Analytica Chimica Acta 748 (2012) 81–88

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

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

A rational design of the multiwalled carbon nanotube–7,7,8,8-tetracyanoquinodimethan sensor for sensitive detection of acetylcholinesterase inhibitors

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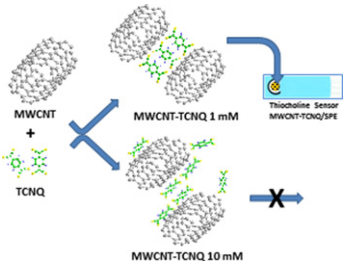
HIGHLIGHTS

- A new concept based on the interaction between MWCNTs and TCNQ was developed.
- Synergistic effect of MWCNT and TCNQ allow thiocholine detection with high sensitivity.
- Low detection limit of 7 ppt for paraoxon-methyl and 0.1 ppb for chlorpyrifos, respectively.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 March 2012
Received in revised form 13 August 2012
Accepted 28 August 2012
Available online 4 September 2012

Keywords:
Carbon nanotube
TCNQ
Acetylcholinesterase
Supramolecular arrangement
Screen printed electrode
Biosensor

ABSTRACT

A new, simple and effective amperometric acetylcholinesterase biosensor was developed using screen-printed carbon electrodes modified with carbon nanotubes (MWCNTs)–7,7,8,8-tetracyanoquinodimethane (TCNQ). The design of the biosensor was based on the supramolecular arrangement resulted from the interaction of MWCNTs and TCNQ. This arrangement was confirmed by spectroscopic and electrochemical techniques. Two different supramolecular arrangements were proposed based on different MWCNTs:TCNQ ratios. The synergistic effect of MWCNTs and TCNQ was, for the first time, exploited for detection of thiocholine at low potential with high sensitivity. The biosensor developed by immobilization of acetylcholinesterase (AChE) in sol–gel allowed the detection of two reference AChE inhibitors, paraoxon-methyl and chlorpyrifos with detection limits of 30 pM (7 ppt) and 0.4 nM (0.1 ppb), respectively. Efficient enzyme reactivation was obtained by using obidoxime.

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1. Introduction

Pesticide residues represent a major concern due to their high toxicity toward humans and animals and therefore a fast detection at low concentrations is of great importance [1]. To solve this problem several methods have already been proposed including electrochemical biosensors [2,3]. The biosensors for pesticide detection have the advantage of a high sensitivity and could be easily integrated in portable devices offering the possibility of on site measurements. The poisoning effect of organophosphate and carbamate pesticides is based on the inhibition of the acetylcholinesterase (AChE) by phosphorylation or carbamoylation of the active site [2]. The enzymatically generated thiocholine (TCh) is an electro-active compound, which can serve as an indicator to reflect the inhibition effect of pesticides. On unmodified carbon-based electrodes, TCh is oxidized at a potential as high as +700 mV *vs.* Ag/AgCl. Such a high value leads to a high background current and can cause interferences from other electroactive species

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http://dx.doi.org/10.1016/j.aca.2012.08.045

present in the sample. In order to reduce the oxidation potential of TCh, various mediators were already reported in the literature, such as potassium ferrocyanide [4], cobalt phthalocyanine (CoPC) [5], Prussian blue [6], cobalt hexacyanoferrate [7]. Highly conductive nanomaterials such as carbon nanotubes (CNTs), were also reported to decrease the TCh oxidation potential [8,9]. The use of immobilized multi-walled carbon nanotubes (MWCNTs) and AChE in polyelectrolyte multilayers has reduced the working potential to +150 mV *vs.* Ag/AgCl [8]. Also, mixtures of CNTs and imidazolium-based ionic liquids allowed detection of TCh at +50 mV *vs.* Ag/AgCl, with high sensitivity and detection limits between 0.05 and 0.08 mM TCh [10]. The sensors were further used for the detection of chlorpyrifos with a detection limit of 4 nM [11].

The discovery of the highly conducting 7,7,8,8-tetracyanoquinodimethane (TCNQ) paramagnetic salt constituted an important advancement in the development of biosensors based on AChE inhibitions [12]. Thus, several groups have used TCNQ together with various types of carbonaceous materials obtaining a significant signal enhancement for the oxidation of TCh compared with the unmodified electrodes [13–15]. However, studies on CNTs and TCNQ were scarcely reported, and they refer only to spectroscopic characterizations [12,16].

The CNTs modification with TCNQ can be made in different ways. Both doping and deposition can be envisaged as possible routes. The main advantage of noncovalent adsorption (physisorption) over covalent one (chemisorption) is that the former may modify the electronic properties of CNTs in a sizable way. However, it does not drastically change its atomic structure. Noncovalent adsorption is, in principle, easily reversible.

Since there are no chemical bonds between the adsorbed molecule and the CNTs, the chemical and mechanical properties of CNTs are only weakly perturbed [17]. Not only density functional theory and semi-empirical methods [18] but also *Ab initio* calculations demonstrated that bridge configurations represent the energetically most favorable physisorbed structures. The TCNQ–CNTs binding energy increases as a function of the nanotube radius. In accordance to this model, encapsulated systems exhibited similar binding energies [19]. The electronic charge transfers always occurs from the CNTs toward the molecule, being proportional to the TCNQ–CNTs binding energy [17]. Similar conclusions were achieved using density functional theory and semi-empirical methods [18]. Raman investigations provided evidence for this type of interaction [12]. X-ray photoelectron spectroscopy and near-infrared data have also been reported for the same purpose [16].

The biosensor analytical performance could be dramatically affected by the AChE immobilization procedure. Results using various immobilization methods: direct adsorption [20], cross-linking on chitosan–MWCNTs composite *via* glutaraldeyde [21] mixing the enzyme in polymers [22] or MWCNTs/sol–gel route [23] confirmed this fact.

This study aimed to investigate MWCNTs–TCNQ as electrode materials for the (bio)sensors development. Spectroscopic investigations were carried out in order to identify the interactions between TCNQ and MWCNTs. Electrochemical experiments allowed us to characterize and optimize the TCh sensor. In order to stabilize the catalytic activity of the AChE and prevents enzyme leaching a sol–gel network was selected for immobilization. Paraoxon-methyl and chlorpyrifos were selected as reference pesticide for testing the biosensor.

2. Experimental

2.1. Materials and reagents

Acetylcholinesterase (from electric eel, 425.94 IU mg^{-1}) acetylthiocholine chloride (ATCh, purity ≥ 99%), multiwalled carbon nanotubes (purity ≥ 95%, outside diameter = 40–60 nm, inside diameter = 5–10 nm, length = 0.5–500 μm), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium chloride, sodium phosphate dibasic, potassium phosphate monobasic, 7,7,8,8-tetracyanoquinodimethane (TCNQ), Nafion (∼5% in a mixture of lower aliphatic alcohols and water), obidoxime (1,1′-[oxybis(methylene)]bis 4-[(E)-(hydroxyimino)methyl]pyridinium), acetonitrile (ACN, purity ≥ 99.9%), tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMOS), polyethylene glycol (PEG600) were purchased from Sigma–Aldrich. Paraoxon-methyl (O,O-dimethyl O-(4-nitrophenyl) phosphate, purity ≥ 96%) was purchased from Fluka and chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate, purity ≥ 99.9%) was purchased from Riedel-de Haën. The phosphate buffer (PBS) solution 0.1 M, containing 0.1 M KCl, and the 0.1 M ATCh stock solution were prepared in ultrapure water. The AChE stock solution (66 IU mL^{-1}) was prepared in 0.1 M PBS, pH 8. The paraoxon-methyl and chlorpyrifos dilutions were each prepared in ultrapure water from a 10^{-4} M stock solution prepared in acetonitrile. The ATCh and pesticide dilutions were prepared directly from stock solutions prior to their use in measurements.

2.2. Instruments

Cyclic voltammetry, chronoamperometry measurements and electrochemical impedance spectroscopy (EIS) were carried out using a potentiostat AUTOLAB PGSTAT-12 (Eco Chemie, Utrecht, The Netherlands) and the software used to gather data was GPES 4.9. The screen-printed electrodes (SPE, Dropsens 110) consist of a three-electrode system having a carbon working electrode (4 mm diameter), a silver pseudo-reference electrode and a carbon counter electrode. Before each experiment a film of silver chloride on the silver pseudo-reference electrode was electrochemically deposited from a phosphate buffer solution in the presence of 0.1 M KCl, according to procedure reported by Arduini [24]. All experiments were carried out at room temperature (22 °C), using a 10 mL cell. Raman spectra were recorded using a Horiba Jobin Yvon-Labram HR spectrophotometer. DR-UV–vis spectra were recorded with Analytik Jena Specord 250 spectrophotometer. Elemental analysis was performed with EuroEA 3000 elemental analyzer (Euro Vector Instruments, Italy).

2.3. Modification of the screen-printed electrodes

Before any modification the SPEs were electrochemically pretreated by application of a potential of 1200 mV in sulfuric acid 0.1 M, for 2 min. Three types of screen-printed modified electrodes were prepared: SPEs modified with MWCNTs (MWCNTs/SPE), SPEs modified with TCNQ (TCNQ/SPE) and SPEs modified with MWCNTs–TCNQ (MWCNTs–TCNQ/SPE). For TCNQ/SPE, a 1 mM TCNQ solution was prepared in acetonitrile (solution A). In the case of MWCNTs/SPE and MWCNTs–TCNQ/SPE, 2 mg of MWCNTs were suspended in 100 μL acetonitrile (solution B), respectively in 100 μL TCNQ 1 mM (solution C), by sonication for 45 min in a 0.5 mL tube. A volume of 5 μL solution A, B or C were mixed with 45 μL ACN then, 5 μL from each mixture were casted on the working electrodes and left overnight to dry at 4 °C in a desiccator. TCNQ loading was 0.8 μg cm^{-2} for TCNQ/SPE and MWCNTs–TCNQ/SPE. In order to avoid the TCNQ leaching, Nafion was used only for preparation of TCh sensors. Modified electrodes with Nafion were prepared in a similar manner by replacing the ACN with Nafion (5%).

2.4. Preparation of the biosensors

The sol–gel matrix for AChE immobilization was prepared according to a method reported earlier [11] by mixing 100 μL TMOS

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