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Voltammetric sensor based on carbon paste electrode modified with molecular imprinted polymer for determination of sulfadiazine in milk and human serum

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ABSTRACT

A new sensitive voltammetric sensor for determination of sulfadiazine is described. The developed sensor is based on carbon paste electrode modified with sulfadiazine imprinted polymer (MIP) as a recognition element. For comparison, a non-imprinted polymer (NIP) modified carbon paste electrode was prepared. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods were performed to study the binding event and electrochemical behavior of sulfadiazine at the modified carbon paste electrodes. The determination of sulfadiazine after its extraction onto the electrode surface was carried out by DPV at 0.92 V vs. Ag/AgCl owing to oxidation of sulfadiazine. Under the optimized operational conditions, the peak current obtained at the MIP modified carbon paste electrode was proportional to the sulfadiazine concentration within the range of 2.0×10^{-7}–1.0×10^{-4} mol L^{-1} with a detection limit and sensitivity of 1.4×10^{-7} mol L^{-1} and 4.2×10^{5} μA L mol^{-1}, respectively. The reproducibility of the developed sensor in terms of relative standard deviation was 2.6%. The sensor was successfully applied for determination of sulfadiazine in spiked cow milk and human serum samples with recovery values in the range of 96.7–100.9%.

1. Introduction

Sulfonamides are commonly known as sulfa drugs and belong to a large group of synthetic antibacterial compounds [1]. Despite the discovery and wide use of other antibiotics, sulfonamides are the most widely employed as antibacterial agents in both human and veterinary medicine [2] due to their low cost and high efficiency in the treatment of bacterial diseases. The mechanism of sulfonamide action is based on inhibition of *p*-aminobenzoic acid conversion, interrupting bacterial utilization of these compounds in the synthesis of folic acid and ultimately of purine and DNA [3]. If these antibiotics have been improperly used, there is a risk of resistance of bacteria to these drugs [4]. Moreover, sulfonamide residues in treatment of animals may cause allergic or toxic reaction to consumer of animal products. To protect consumers from risks related to sulfonamide residues, the European Union Council Regulation sets a safe limit that the combined total residues of all sulfonamide based drugs should not exceed 100 ng g^{-1} in animal origin foodstuffs [5]. One of the few sulfonamide drugs that have been used widely is sulfadiazine. The maximum sulfadiazine residue limit tolerated by international regulations for milk is 0.07 ppm [6,7].

Several methods have been reported for the determination of residual sulfadiazine in various samples, including spectrophotometry [8], different chromatographic methods (HPLC, LC/MS, GC, and TLC) [9,10], capillary electrophoresis [11] and biosensing [12]. These techniques are accurate, precise and robust, but some of them require tedious procedures for clean up and enrichment of sulfadiazine. A viable alternative to determination of sulfadiazine is electroanalytical techniques, which have advantages such as simplicity, portability, and sensitivity that make them very attractive for monitoring of pharmaceutical compounds [13,14]. Voltammetry [15–21], potentiometry [22–25], and amperometry [26–29] methods have been widely applied for the determination of sulfadiazine. So far, the evaluation of the electrochemical behavior and quantification of sulfadiazine by electrochemical methods have been performed using several working electrodes such as glassy carbon (GC) [18], diamond [26], bismuth-film [15], modified glassy carbon [16], boron-doped diamond [19], antimony film [21] and multiwall carbon nanotubes/ionic liquid modified electrodes [29]. The development of new electrochemical methods using carbon paste electrode has been reported in the field of pharmaceutical analysis [30]. Carbon paste electrode can be used as working electrode for determination of both oxidizable and reducible groups in pharmaceutical analytes. It has received considerable attention due to advantages such as easy preparation, biocompatibility, non-expensive, renewable surface, high stability and wide operation potential window. Furthermore, incorporation of modifiers into carbon paste material makes it attractive in the electroanalytical applications [31]. The performance of chemically modified carbon paste electrodes depends on the properties of the modifier that affect the selectivity of the electrodes toward analytes.

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One of the selective materials for the recognition and separation of drugs from their matrices is molecularly imprinted polymer (MIP). MIPs are made by crosslinked polymers in the presence of "print" molecule (template). After removal of the template, the polymer can be used as a selective binding medium for the template or structurally related compounds. The electron transfer of an MIP with direct bare electrode is difficult to achieve due to burying of the electroactive center in the polymer. The MIP can be used as a modifier in preparation of chemically modified carbon paste electrodes. So, in the MIP modified electrode with carbon paste, the direct electron transfer to electroactive molecule accelerates. Thus, the production of composites of molecularly imprinted carbon paste electrodes exhibits both predetermined selective molecular recognition properties and high electrical conductivity. Besides, the electrode surface is easily renewable after smooth polishing it on a weighting paper [31]. Various composite electrodes involving MIPs were used as the working electrodes [32–36]. To the best of our knowledge, there is no previous report on voltammetric determination of sulfadiazine by using the MIP modified carbon paste electrode. As demonstrated by the obtained results, combination of sulfadiazine extraction onto the surface of the electrode with DPV detection method made the electrode as a sensitive and selective sensor for sulfadiazine determination in complex matrices. Additionally, simplicity, low cost and environmental friendly material used in fabrication of the electrode make it attractive sensor in electrochemical analysis. The MIP modified carbon paste electrode was applied for determination of sulfadiazine in human serum and milk samples.

2. Experimental

2.1. Apparatus

All electrochemical measurements were performed with a Metrohm 746 VA trace analyzer and a 747 VA stand (Herisau, Switzerland) at room temperature. A three electrode cell configuration was used with MIP or NIP modified carbon paste electrode as the working electrode, a platinum bar as the counter electrode and a saturated Ag/AgCl electrode as a reference electrode. The pH was adjusted using a corning 125 pH-meter (Beckman, USA) with a combined glass electrode.

2.2. Materials and reagents

(4-amino-N-2-pyrimidinyl)benzene sulfonamide (Sulfadiazine) and 2-azobisisobutyronitrile (AIBN) were supplied by Sigma-Aldrich (Munich, Germany). Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were obtained from Merck (Darmstadt, Germany). Graphite powder was provided from Fluka (Busch, Switzerland). Other chemicals were of analytical grade and purchased from Merck. A standard stock solution (5.0×10^{-3} mol L^{-1}) of sulfadiazine was prepared daily by dissolving an accurate amount of the bulk drug in dilute alkaline solution. Other concentrations of the drug were prepared in a volumetric flask by diluting the stock solution with the buffer. All aqueous solutions were prepared with doubly distilled water obtained from AquaMax water purification system (Young-Lin Corp., Korea). Britton-Robinson buffer (BR; 0.05 mol L^{-1} in acetic, orthophosphoric and boric acids), acetate buffer (0.05 mol L^{-1} in acetic acid and sodium acetate) and phosphate buffer (PB; 0.05 mol L^{-1} in phosphoric acid and sodium phosphate) were prepared and adjusted to the required pH with NaOH solution and used as supporting electrolyte.

2.3. Procedures

2.3.1. MIP preparation

General procedure to prepare imprinted polymer was as follows:

The template molecule (sulfadiazine; 0.5 mmol) was mixed thoroughly with the functional monomer MAA (2.0 mmol) in 20 mL acetonitrile in a 50.0 mL screw capped glass tube and was stirred for 45 min to prepare pre-complex of monomer-template molecule. The cross-linker EGDMA (10 mmol) and the initiator AIBN (30 mg) were then added to the above solution. After sonication of the mixture for 10 min to obtain a homogeneous solution, it was deoxygenated with a stream of nitrogen gas for 10 min. Finally, the tube was sealed under the nitrogen atmosphere and was then placed in an oil bath at 60 °C for 24 h. The resultant polymeric particles were washed with methanol to remove unreacted monomers. Then, the template molecule was removed from the polymer with MeOH/acetic acid (90/10; v/v) solution in a Soxhlet extraction system during 24 h. The removal of the template from the MIP particles was ensured by measuring the absorbance of the washout solution at 246 nm. After drying the polymer, it was sieved and the particle size fractions of 32–63 μm were collected. For comparison, the NIP was prepared using the same procedure in the absence of the template molecule.

2.3.2. Preparation of the MIP modified carbon paste electrode

For preparation of the carbon paste electrode, n-eicosane as a binder was melted at 45–50 °C and mixed thoroughly with graphite powder in a ratio of 3:1 (w/w). A portion of this paste was tightly packed into the end of a 1 mL propylene tube (3 mm, i.d.) equipped with a copper wire through the paste and was left to dry in an ambient condition. The remaining paste on the surface of the electrode was removed by polishing it onto a weighting paper and subsequently rinsed with water. The modified carbon paste electrodes were prepared in a similar manner, except for adding an appropriate amount of the MIP or NIP to the graphite powder. After each measurement, the surface of the electrode was renewed by pushing the paste out of the packing tube and polishing the new surface on a weighting paper followed by washing with water.

2.3.3. General procedure for sulfadiazine determination using the modified carbon paste electrodes

The modified electrode was incubated in the sulfadiazine solution prepared in PB at pH 3.5 for 10 min under stirring in an open circuit. The electrode was then rinsed with doubly deionized water to remove any weakly adsorbed analyte, and was transferred into a voltammetric cell containing 0.05 mol L^{-1} PB at pH 6.5. Determination of sulfadiazine was performed by DPV method at 0.92 V (vs. Ag/AgCl) owing to oxidation of sulfadiazine. For recording the differential pulse voltammograms, the potential was scanned from 0.5 to 1.2 with a scan rate of 12 mV/s and pulse amplitude of 90 mV. The average oxidation peak height was used for construction of the calibration curve. The results are reported based on triplicate analysis.

2.3.4. Determination of sulfadiazine in real samples

The human serum was obtained from the local hospital and found to be free of sulfadiazine. 0.4 mL of sulfadiazine in several concentrations was added to 0.6 mL of the human serum and stirred for 10 min. Then, 0.5 mL acetonitrile was added to the above mixture and the precipitated proteins were separated by centrifuging at 12,000 rpm. 0.5 mL of the clear supernatant was transferred into the 25 mL volumetric flask and diluted with the PB at pH 3.5. Determination of sulfadiazine was carried out with the same procedure described in Section 2.3.3. The recovery of the drug in the spiked serum was obtained by standard addition method based on triplicate analysis. The same procedure was used for preparation and determination of sulfadiazine in cow milk sample.

3. Results and discussion

3.1. Electrochemical behavior of sulfadiazine on the modified carbon paste electrodes

Preliminary study on the electrochemical behavior of 1.0×10^{-4} mol L^{-1} sulfadiazine in BR solution of pH 6.5 on the bare carbon paste electrode was performed by cyclic voltammetry in the potential