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Simultaneous determination of salbutamol and propranolol in biological fluid samples using an electrochemical sensor based on functionalized-graphene, ionic liquid and silver nanoparticles

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ABSTRACT

A simple, rapid, and low-cost electroanalytical method was developed using a glassy carbon electrode modified with functionalized-graphene (FG), ionic liquid (IL) and silver nanoparticles (AgNPs) for simultaneous detection of salbutamol (SAL) and propranolol (PRO). The electrochemical behaviour of the electrodes was investigated by cyclic voltammetry and square wave voltammetry (SWV) under optimised conditions. Using SWV, the AgNP-IL-FG-NF/GCE sensor showed a linear response from 79 nmol L^{-1} to 2.9 μmol L^{-1} for SAL and from 0.1 to 2.9 μmol L^{-1} for PRO with limits of detection of 13 and 17 nmol L^{-1} for SAL and PRO, respectively. The proposed sensor showed good stability, repeatability and reproducibility, and was applied successfully to the simultaneous determination of SAL and PRO in biological fluid samples. The proposed method proved to be excellent, being therefore a reliable alternative method for the simultaneous determination of SAL and PRO.

1. Introduction

Salbutamol (SAL) also known as albuterol ((*RS*)-4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol – Fig. 1A) is a drug widely used in the treatment of asthma, chronic pulmonary disease and in the control of blood potassium levels. SAL can cause side effects, such as headache, dizziness, shakiness, and fast heart rate [1–3], and if administered in excess or if consumed improperly can cause serious health problems, including aggravating bronchospasm, irregular heartbeat, and low blood potassium levels [1, 4, 5].

Propranolol (PRO) (1-isopropylamino-3-(naphthalen-1-yloxy) propan-2-ol – Fig. 1B) is a beta-adrenergic blocking agent [6–8]. This drug is most frequently prescribed for the treatment of high blood pressure, chronic angina pectoris, prophylaxis and treatment of cardiac arrhythmias, prophylaxis of myocardial reinfarction and treatment of tremors [7, 8]. PRO may cause adverse reactions, such as congestive heart failure, aggravation of atrioventricular conduction disorders, bronchospasm, severe bradycardia and hypotension [7, 8].

Although the simultaneous use of these drugs (SAL and PRO) is not common because PRO is a non-cardioselective beta-blocking agent and the risk of its use together with SAL may outweigh the benefits for patients with asthma, their consumption should be avoided or supervised by a doctor [9–11]. In the literature, some cases of symptomatic overdose with SAL are reported, where PRO was used as an antidote and not as an anti-asthmatic drug [12]. Ramoska et al. [13] reported the use of PRO in the treatment of SAL poisoning in two asthmatic patients, in which case PRO was used to minimise the effect caused by SAL. In another study, Kupeli [14] reported the use of PRO for infantile haemangiomas, but only 3 out of 14 patients presented bronchospasm and were treated with SAL. Thus, even though SAL and PRO are not found in together in pharmaceutical formulations, they can be co-administered in clinical treatments [12, 13] and in other applications [15–17]. Therefore, the simultaneous determination of SAL and PRO in biological fluids is very important for physiological pharmacokinetics and clinical diagnosis [12, 13]. In view of the applicability of these drugs in extreme cases, whether due to misuse or in poisoning, their quantification in biological fluid samples is of paramount importance. In the literature, some procedures have been reported for the simultaneous determination of these classes of drugs [18, 19]. Among these analytical procedures, electroanalytical techniques have shown great advantages compared to other traditional techniques such as chromatography and spectrophotometry in the analysis of biological fluid samples [20–22]. Their advantages are greater simplicity, analysis in real time, low cost and short analysis time. Considering the necessity for increasingly sensitive and selective electrochemical sensors, an analytical method that has been highlighted is the use of modifiers immobilised on conventional electrode surfaces, such as carbon nanostructures, e.g. carbon black, carbon nanotubes and graphene [23,

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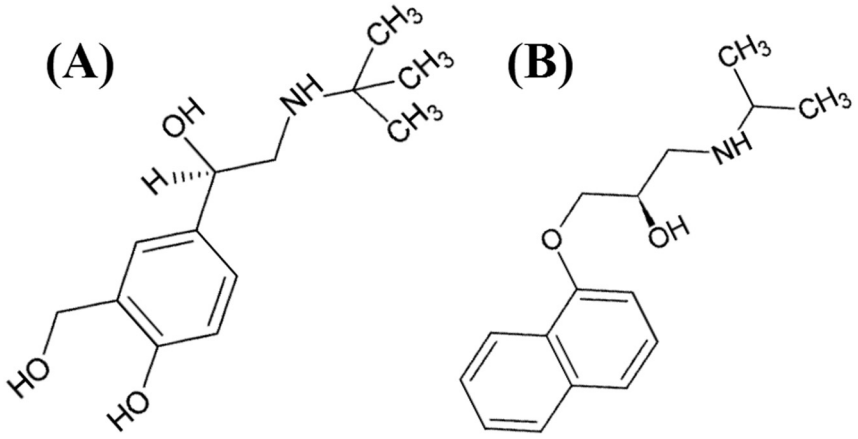

Fig. 1. Chemical structures of (**A**) salbutamol and (**B**) propranolol.

24]; polymers, e.g. PDDA, chitosan and nafion (NF) [24, 25]; ionic liquids (ILs) [26]; and metallic nanoparticles, e.g. Au, Cu, Pt and Ag [23, 27], which can be used to improve the performance of the sensor and induce catalytic and/or electrocatalytic effects [28–30].

Functionalised carbon nanomaterials such as functionalized-graphene (FG) have shown to be been promising due to their properties of better dispersibility in aqueous media, which facilitates their use in the preparation of films, and also that they interact with other modifiers, thus increasing the performance of electrochemical sensors for the determination of analytes of interest [30, 31]. In addition, the use of metallic nanoparticles incorporated on the surface of an electrode can provide several advantages such as catalytic effects and greater effective sensor surface area [23, 32].

In the present work, we prepared and characterised a glassy carbon electrode (GCE) modified with FG, IL (1-butyl-3-methylimidazolium tetrafluoroborate) and silver nanoparticles (AgNPs) for the simultaneous determination of SAL and PRO in biological fluid samples.

2. Experimental

2.1. Reagents and solutions

SAL, PRO, bovine serum, nafion (NF) and 1-butyl-3-methylimidazolium tetrafluoroborate (IL) standards were purchased from Sigma-Aldrich (São Paulo, Brazil); NaOH, reagents for phosphate buffer (H_3PO_4, KH_2PO_4, K_2HPO_4, and K_3PO_4) and KCl were acquired from Acros. Graphene (GR) was acquired from Graphene Supermarket (New York, USA). The solutions were prepared using ultrapure water with resistivity not less than 18 MΩ cm obtained from a Millipore Milli-Q system (Billerica, USA). Stock solutions of SAL and PRO, both at 0.1 mol L^{-1} were prepared directly in ultrapure water.

2.2. Preparation of urine and serum samples

Synthetic urine samples were prepared as previously reported by Laube et al. [33] with the addition of 0.20 mmol L^{-1} KCl, 0.18 mmol L^{-1} NH_4Cl, 0.10 mmol L^{-1} NaCl, 0.10 mmol L^{-1} $CaCl_2$, 0.15 mmol L^{-1} KH_2PO_4 and 0.18 mmol L^{-1} urea in a 25 mL volumetric flask. The serum (bovine serum) samples were diluted 10 times with ultrapure water, and these samples were evaluated at two known concentrations of SAL and PRO (0.60 and 2.0 μmol L^{-1}, respectively), and analysed by recovery percentage.

2.3. Synthesis of the FG

Synthesis of the FG was performed as previously reported by Santos et al. [24]. In short, 100 mg of graphene was submitted to chemical treatment using a concentrated solution of 1:1 (*v*/v) H_2SO_4/HNO_3 and, this suspension was stirred for 12 h at 25 °C. The obtained FG suspension was filtered and washed with ultrapure water to about pH 7.0 and dried at 100 °C.

2.4. Synthesis of the AgNPs

Preparation of the AgNPs was performed as previously reported by Wong et al. [23]. Initially, solutions of 5.0×10^{-3} mol L^{-1} $NaBH_4$ and 1.0×10^{-4} mol L^{-1} $AgNO_3$ were prepared. Then, 1 mL of the $NaBH_4$ solution and 49 mL of the $AgNO_3$ solution were transferred individually to two flasks, and left in an ice bath for 30 min. Finally, the $NaBH_4$ solution was added dropwise under stirring, thereby forming a yellowish colloidal suspension, which was stirred for 30 min and stored in an amber flask.

2.5. Preparation of the AgNP-IL-FG-NF/GCE sensor

The surface of the GCE was initially cleaned with a polishing cloth and 0.5 μm alumina slurry, followed by ultrasonic cleaning with ethyl alcohol and ultrapure water for 2 min. Under optimised conditions, the dispersion of AgNP-IL-FG-NF was prepared using 1.0 mg of FG, 1.0 mg of IL (1-butyl-3-methylimidazolium tetrafluoroborate), 10 μL of 0.25% (v/v) NF solution, 250 μL of AgNPs, and 740 μL of ultrapure water. The reagents were subjected to ultrasonic agitation for 40 min in order to obtain a homogeneous dispersion. Next, an 8 μL aliquot of the dispersion was dropped onto the GCE surface and dried at room temperature for 3 h, to obtain the GCE modified with AgNP-IL-FG-NF film.

2.6. Apparatus and reference method

Electrochemical experiments were performed by employing a Metrohm/Eco Chemie Autolab PGSTAT12 potentiostat/galvanostat electrochemical system controlled by GPES 4.9. Voltammetric measurements were carried out in a three-electrode cell, with an Ag/AgCl (3.0 mol L^{-1} KCl) electrode as the reference electrode, a platinum foil (0.5 cm^2) as the auxiliary electrode and a GCE (Ø = 3 mm) or an AgNP-IL-FG-NF/GCE working electrode. The morphological characterisations of the FG and AgNPs were evaluated by images acquired by field emission gun scanning electron microscopy (FEG/SEM, FEI Magellan 400 L). The film thickness on the electrode surface was evaluated using a Hirox, model KH-7700 digital microscope.

UV–Vis spectrophotometer (Shimadzu model UV 2550) with a quartz cuvette (optical path length of 10 mm) was used as comparative spectrophotometric method [34, 35].