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Doubly imprinted polymer nanofilm-modified electrochemical sensor for ultra-trace simultaneous analysis of glyphosate and glufosinate

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ABSTRACT

A rapid, selective, and sensitive double-template imprinted polymer nanofilm-modified pencil graphite electrode was fabricated for the simultaneous analysis of phosphorus-containing amino acid-type herbicides (glyphosate and glufosinate) in soil and human serum samples. Since both herbicides respond overlapped oxidation peaks and only glyphosate is prone to nitrosation, n-nitroso glyphosate and glufosinate were used as templates for obtaining the well-resolved quantitative differential pulse anodic stripping voltammetric peaks on the proposed sensor. Toward sensor fabrication, a nano-structured polymer film was first grown directly on the electrode via initial immobilization of gold nanoparticles at its surface. This was followed by linking of monomeric (N-methacryloyl-L-cysteine) molecules through S–Au bonds. Subsequently, these molecules were subjected to free radical polymerization, in the presence of templates, cross linker, initiator, and multiwalled carbon nanotubes as pre-polymer mixture. The modified sensor observed wide linear ranges (3.98–176.23 ng mL^{-1} and 0.54–3.96 ng mL^{-1}) of simultaneous analysis with detection limits as low as 0.35 and 0.19 ng mL^{-1} ($S/N=3$) for glyphosate and glufosinate, respectively, in aqueous samples. The respective oxidation peak potentials of both analytes were found to be substantially apart by 265 mV. This enabled the simultaneous determination of one target in the presence of other, without any cross reactivity, interferences, and false-positives, in real samples.

1. Introduction

Occurrence and accumulation of herbicides, and their metabolites, in soil have deleterious effect on the environment. Particularly, organophosphates and organophosphonates constitute one family of the most commonly applied pesticides in agriculture. For example, glyphosate [N-(phosphonomethyl) glycine, GLY] and glufosinate [(DL-homoalanine-4-yl)-methylphosphinic acid, GLU] are known as non-selective and post-emergence contact herbicides. These herbicides possess amino acid-like structures (Stalikas and Konidari, 2001) and interfere with the formation of amino acids and other chemicals in plants (Cikalo et al., 1996; Kataoka et al., 1996). GLY and GLU have similar structures, yet they are different in their mode-of-action (Hoerlein, 1994). Because of moderate toxicity to animals and humans, these have extensively been used as herbicides worldwide. However, if ingested over a period of time, GLY and GLU may affect the central nervous system, resulting in respiratory, myocardial, and neuromuscular malfunctions, which can even lead to death (Fujii et al., 1996; Richard et al., 2005; Walsh et al., 2000). Therefore, many authorities have suggested the maximum residue levels (MRLs) of these compounds in water and agricultural products. The MRLs of GLY and GLU in most crops listed by the United Nations Food and Agricultural Organization are 0.1–5.0 mg/kg and 0.05 mg/kg (FAO, 2013 (http://www.codexalimentarius.net/download/report/655/al29_24e.pdf (2006))), respectively. Currently, GLY is in the list of the U.S. national primary drinking water contaminants with a maximum contaminant level of 0.7 mg L^{-1}. The European Union limit of any pesticide in drinking water has been set to 0.1 μg L^{-1}, irrespective of their toxicological effects (Stalikas and Pilidis, 2000). In view of the fact that real samples are sufficiently diluted to mitigate the matrix effect, there is an urgent need for the simultaneous monitoring of GLY and GLU in the dilute environmental and biological samples, at trace level.

Most of the methods reported for GLY and GLU simultaneous determination necessarily involved sample derivatization. These include gas chromatography (Hori et al., 2003; Motojyuku et al., 2008; Tseng et al., 2004) liquid chromatography (Hanke et al., 2008; Hao et al., 2011), high performance liquid chromatography (Khrolenko and Wieczorek (2005); Moye et al., 1983), and capillary electrophoresis (Chang and Wei, 2005; Corbera et al., 2005; Goodwin et al., 2003; Jiang and Lucy, 2007; See et al., 2010) techniques. However, in order to avoid costly instrumentations, electrochemical sensors have alternatively been used for on-site cost-effective and rapid analysis of these pesticides (Khenifia et al.,

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2009; Simoes et al., 2006; Songa et al., 2009a, 2009b; Wei et al., 2013). Despite the fact that the simultaneous analysis of GLY and GLU requires a chemical alteration in one of the pesticides and it is inevitable prior to the analysis stage via any method, electrochemical sensors reported hitherto still lacked selectivity in complex matrices. Therefore, the development of simple, selective, and sensitive electrochemical sensors that can afford analysis of real samples without any cross-reactivity and false positives is warranted.

Nowadays, imprinting two or more targets (print molecules) in a single molecularly imprinted polymer (MIP) format is an upcoming technology (Dai et al., 2013; Guo and Guo, 2013; Jing et al., 2010; Matsui et al., 2006; Prasad et al., 2013; Sreenivasan, 2001; Suedee et al., 2008; Tiwari et al., 2011; Xin et al., 2013). This saves both time and labor as compared to traditional imprinting. Molecularly imprinted electrochemical sensors apparently combine the characteristics of electrochemical detection and molecular imprinting technology. This might offer high selectivity, simple operation, and low cost (Blanco-López et al., 2004; Li et al., 2011). Recently, nanomaterials have been used in a wide range of applications as a material foundation of chemosensors. Such sensors have exhibited various degrees of success in the improvement of detection sensitivity and selectivity (Guan et al., 2008). Especially, gold nanoparticles (GNPs) have potential applications in the construction of highly selective electrochemical sensors due to their advantages of enhanced diffusion, good stability and biocompatibility. Furthermore, GNPs might serve as “electron antennae” for channeling electron transport between the electrode and the electro-active species (Atta et al., 2012). As a fascinating branch, polymers, artificially decorated onto GNPs in physical or chemical manners, show much potential with higher value in advanced material science (Li et al., 2009).

Most surprisingly, only MIP for GLY is reported (Jenkins et al., 2001; Zhao et al., 2011), whereas GLU selective MIP is yet to be developed. Furthermore, the double imprinting of these herbicides in a single polymer format is, hitherto, not attempted. The prime concern, while developing a double-templated MIP for the development of an electrochemical sensor, is the occurrence of an almost overlapped oxidation current involving identical electron-transfer route of both templates. As a remedial, we have resorted to exploit derivatization selectively that yielded N-nitroso derivative of GLY (NGLY); whereas GLU fortuitously remained underivatized in the herbicide mixture. The reason being GLU is a primary amine that does not favor nitrosation. On the other hand, GLY is a secondary amine that could be derivatized readily as nitrosoalkylamines—a more stable product (Zhao et al., 2007). In this work, the pencil graphite electrode (PGE) surface was first decorated with GNPs followed by the self assembly of cysteine containing monomer molecules. Subsequently, the modified surface was subjected to activator generated by electron transfer for atom-transfer radical polymerization (AGET-ATRP) of the pre-polymerization mixture (containing both NGLY and GLU as templates, ethylene glycol dimethacrylate (EGDMA) as cross-linker and chloroform as initiator) in the presence of heterogeneously dispersed multi-walled carbon nanotubes (MWCNTs). Herein, the concerted effect of GNPs and CNTs dispersants in MIP layer might produce nanohybrids to exhibit an excellent electron-transfer capability for the oxidation of herbicides, with improved electrocatalytic activity (Rajabzade et al., 2012).

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical reagent grade, and used without further purification. Demineralized triple distilled water (conducting range $0.06–0.07 \times 10^{-6}$ S cm^{-1}) was used throughout the experiment. Methacryloyl chloride (MC), chloroauric acid ($HAuCl_4 \cdot H_2O$)), 2-2′ azobis(isobutyronitrile) (AIBN), and L-cysteine hydrochloride monohydrate (Cys), were purchased from Loba Chemie (Mumbai, India) and Spectrochem Pvt. Ltd. (Mumbai, India). Sodium nitrite and ammonium sulfamate, were purchased from S.D. Fine Chem. Pvt. Ltd. (Boisar, India). Solvents dimethylsulfoxide (DMSO), dimethylformamide (DMF), acetic acid, acetonitrile (ACN), and triethylamine (TEA), were purchased from Spectrochem Pvt. Ltd. (Mumbai, India). Cupric chloride ($CuCl_2$) and 2,2′-bipyridyl (bpy) were purchased from BDH chemicals (England). EGDMA, GLY, GLU, MWCNTs, and all interferents of GLY and GLU, were provided by Aldrich (Steinheimer, Germany) and Fluka (Steinheimer, Germany). Standard acetate buffers were made using acetic acid and NaOH and their pH values were adjusted with the addition of a few drops of either 0.1 M HCl or 0.1 M NaOH. Stock solutions (500 μg mL^{-1}) of GLY and GLU were prepared in water. All working standards were prepared by diluting stock solutions with water. Human blood serum sample was obtained from the Institute of Medical sciences, Banaras Hindu University (Varanasi, India) and stored in a refrigerator at ~4 °C, before use. Any pretreatment (deproteinization, ultrafiltration, ultrcentrifugation, etc.) of blood serum sample was avoided since this might lead to inaccuracies in the final results. Nevertheless, 50-fold dilution of blood serum was necessary in order to mitigate the matrix effect. Soil sample was collected from a local agricultural land and suspended in water (1.0 g/30 mL), followed with the removal of solid residues, if any, by centrifugation and filtration. Fortuitously, the soil sample solution did not cast any matrix effect on analysis. Therefore, its dilution was not necessary.

Pencil rods (2B), 0.5 mm in diameter and 5 cm in length, were purchased from Hi Par, Camlin Ltd. (Mumbai, India).

2.2. Apparatus

All voltammetric measurements were carried out with a polarographic analyzer/stripping voltammeter [model 264A, EG & G Princeton Applied Research (PAR), USA] in conjunction with an electrode assembly [PAR model 303A] and a *X–Y* chart recorder (PAR model, RE 0089). Chronocoloumetry was performed with an electrochemical analyzer [CH instruments, USA, model 1200A]. All experiments were carried out using a three electrode cell assembly consisting of modified PGE, platinum wire, and Ag/AgCl (3.0 M KCl) as working, counter, and reference electrodes, respectively. For FT-IR (KBr) spectral analysis, a minimum of about 5.0 mg samples were scrapped out from the modified electrode surface. Subsequently, this was mixed with KBr pellets in a dye to form a disc and then subjected to spectral recording using Varian 3100 FTIR (USA). Morphological study of the nanoparticles was made using Tunneling Electron Microscopy (TEM) (Technai-12 FEI, Eindhoven, Netherland). Morphological images of bare and modified PGE surfaces were recorded using scanning electron microscope (SEM), JEOL, JSM model-840A (Netherland). Atomic force microscopy (AFM) using a NT-MDT Microscope (NT-MDT Co., Russia) was performed in the semi contact mode. All experiments were carried out at 25 ± 1 °C. The coating of film over the GNPs-PGE surface was made using an indigenous spin-coater SCU-2008C (Apex Instruments Co., India).

2.3. Synthesis of monomer [N-methacryloyl-L-cysteine (MAC)]

The monomer, MAC, was prepared and characterized as described elsewhere (Utku et al., 2008). For this, Cys (5.0 g, 0.028 mol) and sodium nitrite (0.2 g, 0.028 mol) were dissolved in 30.0 mL potassium carbonate solution (5% v/v) and ice cooled to 0 °C. To this ice cooled solution, MC (4.0 mL, 0.04 mol) was added drop-wise with vigorous stirring for 2 h. Afterward, pH of the solution was adjusted to 7.0 and then extracted with ethyl acetate.