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Selective determination of dimethoate via fluorescence resonance energy transfer between carbon dots and a dye-doped molecularly imprinted polymer

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ABSTRACT

A novel molecularly imprinted fluorescent sensor was developed for dimethoate determination based on fluorescence resonance energy transfer. First, a doped molecular template polymer was prepared by electropolymerization. During dimethoate detection, a competitive reaction occurred between dimethoate and carbon dot labeled dimethoate. Fluorescence resonance energy transfer between residual carbon dots labeled dimethoate in the sensor and methyl red on the doped molecularly imprinted polymer then occurred, enhancing the fluorescence signal of the sensor. The fluorescence intensity decreased when the carbon dots labeled dimethoate molecules were replaced by dimethoate molecules in the samples. Under optimal conditions, good linear correlation was obtained for dimethoate over the concentration range from 6×10^{-10} mol/L to 3.4×10^{-8} mol/L with a detected limit of 1.83×10^{-11} mol/L. This sensor was used to detect for dimethoate in actual samples, for which recoveries ranging from 95.0% to 106.0% were obtained.

1. Introduction

Dimethoate is a pesticide with moderate toxicity. Improper usage can leave dimethoate residue on plants that may cause harm to human health when consumed [1]. Consequently, many countries have strict restrictions on the acceptable level of dimethoate residue in agricultural products. Several analytical methods have been used to analyze dimethoate, such as high-performance liquid chromatography (HPLC) [2,3], HPLC–mass spectrometry (HPLC–MS) [4,5], and electrochemical sensor [6,7]. HPLC and HPLC–MS have high efficiency, selectivity and sensitivity. However, their application is limited by their high cost and long analysis time. The stability and reproducibility of electrochemical sensors do not currently meet the requirements of trace analysis. Therefore, there is great interest in the developing of a sensitive, selective, inexpensive, and simple method for the detection of dimethoate.

Analytical methods based on fluorescent resonance energy transfer (FRET) [8], a mechanism of energy transfer between fluorescent materials, have been extensively studied for sensing applications because of their potential for rapid, convenient, and accurate measurements [9,10]. Carbon dots (CDs) [11] are a new class of fluorescent nanomaterials that are expected to behave as better FRET donors and acceptors than other organic fluorescent molecules [12,13]. The low cytotoxicity, good biocompatibility, and unique optical properties, such as high stability against photobleaching and tunable excitation and emission wavelengths, are also attractive features of CDs [14]. However, research on FRET using CDs is still in the initial stages and there have been few reports [12,13] concerning the potential applications of such materials.

Molecular imprinting [15] is an effective technique to prepare molecularly imprinted polymer (MIP) networks containing recognition sites. Such modified polymers have shown promise for applications in separation [16], food inspection [17], environmental monitoring [18], and biosensors [19]. Research on MIP sensors for pesticide analysis includes development of work on electrochemical sensors [20,21], electrochemical luminescent sensors [22] and fluorescent sensors [23]. While electrochemical sensors have problems with stability and reproducibility, and luminescent sensors require development of chemiluminescent reagents, fluorescent sensors are both easy to develop and can give highly reproducible results. Traditional fluorescent sensors use fluorescent dyes or

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inorganic quantum dots (such as CdTe or CdS) as probes. These probes may have disadvantages in terms of toxicity, and low sensitivity. Such limitations do not apply to CDs, which show promise in fluorescent sensing. The present study is the first report of a molecularly imprinted fluorescent sensor based on CDs operating through a FRET mechanism.

Our molecularly imprinted fluorescent sensor was fabricated for selective determination of dimethoate. A molecularly templated polymer was prepared by electropolymerization of methyl red (MR)-doped *o*-phenylenediamine as a functional monomer and dimethoate as the template. As has been described in the literature [24,25], detection proceeds via four steps: elution, blocking, incubation, and competition (Fig. 1). Dimethoate in the sample is detected when it undergoes a competitive binding reaction with CDs-labeled dimethoate in the MIP, affecting its fluorescence. In this study, when the MIP was exposed to a light source, the fluorescence of MR (donor) was quenched, and energy was transferred to the CDs (acceptor). The fluorescence of the CDs was markedly enhanced by efficient FRET. Competition with dimethoate decreased the amount of CDs-labeled dimethoate bound to the MIP, decreasing the intensity of the FRET signal in a manner that was linearly proportional to the concentration of dimethoate in the sample. The sensor showed high sensitivity, wide linear range, low detection limits, was convenient to use, and was successfully used to determine the content of dimethoate in real samples.

2. Materials and methods

2.1. Apparatus and reagents

Electrochemical measurements were performed with an electrochemical workstation (CHI660D, Beijing Huake Putian Technology Co., Ltd., Beijing, China). The classical three-electrode system consisted of a KCl-saturated Ag/AgCl electrode as the reference electrode, a platinum wire electrode as the auxiliary electrode, and an MIP-modified indium tin oxide (ITO) electrode as the working electrode. UV–vis absorption spectra were recorded with a Shimadzu UV–vis 1700 spectrophotometer (Shimadzu, Tokyo, Japan). Fluorescence and time scan spectra were recorded on an F-2500 photospectrometer (Hitachi, Tokyo, Japan). Fourier transform infrared (FT-IR) spectra were recorded by a FT-IR-8400 FT-IR spectrometer (Shimadzu). Transmission electron microscope (TEM) images of the CDs were acquired using a Tecnai 30F (Philips Electronics N.V., the Netherlands).

Dimethoate, banvel, parathion, azodrin, methamidophos and phosphamidon (Pesticide analytical standards, >99%) were obtained from Shanghai Civi Chemical Technology Co., Ltd. (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), *o*-phenylenediamine and methyl red were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

All the reagents used in the experiments were of analytical grade. All aqueous solutions were prepared with double-distilled water.

2.2. Synthesis of CDs and CDs labeled dimethoate

CDs were synthesized using the one-step hydrothermal method reported in reference [26]. First, sodium citrate (0.15 g), NH_4HCO_3 (1.2 g) and ultrapure water (10 mL) were sealed in a Teflon-equipped stainless steel autoclave. After hydrothermal treatment at 180 °C for 5 h, the autoclave was cooled to room temperature. The CDs were purified via dialysis. The CDs were bonded onto the surface of dimethoate using the cross-linking agents EDC and NHS. CDs solution (2 mL) were mixed with dimethoate (1 mL, 2×10^{-4} mol/L), EDC (0.005 g) and NHS (0.002 g). The resulting mixture was reacted for 3 h. After reaction, the product was purified via dialysis.

2.3. Preparation of electrodes modified with MIP and non-molecular imprinted polymer (nMIP)

The ITO electrode was cleaned via ultrasonication for 5 min each in acetone, ethanol, and double-distilled water. MIP and nMIP were then prepared directly on an ITO electrodes via electropolymerization. The polymer solution contained 6×10^{-4} mol/L *o*-phenylenediamine, 1×10^{-4} mol/L MR and 2×10^{-4} mol/L dimethoate. The MIP was formed on an ITO substrate by 30 cycles of cyclic voltammetry (CV) in the polymer solution by sweeping the potential range from 0 to +0.8 V at a scan rate of 50 mV/s. After electropolymerization, the sensors were washed with methanol for 10 min to remove the imprinting molecules. The nMIP sensor was fabricated using the same method but without dimethoate. Following each use, the template molecules (dimethoate) were removed by washing with methanol for 10 min.

2.4. Masking, incubation, and competition

The MIP sensor was immersed in 10 mL of 0.01 mol/L PBS solution (pH 7.6) containing 3×10^{-3} mol/L dimethoate for 15 min to mask all the vacant binding cavities in the MIP. The sensor was then incubated in 5 mL of 5 μg/mL CDs-labeled dimethoate solution for 10 min to allow the CDs-labeled dimethoate to replace the unlabeled dimethoate. Finally, the competition of the sensor was assessed in 10 mL 0.01 mol/L PBS solution (pH 7.6) containing of 6×10^{-10} mol/L to 3.4×10^{-8} mol/L dimethoate for 10 min.

2.5. Electrochemical and fluorescence measurements

Electrochemical experiments were performed in 3×10^{-4} mol/L of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution containing 0.5 mol/L of KCl. CV was performed over a potential range of 0 to +0.8 V. Electrochemical impedance spectroscopy (EIS) was performed over the frequency range of 0.1–100,000 Hz, using an alternating voltage of 5 mV.

Fluorescence spectra were measured over the wavelength range of 300–700 nm at an excitation wavelength of 258 nm. The excitation and emission slits of the instrument were set to a width of 3 nm.

2.6. Sample treatment

Vegetable (10 g) samples and anhydrous sodium sulfate (5 g) were weighed in a 100-mL beaker, then ethyl acetate (25 mL) was added. The mixture was homogenized with a blender until smooth. After supersonic stirring and filtration, the filtrate was concentrated by rotary evaporation. The residue was diluted with petroleum ether to a volume of 2 mL. After drying the collected liquor under a stream of N_2 to evaporate the solvent, the final residue was diluted with ultrapure water to a volume of 10 mL.

3. Results and discussion

3.1. Characterization of CDs

The optical properties of CDs are affected by particle size. The morphology of the CDs particles is shown in Fig. 2A. The CDs are uniform, mono-disperse and spherical with an average diameter of 1.8 nm. Fig. 2B reveals that the particle size distribution of CDs is between 0 and 10 nm. The functional groups on the