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A simple and fast batch injection analysis method for simultaneous determination of phenazopyridine, sulfamethoxazole, and trimethoprim on boron-doped diamond electrode

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ABSTRACT

A new analytical procedure for fast and simultaneous determination of sulfamethoxazole (SMX), trimethoprim (TMP) and phenazopyridine (FZP) has been developed. The method is based on batch injection analysis with multiple pulse amperometric detection (BIA-MPA) which offers several desirable features, such as simplicity, reduced costs, robustness, good precision (RSD <3.5%, n = 15), high analytical frequency (up to 70 injections h^{-1}) and generation of small volume of waste by analysis. The BIA-MPA procedure provides linear range from 4 to 320, from 2 to 40 and from 1 to 40 mg L^{-1} and limits of detection of 0.20, 0.15 and 0.05 mg L^{-1} for SMX, TMP and FZP, respectively. The results obtained with the proposed method were compared to those obtained by HPLC and similar results were obtained (95% confidence level).

1. Introduction

Sulfamethoxazole (SMX) or 4-amino-N-(5-methylisoxazole-3-yl)-benzenesulfonamide is a synthetic antibiotic derived from sulfanilic acid that act as bacteriostatic and has been used for the treatment of bacterial infections, including urinary tract infections, pneumonia, chronic bronchitis, meningococcal meningitis and toxoplasmosis [1–3]. Trimethoprim (TMP) or 5-(3,4,5-trimethoxybenzyl) pyrimidine-2,4-diamine is an antibacterial drug, derived from diaminopyrimidine class commonly used in the prophylactic treatment of urinary, intestinal and respiratory infections [4,5]. Phenazopyridine hydrochloride (FZP) or 2,6-pyridinediamine-3-(phenylazo) monohydrochloride is a heterocyclic aromatic azo compound with analgesic characteristics. It is commonly used to reduce discomforts related to urinary tract infections (prostatitis, urethritis, cystitis, etc.) or irritations caused by infections, traumas, surgeries, endoscopic procedures, or the passage of sounds or catheters [6–13].

FZP is frequently used in pharmaceutical formulations combined with SMX and TMP at a mass ratio of 8:1.6:1 (SMX:TMP:FZP, respectively) for the treatment of urinary-tract infections followed by pain [7,13]. The presence of both SMX and TMP in medicinal products produces a synergistic antibacterial activity due to blockade of the folic acid metabolism that causes a decrease in protein synthesis by bacteria [1,14]. The additional presence of FZP causes a local anesthetic effect providing pain relief during the treatment [15].

To the best of our knowledge, there are only two works which describe analytical methods for simultaneous determination of SMX, TMP, and FZP. One is based on multivariate spectrophotometric calibration with the aid of partial least-squares (PLS-1) regression analysis [16] and the other on 3RD-derivative and zero-order photodiode-array spectrophotometry [17]. However, these methods usually require laborious sample preparation procedures with consequently low analytical frequency (determinations per hour). Therefore, the development of new analytical methods for simultaneous determination of the three compounds (SMX, TMP and FZP) is of great importance.

Batch injection analysis (BIA) is a non-flow injection system introduced by Wang and Taha [18]. When this system is coupled with multiple pulse amperometric (MPA) detection, simultaneous determinations are possible using a single working electrode and through the injection of a single sample plug (100–150 μL) [19–23]. In BIA systems, sample or standard solutions are injected with the aid of a micropipette (usually electronic), directly on the surface of the working electrode (wall-jet configuration), which is immersed in a large volume of supporting electrolyte [18,24]. This approach presents several desirable characteristics, such as the use of small sample volumes (typically 10–150 μL), high

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sensitivity, low cost, simplicity and the possibility of developing analytical methods with portable characteristics (use in laboratories with minimum of infrastructure or for on-site analysis) [18,24–27]. In addition, after positioning of the three electrodes (working, auxiliary and reference or pseudo-reference) and filling the BIA cell with supporting electrolyte, the system provides more than two hundred injections without replacement of any component of the system.

MPA technique (GPES software — Metrohm Autolab) allows the application of up to 10 potential pulses quickly and alternately as a function of time. The current acquisition can be carried out separately at each potential pulse (acquisition of up to 10 different amperograms simultaneously) [28]. In addition, the technique also allows the selection of a potential pulse for electrochemical cleaning of the working electrode surface during the experiment (similar to pulsed amperometric detection). Thus, more reproducible results can be obtained even in the presence of species which adsorb on the working electrode surface [29,30].

Recently, an option of working electrode which is being increasingly used is boron-doped diamond (BDD) [31–33]. This material presents advantages over conventional solid electrodes in terms of high stability (low adsorption of organic molecules), chemical inertness, wide potential window, good sensitivity and low background current [34,35]. Moreover, its application for simultaneous determinations of different species [36–41] has shown satisfactory results.

In this work, a batch injection analysis system with multiple pulse amperometric detection (BIA-MPA) is proposed for the simultaneous determination of SMX, TMP and FZP in pharmaceutical formulations. The proposed method involves the injection of a sample plug (150 μL) into the BIA cell and the continuous application of five sequential potential pulses to the BDD electrode. FZP is detected at the first potential pulse, FZP + SMX at the second potential pulse and the three compounds (FZP + SMX + TMP) at the third potential pulse. The subtraction between the currents detected at each potential pulse and the use of correction factor allowed the selective determination of the three compounds. Two additional potential pulses were applied in order to avoid working electrode fouling.

2. Experimental

2.1. Reagents and samples

Sulfamethoxazole (SMX) and trimethoprim (TMP) were obtained from Sigma-Aldrich (St. Louis, MO, USA), phenazopyridine hydrochloride (FZP) from Alfa Aesar (Ward Hill, MA, USA), methanol (MetOH) and acetonitrile from Proquimios (Rio de Janeiro, Brazil), phosphoric acid from Impex (São Paulo, Brazil) and sodium hydroxide from Dynamic (Diadema, Brazil). All reagents were of analytical grade and were used without further purification. All solutions were prepared with deionized water (Millipore Direct-Q3) with a resistivity not less than 18 MΩ cm. Phosphate buffer 0.05 mol L^{-1} (pH = 7) in water/MetOH (70:30) medium was used as the supporting electrolyte. The use of an organic solvent (MetOH) in electrolyte composition was required due to the low solubility of SMX and TMP in aqueous solution. SMX, TMP and FZP standards stock solutions were prepared in MetOH and diluted in buffer solution before all experiments. Pharmaceutical samples (tablets) containing SMX, TMP and FZP were obtained from local drugstore (compounding pharmacy). In the sample preparation step, 10 tablets were weighed, ground (to a fine powder) and homogenized. Then, some portions were weighed and dissolved in MetOH (sample stock solution). Subsequently, aliquots of the stock solution were diluted to appropriate concentrations in the supporting electrolyte for subsequent injection into the BIA-MPA system.

2.2. Instruments and apparatus

Electrochemical measurements were performed employing a PGSTAT 128 N potentiostat (Metrohm Autolab B.V.) controlled by GPES 4.9.007 software. A mini Ag/AgCl saturated with KCl [42] and a platinum wire were employed as the reference and auxiliary electrodes, respectively. A thin film of BDD (~1.2 μm) with a doping level of around 8000 ppm deposited on a polycrystalline silicon wafer (0.7 × 0.7 cm) with 1.0 mm of thickness (NeoCoat SA — Eplatures-Grise 17, CH-2300 La Chaux-de-Fonds, Switzerland) was used as the working electrode. Prior the first use (new material), the BDD electrode was anodically pretreated by applying 0.01 A for 1000 s in 0.04 mol L^{-1} Britton–Robinson buffer solution and then cathodically pretreated by applying - 0.01 A for 1000 s in a 0.1 mol L^{-1} H_2SO_4 solution. This pretreatment is similar to that used in previously published works [43,44]. After the first pretreatment, the BDD electrode was pretreated only cathodically once at the beginning of the work day. If the electrode is not used for a few days, both pretreatments (anodic and cathodic) are again required.

BIA measurements were carried out using a homemade cell described in detail in [45]. In brief, in this cell, one piece of BDD material (0.7 × 0.7 cm) is used and the electrode area is delimited by using a rubber O-ring of diameter 0.4 cm (electrode area = 0.13 cm^2). The electrochemical pretreatment of the BDD was performed with the electrode positioned in the BIA cell. All experiments were carried out with the solution into the BIA cell under stirring. A micro DC-motor was adapted to the BIA cell and used for solution stirring [26]. The stirring rate could be easily changed by varying the voltage from a universal AC/DC voltage regulator (3 to 12 V). All studies were performed at a constant stirring rate of 1400 ± 10 rpm (with the application of 5 V). The injections of solutions were performed with a motorized electronic micropipette (Eppendorf Multipette® stream) maintained at a constant distance from the working electrode to Multipette® Combitip® (≈2 mm), as recommended in a previous work [24].

2.3. HPLC analysis

Results obtained with the proposed method for the simultaneous determination of SMX, FZP and TMP were compared to those obtained by HPLC. A Hitachi pump L-2130, Hitachi LC-4250 UV–VIS detector and a Shim-pack CLC-ODS column (25 mm × 4.6 mm, Shimadzu) were used. The mobile phase was composed of 0.05 mol L^{-1} phosphate butter:acetonitrile (40:60, *v*/v; pH = 3.5), and the flow rate was 1.2 mL min^{-1}. The detector was fixed at 280 nm. The retention times were 2.09, 2.88 and 4.45 min for TMP, SMX and FZP, respectively.

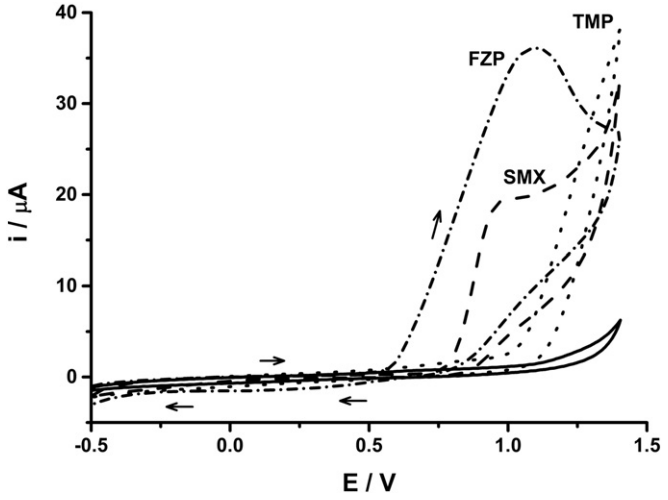

Fig. 1. Cyclic voltammograms obtained at BDD electrode in 0.05 mol L^{-1} of phosphate buffer (pH = 7) in methanol–water (30:70; *v*/v) before (—) and after the addition of 250 mg L^{-1} of FZP (·–·–·), 253 mg L^{-1} of SMX (– – –) or 290 mg L^{-1} of TMP (····). Scan rate = 50 mV s^{-1}; Step potential = 5 mV.