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Simultaneous determination of three species with a single-injection step using batch injection analysis with multiple pulse amperometric detection

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ABSTRACT

In this work, the possibility of simultaneous determination of three compounds with a single-injection step using batch injection analysis with multiple pulse amperometric detection (BIA–MPA) is demonstrated for the first time. A sequence of three potential pulses (+1.25 V, +1.60 V, and +1.80 V) was applied with the acquisition of three separate amperograms. 8-Chlorotheophylline was detected selectively at +1.25 V, both 8-chlorotheophylline and pyridoxine at +1.60 V and 8-chlorotheophylline, pyridoxine, and diphenhydramine at +1.80 V. Subtraction between the currents detected at the three amperograms (with the help of correction factors) was used for the selective determination of pyridoxine and diphenhydramine. The proposed method is simple, inexpensive, fast (60 injections h^{-1}), and present selectivity for the determination of the three compounds in pharmaceutical samples, with results similar to those obtained by HPLC (95% confidence level).

1. Introduction

Diphenhydramine (DIP) and 8-chlorotheophylline (CTP) are two active pharmaceutical ingredients that compose, in equimolar ratio, the salt known as dimenhydrinate, which is usually used to avoid motion sickness associated with nausea and vomiting [1]. The presence of DIP provides a reduction in symptoms of nausea and vomiting and CTP reduces the side effects induced by DIP (drowsiness) [2]. When dimenhydrinate (DIP+CTP) is used to control nausea and vomiting in early pregnancy, better effectiveness was observed if a third active ingredient, pyridoxine or vitamin B_6 (PYR), was added to the pharmaceutical formulation [3]. Studies demonstrate that PYR is recommended for controlling nausea and vomiting in pregnant women [4]. Probably, synergistic effects are observed when medications containing the three active ingredients (DIP+CTP+PYR) are administered simultaneously.

According to our knowledge, there is no previous work that reports an analytical method for simultaneous determination of DIP, CTP, and PYR. Only methods for simultaneous determination of DIP and CTP were found [5,6]. Therefore, the development of new analytical method for simultaneous determination of the three compounds (DIP, CTP, and PYR) is of great importance.

Boron-doped diamond (BDD) is a modern carbon based material that is well suited for electroanalytical applications due to its excellent electrochemical properties, such as low and stable background currents, low sensitivity to dissolved oxygen, a wide working potential window in aqueous solutions and a long term stability [7–9]. These properties are especially useful for electrochemical determination of analytes with high oxidation potentials [10–12]. Recently, the use of BDD electrode has been reported for the determination of organic compounds in pharmaceutical [7–14], biological [14,15], and food [8,16,17] samples.

Batch injection analysis (BIA) is a hybrid system between flow injection and batch experiments [18]. In a BIA system with amperometric detection, a small volume (50–100 μL) of sample or standard solution is directly injected (generally using an electronic micropipette) onto the surface of the working electrode which is immersed in a large-volume of blank solution (wall-jet configuration). The detector records a transient peak-shaped response that reflects the passage of the sample zone over electrode surface (similar to flow injection analysis system-FIA). The height of the peak is proportional to the concentration of the target analyte in the injected solution [19–21]. In BIA systems, tubes and mechanically moving parts used in FIA systems can be replaced by a unique battery-powered instrument (electronic micropipette) [22]. Due to this, BIA systems can easily be used in laboratories

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with minimum of infrastructure or for on-site analysis [23–26]. Recently, our group demonstrated that a BIA system with multiple pulse amperometric detection can be used for simultaneous measurement of two species [27]. An aliquot of sample solution was directly injected onto a single working electrode and the two compounds were selectively detected. A simple correction factor [25,28,29] was necessary to use to achieve this purpose.

In this work, for the first time, a batch injection analysis system with multiple pulse amperometric detection (BIA-MPA) using a single working electrode for simultaneous determination of three compounds (CTP, DIP, and PYR) is proposed. The purpose was achieved by continuous application of three potential pulses (acquisition of three separate amperograms) and a fast (60 s) and single-injection step.

2. Material and methods

2.1. Reagents and samples

Highly-pure deionized water ($R \geq 18$ MΩ cm) obtained from a Millipore Direct-Q3 water purification system (Bedford, MA, USA) was used to prepare all aqueous solutions. Acetic, phosphoric, and sulfuric acids were purchased from Synth (Diadema-Brazil), sodium hydroxide from Dinamica (Diadema, Brazil), ethanol from Química Moderna (Barueri, Brazil), diphenhydramine (DIP), and pyridoxine (PYR) from Sigma-Aldrich (St. Louis, United States) and 8-chlorotheophylline (CTP) from Alfa Aesar (Ward Hill, USA). All reagents were used without further purification. Stock solutions of CTP, and DIP were prepared just before the experiments by dissolution in a 20% (v/v) ethanol–buffer solution. Stock solution of PYR was prepared by dissolution in water. Standard solutions were prepared by dilution of stock solutions in supporting electrolyte (0.1 mol L^{-1} H_2SO_4).

Pharmaceutical formulations (oral solution) were obtained from local drug store. An adequate amount of the solution was dissolved (under sonication for 10 min) in a 20% (v/v) ethanol-buffer solution. Sample solutions were further diluted in supporting electrolyte for subsequent injection in the BIA system.

2.2. Instrumentation and apparatus

Electrochemical measurements using cyclic voltammetry and multiple-pulse amperometry were performed using a μ-Autolab type III potentiostat (Metrohm Autolab B.V., The Netherlands) interfaced to a microcomputer and controlled by GPES 4.9.007 software. A mini Ag/AgCl (saturated KCl) [30] and platinum wire were employed as reference and auxiliary electrodes, respectively. Boron-doped diamond (BDD) from Neocast SA, La Chaux-de-Fonts, Switzerland was used as working electrode. The working electrode consisted of a thin film of BDD (~1.2 μm of thickness; boron doping level of ~8000 ppm) deposited on a polycrystalline silicon wafer (7×7 mm^2) with 1.0 mm of thickness. Prior to use for the first time (new electrode), the BDD electrode was anodically pretreated by applying +0.01 A for 1000 s in 0.04 mol L^{-1} Britton–Robinson buffer solution. Next, a cathodic pretreatment was performed by applying −0.01 A for 1000 s in a 0.1 mol L^{-1} H_2SO_4 solution [31,32]. After the first pretreatment, the BDD electrode was pretreated only cathodically once at the beginning of the workday. If the electrode is not used for a few days, both pretreatments (anodic and cathodic) are again necessary. All results presented in the proposed work were performed with the same BDD electrode.

BIA measurements were conducted using a cylindrical polypropylene cell (inner diameter=6 cm; height=5 cm) similar to that previously described [26]. In this cell, the BDD electrode was positioned on a hole located at the center of the bottom of the cell. The BDD piece (7×7 mm^2) was hard-pressed on an organic solvent resistant O-ring (internal diameter of 5 mm) precisely positioned over this hole. The O-ring prevents leaks and defines the electrode area (0.2 cm^2). The electric contact was made with a copper board or metal plate (stainless steel) positioned under the BDD piece (fixed with screws). A micro DC-motor (3–24 V) was used for solution stirring [23]. A Teflon rod was adapted on the motor shaft. An adjustable DC power supply (3.0–12 V) or a common battery could be used as a power supply of the micro DC-motor so the stirring rate could be easily changed by varying the voltage (portable characteristics). All studies were performed at a constant stirring rate of 280 ± 10 rpm (with the application of 4.5 V). The injection procedure was performed with a motorized electronic micropipette (Eppendorf Multipette® stream). The distance between the Multipette® combitip® and the working electrode was kept constant (≈ 2 mm) [21] in all injection procedures. All electrochemical measurements were performed at 25 °C (room temperature), in the presence of dissolved oxygen.

Results for the simultaneous determination of CTP, PYR, and DIP by BIA–MPA were compared to those obtained by high performance liquid chromatography (adapted from [33]). The HPLC measurements were performed using a Shimadzu LC-10 VP equipped with an UV–vis detector (SPD-10AV), a LC column (Phenomenex 80A MAX-RP-C12, 250 mm × 4.6 mm, 4 mm), a manual injector (20 μL) and a pump (LC-10AD-VP). The mobile phase consisted of a mixture of acetonitrile – (0.01 M H_3PO_4 – triethylamine pH 2.8) (22:78, v/v). The detector and flow rate were fixed at 229 nm and 1.0 mL min^{-1}, respectively. The retention times were 3.5, 5.3, and 16.7 min for PYR, CTP, and DIP, respectively.

2.3. Procedures

The limits of detection were calculated based on $3S_B/b$, where S_B is the standard deviation of the mean value of ten blank measures and b is the slope of the calibration curve. The precision tests were evaluated by twenty replicate measurements of the same standard solution on the same day (intra-day precision) or by six replicate measurements of a solution with same concentration in six different days (inter-day precision). The linearity of the proposed method was evaluated by analyzing a series of different concentrations ($n=3$) of each drug using calibration curves to calculate correlation coefficients ($r > 0.99$) and intercept values.

3. Results and discussion

The electrochemical behavior of the three target compounds (CTP, DIP and PYR) was investigated by cyclic voltammetry in different electrolyte solutions (0.1 mol L^{-1} H_2SO_4 – pH=1.0; 0.05 mol L^{-1} acetic acid/acetate buffer-pH 4.7 and 0.1 mol L^{-1} phosphate buffers-pH 2.1 and 7.2). Figure 1(I) shows the effects of pH value and electrolyte composition on the oxidation potential of CTP, DIP, and PYR.

As can be observed in Fig. 1(I), well-separated anodic peaks ($\Delta E_p \geq 0.2$ V) of the three compounds were only achieved in solution with pH value around 1 (best condition for simultaneous determination). In a previously published work [5], the stoichiometric determination of dimenhydrinate (CTP and DIP) was achieved by BIA–MPA using acetic acid/acetate buffer solution (pH 4.7) as supporting electrolyte. In this condition, the oxidation peaks of CTP and PYR are very close (Fig. 1(I-c)) and simultaneous determination of CTP and PYR cannot be performed. For this reason, 0.1 mol L^{-1} H_2SO_4 was selected as supporting electrolyte for the following studies.