Sensors and Actuators B 240 (2017) 90–99

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Rapid and simple nanosensor by combination of graphene quantum dots and enzymatic inhibition mechanisms

E. Caballero-Díaz [1], S. Benítez-Martínez [1], M. Valcárcel *

Department of Analytical Chemistry, University of Córdoba, E-14071 Córdoba, Spain

ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form 21 August 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
N-doped graphene quantum dots
Fluorescent sensor
Enzymatic inhibition
Fenoxycarb
Acetylcholinesterase

ABSTRACT

In this paper, we report a simple, rapid and reproducible sensing approach for determination of fenoxycarb pesticide in river water based on the combined use of nitrogen-doped graphene quantum dots (N-GQDs) and acetylcholinesterase (AChE) enzyme as biorecognition element. Nanoparticles were used as fluorescent probes, while AChE was employed for inhibitor screening. The enzyme-generated products quenched the native fluorescence of N-GQDs. When fenoxycarb was introduced into the assay solution, the activity of AChE to convert substrate into products resulted to be reduced and consequently, N-GQD fluorescence was recovered gradually in an inhibitor concentration-dependent manner. Preliminary studies showed that nanoparticles were not directly sensitive to fenoxycarb, however, the inclusion of an enzymatic system in solution allowed for successful determination of pesticide in aqueous samples. Under optimized conditions, fenoxycarb concentrations ranging from 6 to 70 μM exhibited an excellent linear relationship with the inhibition efficiency percentage (R^2 = 0.9941). The proposed biosensor has a limit of detection (LOD) of 3.15 μM, and excellent reproducibility values (RSD <3%) in river water analysis. Additionally, this analytical strategy is rapid and simple, and it provides a promising alternative for assaying AChE activity and screening of its inhibitors.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Fluorescent carbon nanodots have received increasing attention in a wide variety of analytical and biomedical applications owing to their outstanding properties [1]. Among them, graphene quantum dots (GQDs) are characterized by a well-defined structure of nanosheets of sp^2 carbons and exhibit extraordinary luminescent properties due to quantum confinement, edge effects, oxygen-containing groups, structural defects and doping elements. Moreover, their large surface area, chemical inertness, excellent photostability, water solubility and low cytotoxicity make them appropriate candidates for biosensing purposes [2]. Analytical applications of GQDs for optical and electrochemical sensing platforms have been recently reviewed in literature [3].

Organophosphorus and carbamate pesticides are widely used in agriculture because of their relatively low persistence and high effectiveness for insect eradication [4] As a result of their use and release into the environment, these pesticides might suppose a potential risk for human health and environmental compartments. Particularly, ethyl 2-(4-phenoxyphenoxy)ethylcarbamate, commonly known as fenoxycarb, is a general use pesticide belonging to the chemical group of carbamates that acts as an insect growth regulator with juvenile hormone activity [5]. In spite of being considered as one of the least toxic carbamates, there is some evidence suggesting that it might exert harmful effects to some non-target species, such as aquatic invertebrates [6,7]. As a consequence, there is a strict regulation on its conditions of use and concentration levels in environmental matrices and agricultural products. Up to now, the majority of analytical approaches for determining fenoxycarb are based on a previous extraction procedure followed by analysis using enzyme-linked immunosorbent assay (ELISA) [8], capillary electrophoresis [9], high-performance liquid chromatography [10], and primarily, liquid/gas chromatography–tandem mass spectrometry [11–13]. Nevertheless, most of these techniques are time-consuming, expensive and require trained personnel.

Recently, enzyme-based sensors have emerged as simple, inexpensive, selective and sensitive alternatives to the aforementioned techniques [14]. Cholinesterases can be used as recognition elements, principally for assay of their inhibitors. In this line, both organophosphorus and carbamate pesticides are known to inhibit the activity of cholinesterase enzyme, being their mechanism of

* Corresponding author.
E-mail addresses: qa1meobj@uco.es, qa1vacam@uco.es (M. Valcárcel).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.snb.2016.08.153
0925-4005/© 2016 Elsevier B.V. All rights reserved.

action quite similar. Specifically, carbamates are pseudoirreversible inhibitors of cholinesterases that act by binding of carbamate moiety to serine hydroxyl in the esteratic part of the active site of enzyme [15]. Some reports have concentrated on the fabrication of cholinesterase-based biosensors with colorimetric/fluorometric readout [16–18]. To the best of our knowledge, there is only two recent studies on literature that take advantage of the outstanding luminescent properties of fluorescent carbon nanodots for the fabrication of sensing systems based on inhibition of acetylcholinesterase (AChE) [2,19].

Herein, we report the application of GQD-based sensing method for determination of fenoxycarb carbamate in surface waters through its inhibitory activity on AChE enzyme. The native fluorescence of synthesized nitrogen doped-GQDs (N-GQDs) was quenched in presence of products of enzymatic hydrolysis of acetylthiocholine chloride (ATC). After addition of inhibitor, the enzymeís activity decreased and the fluorescence of N-GQDs was progressively recovered in a fenoxycarb concentration-dependent manner. Thus, an increasing concentration of fenoxycarb led to both a higher percentage of enzymatic inhibition and fluorescence recovery. Given that fenoxycarb does not directly interact with N-GQDs, this study proposes a simple and rapid biosensor for fluorimetric analysis of this pesticide by combination of N-GQDs as fluorescence probes and AChE as biorecognition element.

2. Material and methods

2.1. Reagents

Acetylcholinesterase (AChE) from *Electrophorus electricus* (200–1000 units mg^{-1} protein), acetylthiocholine chloride (≥99%) (ATC), Tris buffer substance (pH 7.5), bovine serum albumin (lyophilized powder, ≥96%) and ethyl 2-(4-phenoxyphenoxy)ethylcarbamate (fenoxycarb) were all obtained from Sigma-Aldrich (Madrid, Spain). In order to synthesize N-GQDs, citric acid monohydrate (99.0–102.0%) and ammonia (25%) were acquired from Sigma Aldrich (Madrid, Spain) and Panreac (Barcelona, Spain), respectively. Ethanol solvent to prepare fenoxycarb stock solutions was supplied by Panreac (Barcelona, Spain). Ultrapure water (18.2 MΩ) was produced through a Milli-Q water purification system (Millipore, Bedford, MA, USA) and used throughout all the studies.

2.2. Instruments and apparatus

Fluorescence spectra were recorded by a PTI QuantaMaster™ Spectrofluorometer (Photon Technology International) equipped with a 75 W xenon short arc lamp and a model 814 PTM detection system. The excitation wavelength was 355 nm and the emission spectrum was recorded in the 380–600 nm range with a maximum peak at 440 nm. Fluorescence measurements were carried out in quartz microcuvettes (10 mm light path) from Hellma Analytics (Müllheim, Germany). The system control, data acquisition and data analysis were performed by FeliX32 software.

High-resolution transmission electron microscopy (HRTEM) images were obtained with a JEOL JEM 2010 electron microscope (S.C.A.I., Córdoba, Spain) operating at an acceleration voltage of 200 kV.

Fourier transform mid infrared (FT-MIR) spectra were obtained on a Bruker Tensor 27FT-MIR spectrophotometer using KBr pellets.

Adjustments of pH were achieved using a pH-Meter basic 20 from Crison Instruments (Barcelona, Spain).

XPS measurements were performed on a Phoibos 150 MCD (Specs, Berlin, Germany) using an Al source.

Zeta potential of nanoparticles was obtained using a Zetasizer Nano (Malvern, Worcester, United Kingdom).

2.3. Synthesis of nitrogen-doped graphene quantum dots (N-GQDs)

N-GQDs were synthesized by carbonization of citric acid in presence of ammonia following the experimental protocol described by Tam et al. but including some slight modifications [20]. In short, 12 mL of aqueous solution of citric acid (1 mg mL^{-1}) and 1.5 mL of ammonia (25%) were mixed into a 25 mL teflon lined hydrothermal synthesis autoclave reactor and then heated in an oven for 3 h at 200 °C. After cooling to room temperature, the N-GQD solution was adjusted at pH 9 and then dialyzed in dialysis tubes (MWCO 3.5 kDa) (Sigma Aldrich, Madrid, Spain) against double-distilled water for 4 h with the purpose of removing the excess of reagents and impurities. Prior to analysis, aqueous dilutions (1:25) of N-GQD synthesis solution were prepared and readjusted at pH 6 with formic acid.

2.4. Preparation of stock solutions

Stock solutions of AChE (1 mg mL^{-1}) were prepared in 20 mM Tris buffer solution (pH 7.5) containing 1 mg mL^{-1} of bovine serum albumin (BSA) as specified by the manufacturer. AChE solutions were stored refrigerated in the dark at 2–8 °C until use. ATC solutions (10 mg mL^{-1}) were prepared in ultrapure water and also stored refrigerated at 2–8 °C. Stock standard solution of fenoxycarb was prepared in ethanol at a relatively high concentration (1000 μM) and then, diluted with ultrapure water until obtaining the desired concentrations. The effects of organic solvent on AChE activity were found to be negligible.

2.5. River water samples

The proposed analytical method was finally tested in real samples using river water collected from the Guadalquivir River (Córdoba, Spain). Samples were kept in the dark and adjusted at pH 6.0 with formic acid solution before analysis.

2.6. Sensing approach for fenoxycarb determination

The sensing procedure for fenoxycarb determination was as follows: (1) different amounts of fenoxycarb were added to an aqueous solution containing AChE (100 μL; 1 mg mL^{-1}) and the mixtures were incubated in the dark at 37 °C for 15 min; (2) an aliquot of ATC (140 μL, 10 mg mL^{-1}) was added to the previous solution and then kept in the dark at 37 °C for another 5 min; (3) finally, 500 μL of N-GQD solution (1:25 dilution) were added to each sample which was allowed to stand in the dark for 5 min before fluorescence analysis (the final volume in cuvette was 1 mL).

All optimization studies were conducted in absence of inhibitor, thus, variation in quenching caused by the enzymatic reaction products on the native fluorescence of N-GQDs was monitored and taken as analytical signal. On the other hand, for assays with fenoxycarb, signal was traduced into inhibition efficiency percentage, and the calibration curve was built by plotting these percentages against fenoxycarb concentrations.

3. Results and discussion

3.1. Principle of the sensing mechanism

The sensing approach proposed in this work for determination of fenoxycarb takes advantage of the use of fluorescent N-GQDs to assay the activity of AChE, which is highly influenced by the presence of enzyme inhibitors. The basic principle of biosensor is shown

Download English Version:

https://daneshyari.com/en/article/5009555

Download Persian Version:

https://daneshyari.com/article/5009555

Daneshyari.com