Selective amperometric flow-injection analysis of carbofuran using a molecularly-imprinted polymer and gold-coated-magnetite modified carbon nanotube-paste electrode[☆],[☆☆]

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ABSTRACT

Herein, we propose a new approach for selective determination of carbofuran (CBF) in vegetables, based on a simple flow-injection system using a molecularly-imprinted amperometric sensor. The sensor design is based on a carbon-paste electrode decorated with carbon nanotubes and gold-coated magnetite ($CNTs$-$Fe_3O_4@Au/CPE$) coated with a molecularly-imprinted polymer (MIP) for CBF sensing. The MIP was synthesized on the electrode surface by electropolymerization using a supramolecular complex, namely 4-ter-butylcalix [8] arene-CBF (4TB [8]A-CBF), as the template. We used o-phenylenediamine as the functional monomer. Our results demonstrate that incorporation of the MIP coating improves the electrochemical catalytic properties of the electrode, increases its surface area, and increases CBF selectivity by modulating the electrical signal through elution and re-adsorption of CBF. The imprinted sensor (MIP-$CNTs$-$Fe_3O_4@Au/CPE$) was used in a flow-injection analysis (FIA) system. Experimental conditions were investigated in amperometric mode, with the following optimized parameters: phosphate buffer solution (0.1 M, pH 8.0) as the carrier, flow rate $0.5\ mL\ min^{-1}$, applied potential $+0.50\ V$. When used in the FIA system, the designed imprinted sensor yields a linear dynamic range for CBF from 0.1 to 100 μM ($r^2 = 0.998$) with a detection limit of 3.8 nM ($3S_b$), and a quantification limit of 12.7 nM ($10S_b$). The sensor exhibits acceptable precision (%RSD = 4.8%) and good selectivity toward CBF. We successfully applied the electrode to detect CBF in vegetable samples.

1. Introduction

Carbofuran (CBF) is a broad-spectrum carbamate pesticide used worldwide by the agricultural industry to protect a wide variety of fruit, vegetables, and other crops [1,2]. However, its high toxicity and accumulation in living organisms has resulted in public concerns regarding food safety and human health [3,4]. Owing to its high toxicity, the Acceptable Daily Intakes (ADIs) of CBF as recommended by the Codex Alimentarius Commission is 0–0.002 mg.(kg of body weight)$^{-1}$ [5,6]. To monitor the safety of food supplies, government agencies of most countries have established maximum residue levels (MRLs) of pesticides and have set up crop pesticide-residue monitoring programs. The United States Environmental Protection Agency (EPA) set MRLs for CBF at $0.1\ mg\ kg^{-1}$ in selected agricultural product including green

bean, banana, coffee and rice [7,8]. Therefore, it is necessary to detect and monitor CBF residues in farm products and in the environment [9,10]. Detection of carbamate pesticides is generally carried out using high performance liquid chromatography (HPLC) [11,12], gas chromatography (GC) [13], capillary electrophoresis (CE) [14], mass spectrometry [15], piezoelectric immunosensing [16] and amperometric immunosensor apparatus [3,17]. Although these techniques are sensitive and well-established, the methods somehow require expensive instrumentation as well as complex and time consuming clean-up procedures, which carry risks for analyte loss. This limits their application and makes them one way or another unsuitable for routine field operation [7,11–14]. Moreover, these methods do not easily allow continuous in situ analysis, and often require highly trained operators [11–15]. Consequently, electrochemical techniques are considered

suitable complementary tools for real-time detection of CBF in real samples [2,3]. The use of electrochemical sensors coupled with flow-injection analysis (FIA) is a highly promising approach. It provides numerous advantages, including analysis of samples in real time, versatile instrumentation, low cost, high sample throughput, and offers high sensitivity with modest reagent consumption and waste generation.

Generally, electrochemical detection of pesticides in food analysis applications relies on enzyme bio- and chemical-sensors. Although these two sensor types can be sensitive and selective, they do have drawbacks. For example, biosensors, which normally use acetylcholinesterase (AChE) enzyme immobilized on electrode materials such as glassy carbon [18], gold [19], and platinum [20], are limited by their relatively high cost and by the inherent instability of the enzymes. Enzymes require particularly critical operational and storage conditions, including pH, ionic strength, and temperature requirements. Non-enzyme pesticide sensors provide an attractive alternative to enzymatic biosensors. However, they suffer problems with electrode fouling and lack of selectivity; non-enzyme systems require high potentials for analyte detection and suffer interference from the sample matrix [21]. The most important and challenging aspect in the development of CBF chemical sensors is the enhancement of sensitivity and selectivity [2,21]. Chemically modified nanomaterial-based electrodes have been reported for determination of carbaryl [2,22] and carbofuran [2,23,24]. These electrodes used carbon nanotubes (CNTs) with cobalt phthalocyanine [22], cobalt oxide decorated with reduced graphene oxide (rGO) [2], graphene oxide (GO) with gold nanoparticles (AuNPs) [23], and GO with hemin modified carbon electrodes [24]. Nano-materials play important roles in improving the performances of electrical communications and electrocatalytic activity, and have been applied to decrease surface fouling of electrodes [21,24]. However, analytical features, such as precision, sensitivity, and selectivity need further improvement.

Recently, molecularly-imprinted polymers (MIP) have seen wide application in electrochemical sensors. MIPs provide selective analysis due to their tailor-made affinity and specificity to a target analyte [25,26]. They are suitable to rapidly identify a template molecule, and are easily synthesized and incorporated into an electrode to provide a sensitive and selective electrochemical sensor with reproducible analytical results. However, as a sensing material, the rate of analyte diffusion across the MIP film needs improvement to reduce response time [25]. Furthermore, an efficient signal transducer is needed to effectively convert molecular recognition binding signals into detectable electrical signals, and so to enhance sensitivity of the sensing system [25,26]. The electroconductivity and recognition sites of MIPs are poor. Therefore, various nanomaterials have been extensively used in the development of MIP based sensors to promote electroconductivity, electron transfer and recognition sites owing to their unique properties of high conductivity and large specific surface area allowing for high density of chemical binding and large surface area to the synthesis of MIP [26–28]. In terms of improving the sensitivity of MIP sensors for pesticides and herbicides, nanomaterials such CNTs [26,27], gold nanoparticles [28], AuPt alloy nanoparticles [29], and gold-coated magnetite (Fe_3O_4@Au) nanoparticles [30] were reported. These examples include CBF [26,28], carbaryl [29], diuron [27], and parathion-methyl [30], for glassy carbon (GC) [26,28,30] and carbon-paste [29] electrodes.

Herein, we report a novel CBF sensor, fabricated using nanomaterials comprising CNTs and Fe_3O_4@Au nanoparticles to increase electrode sensitivity, and MIP to provide a specific binding site for the CBF target molecule. The MIP-sensor design is based on a carbon-paste electrode modified with CNTs and Fe_3O_4@Au (CNTs-Fe_3O_4@Au/CPE). A MIP for CBF was synthesized at the electrode surface by electropolymerization with a 4-ter-butylcalix [8] arene–CBF (4TB[8]A-CBF) supramolecular complex as the template, and *o*-phenylenediamine as the functional monomer. Hybrids nanostructure formed with carbon

nanotubes (CNTs) and Fe_3O_4@Au have exhibited a synergic effect leading to significant improvements in the electronic and mechanical characteristics of each single component. In this context, the use of CNTs-Fe_3O_4@Au which possess unique properties including high electrical conductivity, good electrocatalytic activity and large surface area offers significant enhancement in the sensor performance. To our knowledge, this is the first report of using CNTs-Fe_3O_4@Au/CPE to enhance conductivity and electron transfer to achieve the electropolymerization of 4TB[8]A-CBF supra molecule and selective of CBF detection. The synthesized MIP was characterized by Fourier transformed infrared (FTIR) spectroscopy and atomic force microscopy (AFM). The CBF imprint has a porous structure, with cavities formed on the surface of the polymer film during electrodeposition. We applied the imprinted sensor (MIP-CNTs-Fe_3O_4@Au/CPE) to on-line amperometric detection of CBF in a FIA system, and the sensor successfully quantified CBF in vegetable samples. The proposed method is highly feasible, and provides excellent sensitivity, selectivity, and stability with a sample throughput of 61 samples per hour.

2. Experimental

2.1. Reagents and materials

All chemicals were analytical grade. Deionized-distilled water (WaterPro Ps, USA) was used for standard and reagent preparation. *o*-Phenylene diamine ($C_6H_8N_2$), graphite powder, 4-tert-butylcalix [8] arene ($C_{88}H_{112}O_8$) (4TB [8]A, 97%), and hydrogen tetrachloroauric (III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$) were purchased from Acros organics (Geel, Belgium). Carbofuran ($C_{12}H_{15}NO_3$, 98%), sodium acetate ($C_2H_3NaO_2$), acetic acid (CH_3COOH), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$), and sodium phosphate dibasic (Na_2HPO_4) were obtained from Sigma-Aldrich (St. Louis, USA). Carboxylated functionalized multiwall carbon nanotubes (CNTs-COOH, diameter: 15 ± 5 nm, length: 1–5 μm, purity: > 95%) were purchased from Nanolab Inc. (MA, USA). Poly(dimethylsiloxane) or PDMS (Sylgard® 184) were purchased from Dow Corning (Wiesbaden, Germany). A stock solution of 10 mM carbofuran (CBF) was prepared using methanol and then stored in the dark at 4 °C. The hydrolysate of CBF was obtained by dissolving CBF in NaOH (0.1 M), and then placing in a water bath at 60 °C for an hour to ensure complete hydrolysis [23,31,32].

2.2. Apparatus

2.2.1. Cyclic voltammetry

A cyclic voltammetry (CV) study was performed in batches using a self-assembled three-electrode cell, comprising a MIP-CNTs-Fe_3O_4@Au carbon-paste working electrode, an Ag/AgCl (sat.) reference electrode, and a platinum wire counter-electrode. We performed CV measurements using an eDAQ potentiostat (model EA161, Australia) equipped with e-corder (model 210), and e-Chem software v2.0.13. The working electrode was built by packing the paste inside a glass tube (inner diameter 0.2 cm, length 7.5 cm) with a fixed copper wire for the electrical contact. The CPE active surface area was approximately 0.031 cm². Phosphate buffer solution (0.1 M, pH 8.0) was used as a supporting electrolyte for all electrochemical measurements.

2.2.2. Amperometric detection using a simple flow-injection (FI) system

We employed a simple FI system for the amperometric detection of CBF [33,34], using the MIP sensor. The system comprised a Shimadzu pump (model LC-10AD, Japan), a Rheodyne injector (model 7725, USA), fitted with a 100 μL sample loop, and an electrochemical detector (ECD). Amperometric measurements were performed using an eDAQ potentiostat (EA161), equipped with e-corder 210, chart v.5.5.11 software, and an in-house three-electrode thin-layer flow cell. The MIP-CNTs-Fe_3O_4@Au/CPE served as the working electrode, Ag/AgCl as the reference electrode, and a stainless-steel tube as the counter electrode.