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Electrochemical determination of 2-isopropoxyphenol in glassy carbon and molecularly imprinted poly-pyrrole electrodes

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ABSTRACT

A simple, rapid and sensitive electrochemical method using a molecularly imprinted polymer (MIP) based on the electropolymerisation of pyrrole (Py) was developed for the determination of 2-isopropoxyphenol (IPP) in model and real samples. The electrochemical behavior of IPP was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) on bare glassy carbon (GC) electrodes in a Britton-Robinson buffer (pH, 2) solution. IPP exhibited a quasi-reversible behavior on a GC electrode. An anodic peak for IPP showed good linearity over a concentration range from 0.21 to 75 μM (r^2 = 0.999) with a limit of detection (LOD) of 0.21 μM in DPV. For the theoretical design of the MIP, to screen suitable functional monomers and to optimize monomer-template mole ratio, a computational approach was followed using density functional (B3LYP) and Semi-Empirical Parameterized Model number 3 (PM3) models. Pyrrole monomers in the presence of IPP template were electrochemically polymerized using CV on the working electrode. The sensor exhibited an oxidation peak at 0.737 V and an excellent linearity (r^2 = 0.9969) toward increasing concentration of the template over the range 0.09–45 μM with a LOD of 0.09 μM. Intra- and inter-day assay precisions, expressed as %RSD, were overall less than 8.67% for both methods. The result of the selectivity experiment showed that the imprinted sensor has a good response and selectivity toward IPP. The developed sensors were successfully applied for the determination of IPP in real samples recovered from in vitro metabolism of Propoxur (PPX).

1. Introduction

Propoxur (2-isopropoxyphenyl *N*-methyl carbamate, PPX) is known as Baygon. PPX is one of the carbamates that are more frequently used these days for eradication of different species of pests, which have a harmful effect on human being and animals such as flies and mosquitoes [1,2]. World health organization (WHO) classified PPX as a moderately hazardous pesticide compound and daily intake of 0.02 mg/kg was considered harmless to humans [3]. Various routes of exposure to PPX, such as water resources, vegetable, fruit and food consumption, absorption through skin [4] have been reported. PPX, like most of other carbamates, interferes with acetylcholine esterase present in post-synaptic nerve ending of the peripheral and central nervous system and leads to overstimulation of nerve impulses causing a wide range neurological sign and symptoms [1,5].

Previous in vitro study observed that CYP enzymes, in the presence of NADPH, are mainly metabolizing PPX into 2-isopropoxyphenol (IPP), 2-hydroxyphenyl *N*-methylcarbamate, and *W*-hydroxymethyl PPX [6]. Moreover, it was reported that the major metabolite of PPX in mammals is IPP in a rapid metabolic route via depropylation to O-hydroxyl phenyl N-carbamate and then hydrolysis to IPP [1,5,7]. Furthermore, in aquatic media (distilled water, drinking water, rain water, river and sea water) two transformation products were detected from the degradation of PPX being IPP and *N*-methylformamide. PPX degradation was dramatically increased in the presence of irradiation and increasing media pH indicating that hydrolysis of PPX is light and pH dependant [2].

Parent compound or its metabolites in biological samples are mainly responsible for the pesticide toxicity [3,8,9]. A study conducted on Caribbean countries revealed that IPP was observed in seven out of 10 countries with a detection frequency around 30% among the carbamate metabolites searched [8]. A linear relationship between PPX oral ingestion and total IPP amount excreted in urine samples has also been proved. Therefore, IPP can be used as a suitable parameter and biomarker for biological monitoring of PPX exposure toxicity [3,10].

Many analytical techniques have been mentioned in the literature for measuring and monitoring PPX and IPP in different types environmental and biological samples including High Performance Liquid

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Chromatography (HPLC) [7,11,12], Gas Chromatography–Mass Spectrometry (GC–MS) [3,9,13,14], Chemiluminescence (CL) [15]. These methods are labour-intensive, require a great deal of time, high cost and require substantial trained staff. To overcome the shortcomings of the previous methods, an alternative tool should be inexpensive, able to be applied in-field analysis, simple to operate and highly sensitive and selective. Electrochemical techniques have all these properties and can produce methods of choice for the determination of pesticides in various samples. As the presence of pesticide compounds in the environment is a matter of concern for public health, the development of reliable and robust analytical method is imperative for evaluation of human exposure and health risks [14,16].

Electrochemical methods using a DPV technique were developed for measuring PPX using glassy carbon and boron-doped diamond electrodes in various water samples [1]. Additionally, voltammetric behavior of PPX was also studied following its alkaline hydrolysis [17]. To the best of our knowledge no electrochemical method has been reported for the analysis of its major metabolite, IPP. In this work, we developed a voltammetric method for determination of IPP using GC electrode and to achieve greater sensitivity and selectivity, the working electrode was modified with an imprinted polymer (MIP). Modification with MIPs was selected as these can be easily prepared; they are cheap and stable in wide range of chemical and physical conditions [18,19]. The basic principle of molecular imprinting is a selective recognition of target molecule in a network of polymeric matrix via its binding sites mimic biological receptors [20]. Nowadays computational studies are increasingly used for designing MIP. Various quantum chemical methods such as density function theory (DFT) can be adopted to select the best functional monomer for MIP design and are based on the energy of the monomer-template interaction [21–23]. In this study, a DFT-B3LYP with 6-31G, computational approach was applied for the selection of best interacting monomer with IPP and the optimization of the matching monomer-template ratio was done by Semi-Empirical using PM3.

2. Materials and methods

2.1. Chemical and reagents

2-Isopropoxyphenol (IPP), chlorferron (CFN), disulfoton-sulfoxide (DSX), fenamiphos (FNP), strychnine (STN), sodium chloride, glacial acetic acid and pyrrole were obtained from Sigma (Sigma-Aldrich, UK). Phosphoric acid, hydrochloric acid and potassium hydroxide were all purchased from fisher scientific (Fisher Scientific, UK); Britton-Robinson buffer solution was made up with phosphoric acid, glacial acetic acid and sodium chloride; the pH value was adjusted with NaOH and HCl. Fumed silica (particle size0.007 μm) and aluminium oxide (particle size 0.05 μm) used for polishing the glassy carbon electrode were both bought from Sigma-Aldrich (Sigma-Aldrich, UK). Acetonitrile (HPLC grade) used in sonication of the GC electrode was purchased from fisher (Fisher Scientific, UK); Water was purified using an ELGA purification system to a specific resistance 18 MΩ and used to prepare all solutions. Artificial human plasma was purchased from sigma (Sigma Aldrich, UK).

2.2. Instruments and apparatus

Voltammetric experiments were performed using a Metrohm 757 VA Computrace (Metrohm Ltd., UK), data processing was performed using Metrohm version 1.0 Ct757 software (Metrohm Ltd., UK) and run in a personal computer (Compaq® DeskPro, Windows® 95). A conventional three electrode system consisting of a glassy carbon (GC) electrode, as the working electrode, a Ag/AgCl electrode, as reference electrode, and platinum as an auxiliary electrode were used for all the experiments (Metrohm Ltd., UK).

Prior to running all experiments the GC electrode was polished to a mirror-like surface successively with activated aluminium oxide and 0.007 μm silica slurry. The electrode was thoroughly washed with water and then treated with acetonitrile in an ultrasonic bath for about 5 min. Electrochemical experiments were carried out in a 50-mL voltammetric cell at room temperature after an initial purging of the solution under nitrogen gas for 300 s. A digital pH meter (Hanna instrument microprocessor pH 210 m) was used when preparing buffer solutions. An ultrasound bath (Kerry, UK) was used for electrode sonication. A digital pH meter (Hanna instrument microprocessor pH 210 m1ter) was used when preparing buffer solutions. An ultrasound bath (Kerry, UK) was used for electrode sonication.

One hundred micro molar individual standard stock solutions of IPP was prepared in acetonitrile and stored at − 20 °C in the dark bottle. All working solutions were freshly prepared from standard stock solution and kept in plastic bottle at − 4 °C fridge. All measurements were done using CV in a potential window 0.4 to 1 V with sweep rate of 0.1 V/s and DPV scan was run from 0.5 to 1 V with a scan rate of 0.0248 V/s.

2.3. Modification of a GC electrode with a poly-pyrrole imprinted polymer

The electro-polymerization was performed in an electrolyte solution which contains 4 mM phenol, 1 mM DSN, and 100 mM BR buffer solution (pH 2). The copolymerization of the Py and IPP were done by cyclic voltammetry in a potential range of − 0.6 V to + 1 V (vs Ag/AgCl) with a scan rate of 0.1 V/s for 5 scan cycles, after initial purging of the mixture under nitrogen gas for 300 s. The IPP molecules were removed from the polymeric film by immersing the MIP electrode into a stirred mixture of acetic acid and acetonitrile at a ratio of 1:5 (v/v). Finally, the molecularly imprinted GC electrode was then dried under nitrogen gas. The non-imprinted polymer (NIP) was also prepared by following the same electro-polymerization and template removal steps but without the presence of the template molecule, IPP, in the electrolyte mixture.

2.4. Computational approach

All calculations were carried out on a computer with 8 GB memory and an Intel® core™ i5-6200u CPU @ 2.30 GHz. Quantum calculations were carried out using Spartan 14, V1.1.4 software. The electronic binding energies were calculated through Density Functional Theory (DFT) and the geometry optimization was performed at the B3LYP/6-31G level. Finally, the molar concentration ratio between template and monomer was studied using Semi-Empirical model (PM3), developed, in order to select the most appropriate ratio. Using Spartan software, the chemical structure of the template (IPP), monomers and all template-monomer complexes were drawn (each chemical structure representing one mole in the polymerization solution) and calculations for the interaction between the different molecules and complexes created were performed in order to assess their stability and interaction energies.

2.5. In vitro incubation of PPX

Pig liver microsomes prepared and stored from a previous study were utilized for the metabolic studies of PPX. Stored pig liver microsomes (PLM) at − 80 °C were thawed and diluted to 10 mg/L. An incubation mixture consisted of PPX (final concentration of 0.250 mM), PLM (0.5 mg/mL microsomal protein), $MgCl_2$ (10 mM), and NADPH system (40 μL) in a total volume of 0.4 mL potassium phosphate buffer (0.1 M, pH 7.4) following previously published study with minor modifications [24]. NADPH system was daily prepared by adding (10 mM glucose 6-phosphate, 1 mM NADP and 2 U/mL) into 0.1 M potassium phosphate buffer (pH 7.4). Before adding NADPH, the mixture was vortex and incubated at 37 °C for 10 min in a shaking water bath. The incubation mixture begins to react by the addition of NADPH for 1 h. The process was terminated by adding 100 μL ice cold methanol