection of the anti-dementia drugs fortified with tap water. For this purpose, electrocopolymerization of 5,6-bis(octyloxy)-4,7-di(thiophen-2-yl)benzo[c][1,2,5]oxadiazole(BODT) with (2-(((9H-fluoren-9-yl)methoxy)carbonylamino) acetic acid (FMOC) on graphite electrode was successfully achieved and used as an immobilization matrix. Acetylcholinesterase (AChE) and choline oxidase (ChO) enzyme couple was immobilized on copolymer coated graphite electrode via covalent binding with the help of carbodiimide chemistry. Changes in the responses of the proposed biosensor based on AChE inhibition were recorded using acetylcholine as the substrate. The bi-enzymatic biosensor based on conducting copolymer showed good linear detection range between 0.01 and 12.0 mM and a detection limit (LOD) of 0.014 mM to acetylcholine. Surface and electrochemical characterization were performed via Scanning Electron Microscopy (SEM) and cyclic voltammetry (CV) techniques. Moreover, the design biosensor system was tested for the detection of neostigmine and donepezil as pharmaceuticals in fortified tap water samples. Very low detection limits of 0.027 μg/L donepezil and 0.559 μg/L neostigmine were achieved. The analysis of spiked tap water proved the biosensor capability to be used. The results were found to be in good agreement with the ones determined by HPLC/DAD technique.

1. Introduction

Conducting polymers (CPs) have received a great deal of attention due to their unusual electrochemical properties such as electrical conductivity, low energy optical transitions and low ionization energy [1]. With their valuable optical and electronic properties as well as good mechanical properties, conjugated polymers have appeared as an attractive field in several application areas [2–5]. The ability to be used conducting polymers as an excellent immobilization platform for biomolecules deposition makes them considerable candidates for biosensor construction. CPs are preferred to produce a compatible microenvironment for biological reactions to mimic the naturally occurring environments of biomolecules and also enable structural and electronic modifications to accomplish desired properties [6]. Moreover, biosensors prepared using conducting copolymers have been proposed for the alternative to the traditional methods due to their number of favorable properties [7,8]. The surface characteristic of biosensor has been improved greatly after forming a two different substructure instead of separate layers. The main motivation for the construction of copolymer based biosensors is not only to show better properties than that of homopolymers but also to develop a sensitive, simple and selective tool for monitoring different kind of analytes. By this way, well organized matrices with adjustable morphology can be arranged offering extensive stability of the enzymes incorporated.

Reversible AChE inhibitors are used as pharmaceuticals (anticholinesterases) for different human diseases which are in relation to a deficit with acetylcholine (ACh). Some of them, which are containing a quaternary ammonium group such as neostigmine and pyridostigmine cannot penetrate the cell membrane or the blood–brain barrier. They act predominantly on peripheral

* Corresponding authors at: Middle East Technical University, Department of Chemistry, 06800 Ankara, Turkey. Tel.: +90 3122103251; fax: +90 3122103200 (L. Toppare). Tel.: +49 5313915960; fax: +49 5313915799 (M. Kolb).
[1] Tel.: +90 3122103251; fax: +90 3122103200.
[2] Tel.: +49 5313915960; fax: +49 5313915799.

http://dx.doi.org/10.1016/j.jelechem.2014.10.007
1572-6657/

nicotinic and muscarinic receptors and have direct action on skeletal muscle. Thus neostigmine is mainly in use for antagonism of neuromuscular blocking and myasthenia gravis [9]. Lipid soluble agents as physostigmine and donepezil (log KOW 4.9) [10] however can cross the blood–brain barrier and enter the CNS. Donepezil is one representative which is approved for the treatment of mild to moderate Alzheimer's diseases [11]. As already reported for many pharmaceuticals [12], anticholinesterases may enter the aquatic environment via wastewater discharges. In 2010, 35.6 million people were estimated to suffer from dementia worldwide [13]. This number is expected to nearly double in every 20 years. Therefore there is an indication that also the use of antidementia drugs will increase and consequently water pollution with these pharmaceuticals is expected to increase. Therefore foresight by monitoring of water compartments is indicated. A high sensitive and quick analytical method would be needed in order to detect and to monitor the presence of these substances in water compartments. So far many methods were already published for the determination of the anticholinesterases donepezil and neostigmine in biological samples like human plasma, blood, urine and drug samples using the chromatographic methods GC and HPLC combined with different extraction and clean-up methods [14–24]. These methods need high sophisticated technique equipment and are time consuming. Thus, the development of enzyme-based biosensors appears as a promising alternative to the classical methods owing to their simple measurement procedure, short response time, sufficient sensitivity and selectivity. For these reasons, biosensors based on the inhibition of cholinesterase enzyme are attractive for the detection of various analytes. So far some publications on the analysis of pharmaceuticals using biosensors have been published. Du group [25] reported that a biosensor to detect neostigmine and galantamine using a platform of sol–gel derived silicate network incorporating gold nanoparticles. Due to the inhibitions of medicines, the electrochemical response of substrate on AChE-sensor was investigated. The inhibition curves showed good correspondence with the results by UV spectrophotometry assay. The constructed sensor exhibited high sensitivity, low cost and simplified procedures. Ozturk et al. [26] reported that fluorescent pH-sensitive dyes entrapped in PVC matrix were used to construct an AChE optical biosensor. The developed sensor was utilized for both neurotransmitter and inhibitor analyses. Sensor properties like response time or repeatability were evaluated. Determination of ACh and donepezil was accomplished by the use of target biosensor design. Zamfir et al. [27] also reported that an acetylcholinesterase biosensor was developed for the detection of two carbamate therapeutic drugs based on an ionic liquid gel thiocholine sensor. The proposed biosensor exhibited very low detection limits of neostigmine. It also tested on the analysis of spiked tap water which had a good recovery degree.

Over the last decades, acetylcholinesterase biosensors have emerged as an ultra-sensitive and rapid technique for toxicity analysis in environmental monitoring, food and quality control [28]. A variety of different immobilization methods have been described for the construction of amperometric acetylcholine sensors. Two enzymes acting in an enzymatic reaction sequence is employed commonly. In the first reaction, acetylcholine is converted to choline due to the hydrolysis of AChE followed by a second reaction where ChO oxidized choline yielding betaine. Meanwhile, oxygen is converted to hydrogen peroxide upon a certain applied potential. By this way, electrochemical current is recorded as a sensor response.

Herein, a highly efficient and sensitive biosensor based on a novel conducting copolymer was developed for the detection of pharmaceuticals in the tap water. For this purpose, a conducting polymer, poly[(5,6-bis(octyloxy)-4,7-di(thiophen-2-yl)benzo[c][1,2,5] oxadiazole] poly(BODT) and poly[(2-(((9H-fluoren-9-yl)methoxy) carbonylamino) acetic acid] poly(FMOC) were synthesized electrochemically and utilized as immobilization platform of the target biomolecules. Choline oxidase (ChO) and acetylcholinesterase (AChE) enzymes were immobilized on the graphite electrode with the help of 1-ethyl-3-(3-dimethylamiopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) as the cross linking agents. Electrocopolymerization was used to enhance both the electron transfer during amperometric measurements and durability and sensitivity of the biosensor with the use of functional groups of polymers. During immobilization, enzyme molecules were covalently attached on the electrode surface with the help of carboxylic acid groups in the polymer backbone and amine groups in the enzyme couple which provide the superior stability and activity of the biosensor. Scheme 1 displays the fabrication of the amperometric biosensor. Then, optimization and characterization studies were carried out for the proposed biosensor. The goal of this work was to develop a sensitive amperometric biosensor for measurements of pharmaceuticals on the basis of their inhibitory effect on AChE activity. After investigation of the experimental conditions related to the performance of the fabricated biosensor, donepezil, and neostigmine in tap water samples were analyzed with the proposed sensor (poly(BODT-co-FMOC)/AChE-ChO). In order to validate the accuracy of the biosensor spiked tap water samples were analyzed in parallel with HPLC/DAD after enrichment with solid phase extraction (SPE).

2. Experimental

2.1. Materials and methods

Neostigmine bromide (98%) (NEO), donepezil hydrochloride monohydrate ($\geqslant$98%) (DON) were purchased from Sigma–Aldrich Co. LCC. (St. Louis, USA). Acetonitrile and methanol (Hipersolv Chromanorm®, gradient grade) were obtained from VWR International GmbH (Darmstadt, Germany), pure water for HPLC was prepared using Seralpur Pro 90 ultrapure water system (Seral Elrich Alhäuser GmbH (Bansbach-Baumbach, Germany). Stock solutions were prepared by solving 10.03 mg NEO and 7.37 mg DON in 10 mL methanol, respectively.

Acetylcholinesterase (AChE, EC 3.1.1.7, 518 U/mg from Electrophorus electricus (electric eel)), acetylcholine chloride, N-hydroxysuccinimide (NHS), $NaClO_4$, $LiClO_4$, choline oxidase (ChOx, EC 1.1.3.17, 14 U mg^{-1} from Alcaligenes sp) and FMOC was purchased from Sigma–Aldrich Co. LCC. (St. Louis, USA). N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) was purchased from Fluka (Buchs, Switzerland). Dichloromethane (DCM), acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). All chemicals needed for the syntheses of monomers were purchased from Aldrich. All chemicals were analytical grade.

For the amperometric studies and cyclic voltammetry measurements Palm Instrument (PalmSens, Houten, The Netherlands) was used. All electrochemical measurements were performed in a three electrode cell consisting of a graphite electrode (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) as the working electrode, platinum electrode (Metrohm, Switzerland) as the counter electrode and a Ag wire as the reference electrode.

2.2. Synthesis of the (5,6-bis(octyloxy)-4,7-di(thiophen-2-yl)benzo[c][1,2,5]oxadiazole) poly(BODT))

Synthesis and characterization of the monomer, BODT were achieved according to literature [29]. Alkyl chain was introduced to pyrocathecol unit to overcome solubility problem. Nitration of 1,2-bis(octyloxy)benzene was performed via electrophilic aromatic