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Evaluation of boron-doped diamond electrode for simultaneous voltammetric determination of hydrochlorothiazide and losartan in pharmaceutical formulations

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ABSTRACT

A method for the simultaneous determination of hydrochlorothiazide (HCTZ) and losartan (LOS) in pharmaceutical formulations using differential-pulse voltammetry (DPV) was developed. Two very well-resolved and reproducible oxidation peaks of HCTZ and LOS, with separation of 0.23 V, were obtained in Britton–Robinson (BR) buffer (pH 9.5) using an anodically pretreated boron-doped diamond electrode. Under the optimum analytical experimental conditions, the voltammetric method exhibited linear responses for simultaneous determination of HCTZ and LOS in the concentration range 3.0×10^{-6} to 7.4×10^{-5} mol L^{-1} for both compounds, with detection limits of 1.2×10^{-6} mol L^{-1} and 9.5×10^{-7} mol L^{-1}, respectively. The proposed method was successfully applied in the simultaneous determination of LOS and HCTZ content in pharmaceutical formulations, whose accuracy was attested by good agreement of the results (paired *t*-test at a 95% confidence level) with those obtained using high performance liquid chromatography (HPLC).

1. Introduction

Losartan potassium (LOS) and hydrochlorothiazide (HCTZ) are drugs widely used for the treatment of hypertension and cardiovascular diseases [1], used separately or together in a combined pharmaceutical formulation. LOS is an angiotensin II antagonist; it reduces hypertension by suppressing the effects of angiotensin II of rennin angiotensin-aldosteron system. On the other hand, HCTZ is a thiazide diuretic compound, which increases the renal excretion of water and electrolytes [1].

As it is known, some hypertensive patients require two treatment with drugs presenting complementary mechanism of action with the aim to diminished their blood pressure. LOS and HCTZ in a combined dosage are preferred first-line treatment for most patients, because both compounds have been found to be more effective in the treatment of hypertension in patients whose blood pressure is not adequately controlled by an individual drug. Due to the frequency that LOS and HCTZ are prescribed, it is very interesting the development of sensitive and selective analytical methods for the simultaneous quantification of these compounds in pharmaceutical formulations as quality control.

There have been several reports on the individual or simultaneous determination of LOS and HCTZ in biological materials and tablets, including the use of chromatography [2–10], capillary electrophoresis [11], spectrophotometry [12,13] and conductometry [14]. The official method recommended by the United States Pharmacopeia [15] for the individual determination of LOS and HCTZ involves the use of high performance liquid chromatography (HPLC), but there is not yet a method for LOS–HCTZ mixture in any pharmacopeia. On the other hand, high performance liquid chromatography methods have been developed for simultaneous LOS and HCTZ determination in pharmaceutical formulations [8,16,17]. However, usually these methods require the use of organic solvents, extensive preliminary sample pretreatment, time-consuming derivatization steps and high implementation costs, thus justifying the need for reliable, low cost and simpler methods.

Voltammetric analysis, being simple, rapid, highly sensitivity, selective and economical, constitutes a highly convenient alternative approach for individual and simultaneous determination of several compounds [18–22]. There are available few studies on the individual voltammetric determination of LOS and HCTZ. Hanging

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mercury drop electrode (HMDE) [23] has been employed for individual quantitative determination of LOS in bulk and pharmaceutical products. Glassy carbon electrode (GCE) [24], GCE modified with multiwalled carbon nanotubes (GCE/MWCNTs) [25], ferrocenedicarboxylic acid modified carbon paste electrode (CPE/FDC) [26], and multiwall carbon nanotubes/silicone rubber composite electrode (SRE/MWCNTs) [27] were employed for individual determination of HCTZ in pharmaceutical and urine samples. However, to the best of our knowledge there are no published reports regarding an electroanalytical method for simultaneous determination of LOS and HCTZ in pharmaceutical forms or biological fluids.

Boron-doped diamond electrode (BDDE) is particular useful as an electrode material [21,22,28–30], due to an electrochemical stability in both alkaline and acidic media, a very low and stable background current, and a very wide potential range, which can be larger than 3.5 V [31–33]. The analytical performance of BDDE depends on their surface termination (oxygen or hydrogen) [34–38]. The anodic pretreatment of its surface is known to change the diamond surface from hydrophobic to hydrophilic by introducing oxygen functional groups onto the surface and the cathodic pretreatment changes the diamond surface from hydrophilic to hydrophobic by introducing hydrogen functional groups.

In this work, the simultaneous voltammetric determination of HCTZ and LOS using differential-pulse voltammetry (DPV) and an anodically pretreated BDDE without the need for prior separation step has been proposed. This method was applied in the simultaneous determination of HCTZ and LOS in pharmaceutical formulations. The obtained results were compared with those from HPLC [6].

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade (LOS, HCTZ, sulfuric acid from Sigma–Aldrich; boric acid, acetic acid orthophosphoric acid, and sodium hydroxide from Merck) and all the solutions were prepared with ultra-purified water supplied by a Milli-Q system (Millipore®) with resistivity greater than 18 MΩ cm.

Commercial pharmaceutical samples used in this studies were: hydrochlorothiazide tablets (EMS Pharma, Brazil, labeled 25 mg HCTZ per tablet), losartan tablets (Medley Pharmaceuticals Ltd., Brazil, labeled 50 mg LOS per tablet), and hydrochlorothiazide:losartan tablets (Medley and EMS, labeled 12.5:50 mg HCTZ:LOS per tablet). These samples were purchased from local drugstore in city of Londrina in Brazil.

A Britton–Robinson (BR) buffer was chosen as supporting electrolyte (as reported further below): 0.04 mol L^{-1} in acetic, orthophosphoric, and boric acids, with pH adjusted to 9.5 with a 2.0 mol L^{-1} NaOH solution.

A 1.0×10^{-2} mol L^{-1} stock solution of LOS was prepared before use in a BR buffer solution (pH 9.5). A 1.0×10^{-2} mol L^{-1} stock solution of HCTZ was prepared in a BR buffer solution (pH 9.5) containing 30% acetone (v/v). Both LOS and HCTZ working solutions were prepared by appropriated dilution of these stock solutions with the BR buffer solution (pH 9.5).

2.2. Apparatus

The voltammetric experiments at BDDE were performed using a PalmSens potentiostat/galvanostat controlled with the PalmSens PC software. The pretreatment of the BDDE was carried out in an MQPG-01 potentiostat (Microquímica). All the electrochemical experiments were conducted in a three-electrode single-compartment glass cell, including a Pt wire as auxiliary electrode, an Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode (which hereinafter all working electrode potentials are referred) and a BDDE (8000 ppm; 0.26 cm^2 exposed area; Adamant, Switzerland) or glassy carbon electrode (GCE) (Tokay Carbon Co., Japan) as working electrode. Detailed information on the preparation of the boron-doped diamond films is reported elsewhere [39]. Prior as working electrode. Diamond film was synthesized on a silicon substrate by Hot Filament Chemical Vapor Deposition (HFCVD) technique, where the gaseous phase consisted of methane with excess hydrogen gas and trimethylboron as doping. Detailed information on the preparation of the diamond films was reported elsewhere [39]. Prior to the experiments, the BDDE was anodically pretreated in a 0.5 mol L^{-1} H_2SO_4 solution by applying 0.5 A cm^{-2} during 40 s; thus, the BDDE surface was made predominantly oxygen terminated. The selection of this pretreatment procedure is discussed in detail in Section 3.1.

The GCE (5 mm diameter) was carefully polished to a mirror finish, starting with metallographic abrasive paper (no. 6) and finishing with slurries of 0.3 and 0.05 μm alumina. After being rinsed with doubly distilled water, sonicated for 5 min in absolute ethanol and then in ultrapure water, the polished GCE was dried at room temperature.

All experiments were carried out at an ambient temperature of 25.0 ± 0.5 °C. The pH was measured at 25.0 ± 0.5 °C using a pH-meter (Hanna Instruments), model HI-221, employing a combined glass electrode with an Ag/AgCl (3.0 mol L^{-1} KCl) external reference electrode.

The determination of HCTZ and LOS by HPLC were carried out using an LC-20AT Shimadzu system, with a UV–vis diode array detector set at 254 nm. The chromatographic separation conditions were carried out in according to previous work [6]. The separation of LOS and HCTZ was accomplished on a Shimadzu CLC-ODS (M) column (250 mm × 4.6 mm; 5 μm) at 35 °C. The phase mobile consisted of an acetonitrile–phosphoric acid 0.1% (m/v) (40:60, v/v) at a flow rate of 1.0 mL min^{-1}, while the injection volume was 20 μL.

2.3. Analytical procedures

Cyclic voltammetry (CV), differential-pulse voltammetry (DPV), and square-wave voltammetry (SWV) were employed to investigate the electrochemical behavior or quantification of HCTZ and LOS compounds.

Analytical curves were obtained by addition of aliquots of the previously prepared HCTZ and LOS standard solutions into the measurement cell containing 10.0 mL of the BR buffer solution (pH 9.5). Square-wave and differential pulse voltammograms were obtained after each aliquot addition. Thus, the analytical parameters were compared and the best results were used to quantify simultaneously both compounds in commercial pharmaceutical samples. All measurements were carried out in triplicate for each concentration. Detection limit (LOD) was calculated as three times the standard deviation for the blank solution divided by the slope of the analytical curve.

To prepare the solutions of the commercial pharmaceutical samples of HCTZ and LOS, 10 tablets of each pharmaceutical formulation were reduced to a homogeneous fine powder in a mortar. A suitable amount of this powder was weighed and transferred to 10 mL calibrated volumetric flasks containing BR buffer solution (pH 9.5) and 30% acetone (v/v). After, suitable aliquots of the supernatant were transferred to 10 mL calibrated flasks and completed to volume with the BR buffer solution (pH 9.5). An aliquot of each sample solution was directly transferred to the electrochemical cell containing the supporting electrolyte, after which the voltammograms were obtained. The HCTZ and LOS concentrations in each sample solution