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Electrocatalytic oxidation and voltammetric determination of levodopa in the presence of carbidopa at the surface of a nanostructure based electrochemical sensor

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ABSTRACT

In the present paper, the use of a carbon paste electrode modified by meso-tetrakis(3-methylphenyl) cobalt porphyrin (CP) and TiO_2 nanoparticles for the determination of levodopa (LD) and carbidopa (CD) was described. Initially, cyclic voltammetry was used to investigate the redox properties of this modified electrode at various scan rates. Next, the mediated oxidation of LD at the modified electrode was described. At the optimum pH of 7.0, the oxidation of LD occurs at a potential about 150 mV less positive than that of an unmodified carbon paste electrode. Based on differential pulse voltammetry (DPV), the oxidation of LD exhibited a dynamic range between 0.1 and 100.0 μM and a detection limit (3σ) of 69 ± 2 nM. DPV was used for simultaneous determination of LD and CD at the modified electrode, and quantitation of LD and CD in some real samples (such as tablets of Parkin-C Fort and Madopar, water, urine, and human blood serum) by the standard addition method.

1. Introduction

Patients suffering from Parkinson's disease have a significant depletion of dopamine (DA) in their brains. DA cannot be administered directly because this neurotransmitter cannot cross the blood–brain barrier into the central nervous system and cannot be employed to restore its normal level. Levodopa (LD) (a precursor of DA) is an important neurotransmitter, which is used for the medication of neural disorders such as Parkinson's disease (Barnes, 1988). After administration, LD is converted into DA through enzymatic reaction catalyzed by dopadecarboxylase. Some side effects of systemic DA can appear if LD is taken at high dosages because of the metabolism of LD being extracerebral. For better therapeutic effect and lower toxicity, carbidopa (CD) is administered in association with LD in pharmaceutical formulation containing 10–25% of CD. This catecholamine has inhibition effect on the decarboxylase activity (Reynolds, 1993). Therefore, by administering LD combined with CD, the concentration of DA is controlled at appropriate level and this combination also results in reduction of side effects and improvement of therapy. This is why the development of a method for the simultaneous determination of LD and CD has appeared to be of great importance because of their coexistence in pharmaceutical preparations (Nutt and Fellman, 1984; Gomes and Soares-da-Silva, 1999; Olanow et al., 2001).

Different analytical methods have been employed for the determination of LD and CD in raw material, pharmaceutical formulations and biological fluids, mainly by fluorescence spectrometry (Kim et al., 2008), spectrofluorimetric (Madrakian et al., 2009), spectrophotometry (Chamsaza et al., 2007), high performance liquid chromatography (Li et al., 2010; Muzzi et al., 2008) and capillary electrophoresis (Zhao et al., 2007). As with other catecholics derivates, these drugs contain electroactive groups and can be electrochemically oxidized on carbon, platinum or gold electrodes. This has enabled the electrochemical characterization and determination of LD (Shahrokhian and Asadian, 2009) and CD (Yaghoubian et al., 2009a, 2009b). However, few electrochemical methodologies have been developed for simultaneous determination of these drugs, probably due to their similar structural patterns and electrochemical responses showing dramatic overlapping when using conventional electrodes.

Carbon paste electrode (CPE) is a special kind of heterogeneous carbon electrode consisting of mixture prepared from carbon powder and a suitable water-immiscible or non-conducting binder (Mazloum-Ardakani et al., 2010a, 2010b, 2010c, 2011; Trnkova et al., 2011).

The use of carbon paste as an electrode was initially reported in 1958 by Adams (Adams, 1958). In afterward researches a wide variety of modifiers (Shahrokhian and Khafaji, 2010; Martinez

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et al., 2010; Beitollahi et al., 2008; Mazloum-Ardakani et al., 2010a, 2010b, 2010c; Kozan et al., 2010) have been used with these versatile electrodes. CPEs are widely applicable in both electrochemical studies and electroanalysis thanks to their advantages such as very low background current (compared to solid graphite or noble metal electrodes), facility to prepare, low cost, large potential window, simple surface renewal process and easiness of miniaturization (Patil et al., 2009). Besides the advantageous properties and characteristics listed before, the feasibility of incorporating different substances during the paste preparation (which results in the so-called modified carbon paste electrode) allows the fabrication of electrodes with desired composition, and hence, with pre-determined properties (Raoof et al., 2006).

The development of nanoscience has been growing very fast over the last few years. Traditional areas of technology have been boosted due to the great advantages that nanoparticulate based materials may offer. In the field of electrochemistry, these advantages are reached by both the enhancement of the total rate of diffusion processes due to the surface to volume ratio (Vidotti et al., 2007) and/or leading to a higher number of molecules that can be attached or adsorbed on the inorganic nanoparticles (Daniel and Astruc, 2004).

TiO_2 is widely used in cosmetics, solar cells, batteries, additives in toothpaste and white paint, and others. Recently, there has been a considerable interest in using TiO_2 nanoparticles as film-forming materials since they have high surface area, optical transparency, good biocompatibility, and relatively good conductivity. Various TiO_2 films were also used to immobilize proteins or enzymes on electrode surface for either mechanistic study of the proteins or fabricating electrochemical biosensors. For example, Durrant and co-workers immobilized a range of proteins into nanoporous TiO_2 film electrodes and successfully used this strategy to develop electrochemical and optical biosensors (Topoglidis et al., 2000, 2001). Luo and co-workers used nanocrystalline TiO_2 films on electrodes to entrap heme proteins such as cytochrome *c*, myoglobin, and hemoglobin, and observed the direct electrochemistry of these proteins (Li et al., 2001). Accumulation and electroactivity of cytochrome *c* in mesoporous layer-by-layer films of TiO_2 and phytate at ITO electrodes was also studied (McKenzie and Marken, 2003). Titania sol–gel matrix films were used to immobilize HRP (horseradish peroxidase), and with the aid of mediators, HRP–TiO_2 film electrodes were used to detect H_2O_2 by amperometry (Yu and Ju, 2002; Xu et al., 2002).

In view of the desirable characteristics of nanoparticles, it is likely that electrochemical processes may occur in a facile manner on nanoparticles modified carbon paste electrodes. Therefore, the present work aims to employ voltammetry for the individual and simultaneous determination of LD and CD at a new synthesized cobalt porphyrin-TiO_2 nanoparticles modified carbon paste electrode (CP-TNMCPE). The electrochemical characterizations of the obtained electrode have been carried out through cyclic voltammetry (CV) and chronoamperometry (CHA). Utilizing the developed method, individual and simultaneous determination of the two compounds has been carried out in pharmaceutical formulations, water, urine and human blood serum samples.

2. Experimental

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT-302N, Eco Chemie, Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the CP-TNMCPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 691 pH/ion meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. LD, CD and all other reagents were of analytical grade from Merck (Darmstadt, Germany). Graphite powder and paraffin oil (DC 350, density = $0.88\ g\ cm^{-3}$) as the binding agents (both from Merck, Darmstadt, Germany) were used for preparing the pastes. The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0–11.0.

2.2. Synthesis of meso-tetrakis(3-methylphenyl)porphyrin cobalt (II)

Meso-tetrakis(3-methylphenyl)porphyrin ($H_2T(3\text{-}CH_3)PP$) was synthesized following a literature method (Gonsalves et al., 1991). Meso-tetrakis(3-methylphenyl)porphyrin (0.038 mmol) and cobalt acetate (0.077 mmol) were refluxed in chloroform overnight under nitrogen. When the reaction was complete, the solvent was removed. The product was washed with water (Wayland et al., 1997).

2.3. Preparation of TiO_2 nanoparticles

Colloidal suspension of TiO_2 nanoparticles was synthesized by mixing titanium tetraisopropoxide, H_2O_2, and H_2O with volume proportions of 12:90:200, respectively. The resulting solution was refluxed for 10 h to promote the crystallinity.

2.4. Preparation of the electrode

The CP-TNMCPEs were prepared by hand mixing 0.01 g of CP with 0.95 g graphite powder and 0.04 g TiO_2 nanoparticles with a mortar and pestle. Then, ~0.7 mL of paraffin was added to the above mixture and mixed for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 10 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, CP modified CPE electrode (CP-CPE) without TiO_2 nanoparticles, TiO_2 nanoparticles paste electrode (TNCPE) without CP, and unmodified CPE in the absence of both CP and TiO_2 nanoparticles were also prepared in the same way.

3. Results and discussion

3.1. Electrochemical properties of modified CP-TNMCPE

To the best of our knowledge there is no prior report on the electrochemical properties and, in particular, the electrocatalytic activity of CP in aqueous media. Therefore, we prepared modified electrodes based on CP-TNMCPE and studied their electrochemical properties in a buffered aqueous solution (pH 7.0) using CV. It should be noted that one of the advantages of CP as an electrode modifier is its insolubility in aqueous media. Experimental results showed reproducible, well-defined, anodic and cathodic peaks with E_{pa} and E_{pc} of 0.50 and 0.34 V vs. Ag/AgCl/KCl (3.0 M), respectively (Fig. 1). The observed peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$ of 160 mV, was greater than the value of $59/n$ mV expected for a reversible system, suggesting that the redox couple of CP in CP-TNMCPE has a quasi-reversible behavior in aqueous medium. The effect of the potential scan rate (ν) on electrochemical properties of the CP-TNMCPE was also studied by CV. Plots of both anodic and cathodic peak currents (I_p) were linearly dependent on ν in the range of 20–700 $mV\ s^{-1}$ (Fig. 1A), indicating that the redox process of CP at the modified electrode is diffusionless in nature.