Journal of Electroanalytical Chemistry 733 (2014) 60–68

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Voltammetric determination of ropinirole in the presence of levodopa at the surface of a carbon nanotubes based electrochemical sensor in pharmaceuticals and human serum

Mirela Sadiković, Biljana Nigović *, Sandra Jurić, Ana Mornar

University of Zagreb, Faculty of Pharmacy and Biochemistry, A. Kovacica 1, 10000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 4 July 2014
Received in revised form 11 September 2014
Accepted 17 September 2014
Available online 28 September 2014

Keywords:
Ropinirole
Levodopa
Carbon nanotubes
Electrochemical sensor
Stripping analysis

ABSTRACT

A nanostructured electrochemical sensor was developed to establish an innovative way to measure ropinirole and levodopa in real pharmaceutical and biological samples, which offers a high selectivity derived from the stable carbon nanotubes-Nafion polymer film. The surface morphology of the modified electrodes was characterized by scanning electron microscopy. The effect of surface modifications, supporting electrolyte, amount of carbon nanotubes suspension, accumulation time and potential were investigated. A sensitive electroanalytical methodology for the simultaneous determination of both drugs co-administrated in advanced Parkinson's disease patients using adsorptive stripping square-wave voltammetry is presented. Under the optimized conditions, ropinirole and levodopa gave a linear response in the range 1×10^{-7}–1×10^{-5} M and 2.5×10^{-7}–1×10^{-5} M with detection limits 1.6×10^{-8} M and 5.2×10^{-8} M, respectively. The method was successfully utilised for their quantification in human serum samples and good recoveries were obtained without interference from endogenous dopamine, uric and ascorbic acid. In addition, the proposed sensor was successfully applied in the independent determination of ropinirole and levodopa content in pharmaceutical formulations, whose accuracy was attested by good agreement of the results with those, obtained using high performance liquid chromatography. The proposed sensor is characterized by high sensitivity and reproducibility, simple fabrication procedure, easy handling, resistance against surface fouling and low cost.

1. Introduction

Parkinson's disease is one of the most common neurodegenerative disorders in humans leading to progressive deterioration of motor function due to loss of dopamine-producing brain cells. Ropinirole (ROP) is a novel nonergoline dopamine agonist indicated for the treatment of early and advanced Parkinson's disease (Scheme 1). It is used as monotherapy in the first stages of the disease [1]. When used as an adjunct to dopamine precursor levodopa (L-dopa) in advanced Parkinson's disease patients with motor fluctuations, ROP reduces daily off time and allows a reduction of L-dopa dose. ROP is also one of the four medications approved by the FDA for the treatment of primary restless legs syndrome affecting up to ten percent of the population [2].

To date, only a few analytical methods have been described for the determination of ROP, such as liquid chromatography-mass spectrometry methods for quantification of ROP in biological fluids [3,4], HPLC with UV detection for drug impurity profiling [5,6] and stability-indicating assays [7], high performance thin layer chromatography [8], capillary zone electrophoresis [9], spectrophotometry [10], spectrofluorimetry [11] and ultra-performance liquid chromatography [12,13]. The official method for quantification of ROP has not yet been approved in European Pharmacopoeia [14]. The literature has only one report for electroanalytical determination of ROP employing glassy carbon electrode (GCE) [15]. However, due to the surface-active properties of drug oxidation product, the adsorption of ROP on the electrode surface could not be used as an effective preconcentration step prior to voltammetric quantification of the drug. Consequently, the obtained concentration range with the GCE was sufficient for measurements of ROP in pharmaceutical formulation, but not in biological samples. Therefore, the development of electrochemical sensors for ROP determination with lower quantification limit without time-consuming surface cleaning as in the case of GCE is of great importance. On the other hand, ROP often coexists with L-dopa in biological fluids as these drugs are co-administrated in advanced Parkinson's disease patients. Surprisingly, up to now, no study has

* Corresponding author. Tel.: +385 1 6394 453; fax: +385 1 4856 201.
E-mail address: bnigovic@pharma.hr (B. Nigović).

http://dx.doi.org/10.1016/j.jelechem.2014.09.020
1572-6657/

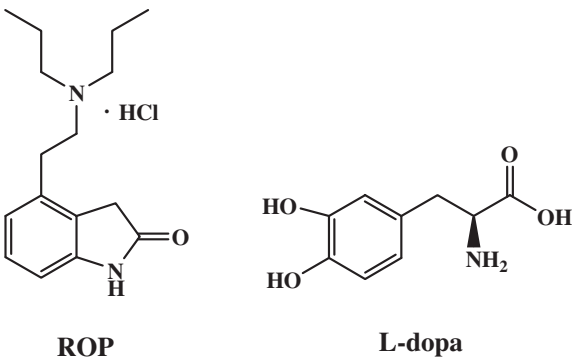
been published in the literature reporting the simultaneous determination of ROP and L-dopa. The monitoring of the therapeutic level concentrations of both drugs in serum samples is a critical subject in clinical medicine to prevent overdose-induced toxicity. The development and application of electrochemical sensors for L-dopa quantification in pharmaceuticals and biological fluids has received considerable interest in recent two years [16–26].

In continuation to our work on analysis of ROP [15], the aim of this study was to construct a simple, stable and sensitive electrochemical sensor for ROP determination. Carbon nanotubes (CNTs) have embarked a new epoch as novel electrode material in pharmaceutical analysis. CNTs can be homogenously dispersed in Nafion (NAF) solution due to hydrophobic side chains [27], while the hydrophilic negatively charged sulfonate group in NAF film enables selective preconcentration of positively charged drug molecules through electrostatic interaction [28,29]. Therefore, the adsorption characteristic of ROP molecule was investigated at the surface of glassy carbon electrode modified with carbon nanotubes and Nafion as cation exchange polymer (CNT-NAF/GCE). The analytical performance of the modified electrode for ROP quantification in the presence of L-dopa was also evaluated. Thus, here we reported for the first time, the development of analytical method for simultaneous determination of ROD and L-dopa. The adsorptive stripping voltammetric method was applied for trace analysis of both drugs in human serum samples. To achieve a better medicinal effect and lower toxicity, it is very important to control the content of ROP and L-dopa in pharmaceutical dosage forms. Therefore, the modified electrode was also used to develop simple, inexpensive, fast and accurate method for direct voltammetric quantification of ROP and L-dopa individually in commercial pharmaceutical formulations. The HPLC was selected as the comparative methods for evaluating the proposed new electroanalytical method.

2. Experimental

2.1. Instrumentation

Voltammetric experiments were carried out using a μ-Autolab potentiostat (Eco Chemie, Utrecht, The Netherlands). The experimental conditions were controlled by General Purpose Electrochemical System (GPES) software, version 4.9. A three-electrode configuration was used with a bare or modified GCE (3-mm diameter, Metrohm, Switzerland) as working electrode, Ag/AgCl (KCl 3 M, Metrohm) reference electrode and a platinum counter electrode. When required, stirring was applied using a computer-controlled stirrer at ca. 300 rpm. All measurements were performed at room temperature (23 ± 2 °C) in a 20 mL electrochemical cell. HPLC analysis were performed using an Agilent 1100 Series LC system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector, controlled by ChemStation software. The scanning electron microscope employed for surface characterisation of the electrodes was a Jeol JSM-7000F model (Jeol Ltd., Tokyo, Japan) with an operating voltage of 5 or 10 kV.



ROP L-dopa

Scheme 1. Chemical structures of ropinirole (ROP) and levodopa (L-dopa).

2.2. Chemicals

Ropinirole hydrochloride (99.4%), kindly donated by Pliva (Zagreb, Croatia), was used as received without any further purification. L-dopa was purchased from Fluka (Laramie, USA). The purity of L-dopa was high enough to comply with the European Pharmacopoeia. Requip® film-coated tablets (GlaxoSmithKline, London, United Kingdom) containing 2.28 mg of ropinirole hydrochloride, equivalent to 2 mg of ROP, and Madopar® tablets (Hoffmann-La Roche, Basel, Switzerland) containing 100 mg of L-dopa and 25 mg of benserazide, were supplied from local pharmacy. Multi-wall carbon nanotubes (purity more than 98%) with o.d. between 6 and 13 nm and tube length from 2.5 to 20 μm as well as Nafion (5 wt% solution in a mixture of lower aliphatic alcohols and water) were from Sigma–Aldrich (Steinheim, Germany). All solutions were prepared from analytical grade chemicals and ultra-pure water obtained by a Milli-Q system (Millipore, Bradford, USA).

Stock solutions of ROP and L-dopa for voltammetric measurements were prepared in ultra-pure water at a concentration of 1.0×10^{-3} M and stored at 4 °C in a refrigerator. Both ROP and L-dopa standard solutions were prepared by appropriate dilution of these stock solutions with the selected supporting electrolyte just before use. The supporting electrolytes were sulfuric acid, hydrochloric acid, acetic acid and Britton-Robinson buffer adjusted to the desired pH with 0.2 M sodium hydroxide solution.

2.3. Preparation of the modified electrodes

A mass of 50 mg of the multi-wall carbon nanotubes was dispersed in 20 mL of concentrated nitric acid. The mixture was then sonicated for 4 h to generate carboxylic acid-functionalized surface. The suspension was filtered through a 200 nm membrane and washed with double distilled water to reach neutral pH. The solid powders were dried under vacuum at room temperature. Then, 1 mg of the functionalized CNTs was dispersed in 1 mL of 0.5% (m/v) Nafion ethanol solution and sonicated for 30 min to form homogenous suspension. The CNT-NAF/GCE was prepared by casting 5 μL of the immobilizing suspension on the polished GCE surface allowing the solvent to evaporate at room temperature. Finally, the modified electrode was carefully rinsed with purified water and scanned by two successive cyclic voltammetric sweeps between 0 and 1.5 V at 100 mV s^{-1} in a blank solution of 0.1 M H_2SO_4 prior to first electrochemical measurements. The surface areas of modified electrodes were obtained by cyclic voltammetry (CV) using 1.0×10^{-3} M $K_3Fe(CN)_6$ as a probe in 0.1 M KCl electrolyte at different scan rates (v). From the slope of the anodic peak current versus $v^{1/2}$ plot, the surface areas of the GCE and the CNT-NAF/GCE was calculated to be 0.057 and 0.298 cm^2, respectively. For comparison, the carbon nanotubes modified GCE (CNT/GCE) and the Nafion modified GCE (NAF/GCE) were also prepared in the same way as described but without the addition NAF and CNTs, respectively.

2.4. Electrochemical measurements

CV was carried out from 0 to 1.5 V with the scan rate varying from 10 to 500 mV s^{-1}. Square-wave voltammetry (SWV) was used for the determination procedures of ROP and L-dopa. The voltammograms were recorded in the SWV mode from 0.4 to 1.5 V either immediately in quiescent solution or after adsorptive