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Molecularly imprinted polymer based electrochemical sensor for the determination of the anthelmintic drug oxfendazole

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ABSTRACT

A molecularly imprinted polymer (MIP) based electrochemical sensor for the determination of the anthelmintic drug oxfendazole (OFZ) was developed. The polypyrrole (PPy) was electrochemically synthesized onto a screen printed carbon electrode (SPCE) surface. The determination of OFZ was accomplished on MIP-SPCE using differential pulse (DP) and square wave (SW) voltammetry in phosphate buffer solution pH 3.8. The MIP exhibited a good selectivity for OFZ with respect to the other benzimidazoles veterinary drugs. The applicability of the method was tested with milk samples, spiked with OFZ at 30.0–250.0 μg kg^{-1} with detection limits of 10.0 and 8.0 μg/kg and the mean recoveries obtained were 101.8 ± 3.8% and 100.8 ± 4.2%, at spiking concentration of 50.0 μg/kg for DPV and SWV, respectively.

1. Introduction

Oxfendazole OFZ (methyl N-[6-(benzenesulfinyl)-1H-1,3-benzodiazol-2-yl] carbamate; Scheme 1) is widely used as anthelmintic for the control of gastrointestinal and lung nematodes in livestock. Moreover, OFZ is the active metabolite of fenbendazole and febantel [1] and the efficacy of these two latter anthelmintics is due partly to the formation of OFZ in the animal's body. The use of OFZ as an orally effective anthelmintic in dairy cattle may result in its presence in milk at low levels. To ascertain the safe use of OFZ in food-producing animals, a sensitive analytical method capable of measuring the drug at low level in milk is required. The analysis of benzimidazoles BZDs in animal tissues and biological fluids by colorimetric [2] fluorometric [3], high-performance liquid chromatographic (HPLC) [4,5], and radioimmunoassay methods [6] has been reported. The colorimetric and fluorometric methods lack the specificity and sensitivity needed for assay in milk. HPLC and ra dioimmunoassay are intended for use in plasma, utilizing minimum sample cleanup. These methods are not applicable to the analysis of trace drug levels in milk, because of the high fat content of raw milk. An HPLC method for the analysis of benzimidazole compounds in animal tissues and milk based on ion-exchange chromatography was also reported [7]. The sensitivity achieved was 50.0 μg/kg in cow milk and 100.0 μg/kg in cattle tissues. The methodologies for the determination of BZDs in biological matrices have been discussed in terms of sample handling, analysis, residues included and sensitivity [8]. It was concluded that the methodology for determination of benzimidazole residues in foods of animal origin needs improvement due the difficult challenge faced, owing to the extensive metabolism of these molecules.

The introduction of MIP materials in the area of electrochemical sensors is emerging fast as a popular tool owing to the growing interest in achieving selective analysis of the target molecules in different fields such as clinical diagnostics, environmental control, food analysis and drug screening. MIPs are synthetic polymers able to selectively recognize a template molecule in an easy and a rapid way. The synthetic procedure is cheap and MIPs are stable under harsh conditions of pH and temperature. Basically, MIPs are prepared by the polymerisation of a suitable monomer and a cross-linker agent in the presence of a template molecule. After polymerisation, the template is removed from the polymeric matrix leaving cavities complementary in size and shape to the template, which should be capable of specific rebinding of the analyte. The electrosynthesis of MIPs has been shown to be a versatile approach in the choice of functional monomers, as shown by the wide range of molecules successfully used to this end. One of the most widely used polymers in electrochemical imprinting is polypyrrole (PPy). PPy is often used in the design of electrochemical biosensors. The Ppy layers could be formed by several different methods: chemical polymerization initiated by oxidators such as

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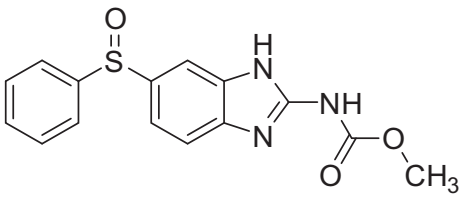

Scheme 1.

$FeCl_3$ or H_2O_2 [9], enzymatic polymerization [10] and electrochemical polymerization [11,12]. Different electrochemical methods were developed in order to form a wide variety of PPy layers; are mainly based on potentiostatic [13], galvanostatic [13,14], or potentiodynamic [11,12] techniques. The potentiodynamic technique seems the most suitable for the formation of stabile conducting polymer layer [12,15] since they allow to increase the concentration of Py monomer at pre-electrode environment during the period when the electrode potential decreases below the potential, which is required for the initiation of the polymerization. Moreover, the potentiodynamic methods allow significant enrichment of PPy layer by entrapped biological compound (e.g. enzyme, antibody, single stranded DNA, etc.) [16–20]. In such way the consumption of expensive biomaterials could be reduced. The potentiodynamic methods based on both potential cycling and potential pulses are mostly applied for the formation of PPy layers [21]. The efficiency of electrochemical polymerization reaction and characteristics of formed PPy layers depend on setting of parameters such as potential sweep rate and vertex potentials. Another controllable and more reliable is another potentiodynamic method, which is based on rectangular potential pulses with fixed potential values [11]. In this method several discreet potential levels, which are the most suitable for polymerization and for relaxation of electrochemical system between polymerization steps, are applied. During the relaxation-step concentrations of pyrrole monomer and materials, which become entrapped within Ppy layer, are restored at pre-electrode environment. A range of electrochemical sensors based on molecularly imprinted PPy have already been used for the determination of bovine leukemia virus glycoproteins [22], benzimidazole [16], bovine hemoglobin [23], paracetamol [19], caffeine [24], epinephrine [25], ascorbic acid [26], and 2,4-dichlorophenoxy acetic acid [27]. Screen-printing permits miniaturization of electrochemical sensors by integrating the reference, auxiliary, and working electrodes on the same chip. SPEs are readily combined with simple, inexpensive, and portable electrochemical instrumentation to make them suitable for on-site determination in real time.

So far no electrochemical methodology has been considered for the determination of OFZ. Considering the advantages of the electrochemical method and the molecular imprinting technique, the key idea of the present work is to construct an electrochemical sensor with high sensitivity and excellent selectivity for OFZ detection. The electrochemical behavior of the MIP for OFZ recognition has been investigated in details. The detection of OFZ was accomplished at MIP-SPCE using DP and SW voltammetry. Furthermore, the MIP-electrode has been successfully used to detect OFZ in milk.

2. Experimental

2.1. Apparatus

Electrochemical measurements were performed with Bio-logic SAS Electrochemical Analyzer, Model SP50, controlled by EC-Lab express Version 5.52 software (Bio-logic SAS, France). Home-made three printed electrodes, a working screen-printed carbon electrode (3.1 mm diameter) printed from a carbon-based ink (Electrodag 421, Acheson); a silver pseudo-reference electrode made from a silver-based ink (Electrodag 477 SS, Acheson) and the auxiliary electrode from a carbon ink, were used. All pH-metric measurements were made on a CG 808 digital pH-meter with glass combination electrode (Schott Gerate, Germany). The electrode was calibrated with commercially available buffer reference solutions.

2.2. Reagents and chemicals

The benzimidazole BZDs anthelmintics (oxfendazole albendazole, mebendazole, and thiabendazole) were from Sigma (St. Louis, USA). The structures of the BZDs studied are shown in Scheme 2. The standard stock solutions were prepared prepared by dissolving in methanol and stored at 4 °C. The working solutions were prepared by diluting the stock solution with the appropriate 0.20 M phosphate buffer solution. Pyrrole (Py) (Sigma–Aldrich) was reagent grade quality and was used as received. The preparation of the aqueous solutions was carried out using ultra pure quality of water. The phosphate buffer solutions were prepared from NaH_2PO_4, Na_2HPO_4 and H_3PO_4, with distilled water. Other chemicals used were of analytical grade, used without further purification.

2.3. Procedure

An aliquot of 200 μL of polymerization solution of $NaClO_4$, M pyrrole and OFZ was dropped onto the exposed area of the strip. The MIP was obtained by the electrodeposition on the surface of the clean SPCE using CV in the potential range between −0.6 and 1.2 V during two cycle (v = 100 mV s^{-1}). The extraction of OFZ template molecule was performed electrochemically by cycling between −0.6 and 1.0 V in 0.2 M phosphate buffer pH 3.8, for ten cycles until all OFZ molecules were stripped from the imprinted PPy film. For comparison, a control NIP-electrode was prepared by the same procedure, only without the addition of template molecule in the polymerization process. An aliquot of 200 μL of the supporting electrolyte solution and sample containing OFZ was added to cover the electrodes, and the voltammograms initiated in the positive direction were recorded. The anodic potential sweep was achieved under different operational parameters. All measurements were carried out at room temperature.

Milk samples were spiked with OFZ at range of concentrations from 30.0 to 250.0 μg kg^{-1}. After vortexing for 45 s, the mixture was centrifuged for 10 min at 5000 rpm to remove milk protein residues and the supernatant was taken carefully. Appropriate volumes of this supernatant were transferred into a volumetric flask and diluted up to the volume with 0.2 M phosphate buffer solution pH 3.8. The voltammetric measurements for these solutions were carried out as described above. Quantifications were performed using the related calibration equations.

3. Results and discussions

3.1. Electrochemical behavior of OFZ at SPCE

No electrochemical data was found in the literature concerning the redox behavior of OFZ neither at the solid nor the mercury electrode. In the first step, OFZ was subjected to CV, DPV, and SWV studies with the aim of a detailed characterization of its electrochemical oxidation behavior on the SPCE. CV for 5.0 μM OFZ in phosphate buffer solution pH 2.0 is shown in Fig. 1, the scanning was started at 0.2 V in the anodic direction. By reversing at +1.20 V, no reduction signal corresponding to the anodic response