A novel way for detection of antiparkinsonism drug entacapone via electrodeposition of silver nanoparticles/functionalized multi-walled carbon nanotubes as an amperometric sensor

Mehdi Baghayeri [a,*], Maliheh Barazandeh Tehrani [b], Amirhassan Amiri [a], Behrooz Maleki [a], Samaneh Farhadi [a]

[a] Department of Chemistry, Faculty of Science, Hakim Sabzevari University, P.O. Box 397, Sabzevar, Iran
[b] Department of Medicinal Chemistry, Faculty of Pharmacy, Pharmaceutical Research Center, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Silver (Ag) nanoparticles were electrochemically deposited on the film of a metformin functionalized multi-walled carbon nanotube modified glassy carbon electrode (Met-MWCNT/GCE), which fabricated an Ag@Met-MWCNT nanocomposite sensor (Ag@Met-MWCNT/GCE) to detect entacapone (ENT). The Ag@Met-MWCNT nanocomposite was characterized by field emission scanning electrochemical microscopy (FESEM), X-ray diffraction (XRD) analysis, FT-IR and electrochemical tests. The modified electrode showed a large electrocatalytic activity for reduction of ENT. This improved activity indicates that Met@MWCNT plays a crucial role in the dispersion and stabilization of Ag nanoparticles on GCE. Under the optimized conditions the linear range for the detection of the ENT was obtained to be 0.05 to 70.0 μM with a low detection limit of 15.3 nM. The proposed sensor can effectively analyse ENT concentration in pharmaceutical formulations and human urine samples, avoiding interference, and is a promising ENT sensor due to good sensitivity, stability and low cost.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The term parkinsonism is used for a motor syndrome whose principal symptoms are tremor at relaxation, lignification, slowing of movement and postural inconstancy. Parkinson's disease (PD) is the most common form of parkinsonism and is usually explained as "primary" parkinsonism, meaning parkinsonism with no external identifiable cause [1]. PD is a degenerative disorder of the central nervous system mainly affecting the motor system. The motor symptoms of PD result from the death of dopamine-generating cells in the substantia nigra, a region of the midbrain [2]. PD is more common in older people, with most cases occurring after the age of 50. Another type of Parkinson's disease which is seen in young adults is called YOPD (young onset PD). In 2013 PD resulted in 103,000 deaths up from 44,000 deaths in 1990 [3–5].

Entacapone (2E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylprop-2-enamide is a member of the class of drugs known as nitrocatechols that functions as a catechol-O-methyl transferase (COMT) inhibitor [6]. It is used in the treatment of PD. When administered in conjunction with dopaminergic agents such as L-DOPA, entacapone prevents COMT from metabolizing L-DOPA into 3-methoxy-4-hydroxy-L-phenylalanine (3-OMD) in the periphery, which does not easily cross the blood brain barrier (BBB) [7–9].

According to above sentences, determination of entacapone (ENT) is quite necessary in the diagnosis and treatment of diseases. Numerous methods for determining ENT have been described in the literature. They include liquid chromatography, spectrophotometry and capillary electrophoresis. However, most of these methods have some disadvantages such as time-consuming, complicated treating process, high costs and low sensitivity [10–15]. Consequently, electrochemical methods have been employed because of the fast response, high sensitivity and low cost. Literature survey shows limited numbers of researches have been reported the determination of ENT using electrochemical methods [16,17]. However these ENT based sensors have some common drawbacks such as poor stability, reusability and slow electron transfer. Therefore, there is a demand for the development of a sensor that is comparatively more stable, reproducible and sensitive for ENT determination in real samples.

The integration of nanotechnology and electrochemistry is expected to produce major advances in the field of electrochemical sensors. During recent years, nanomaterials, such as carbon nanotubes (CNTs) and transition metallic nanoparticles (NPs), have been widely applied in sensors. Nano-scale Metals provide great opportunities for this oxidation reaction and electrodes modified with these materials reveal good performances, such as increasing the surface area and enhancing the mass transport and catalysis. The advantages of Ag nanoparticles such as biocompatibility, catalysis and low toxicity make them suitable for improving properties of composite materials for preparation of modified electrodes [18,19]. However, dispersion of nanoparticles on various

* Corresponding author.
  E-mail address: m.baghayeri@hsu.ac.ir (M. Baghayeri).

supports plays a very important role in fabricating a sensor and aggregation of nanoparticles can prevent their broad applications. Therefore selection of an appropriate support is a key-factor at development of nanoparticle-based sensors [20].

CNTs are receiving more and more research interests since its discovery by Iijima [21]. MWCNT consisting of several layers of graphene [22] has also been widely used in the study of electrochemical sensors due to its wide potential window, interesting electrochemical properties, and good compatibility with biological samples [23]. Functionalization of MWCNTs has particular impact on their electrocatalytic performance. These functional groups can interact with various nano-scale materials to construct nanostructures with appropriate distribution. For example, Han et al. fabricated an electrochemical H_2O_2 sensor based on Ag-MnO$_2$-MWCNTs nanocomposites, which Ag-MnO$_2$-MWCNTs nanocomposites produced good performance toward the detection of H_2O_2 [24]. Li et al. fabricate a novel nonenzymatic sensor of H_2O_2 based on silver nanoparticles and ionic liquid functionalized MWCNTs [25]. Sun et al. synthesized PD NPs on functionalized MWCNTs and study the effects of MWCNTs surfaces for methanol oxidation [26]. Metformin (*N*,*N*-dimethylimidodicarbonimidic diamide) is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function [27]. Because of its numerous nitrogen-containing functional groups, Met appears to be an attractive candidate for interaction with many metal ions to construction of metal nanoparticles [28].

In this paper, we report, for the first time, a highly selective and sensitive electrochemical sensor based on Ag@Met-MWCNT modified GCE for ENT detection at ultra-trace level (nM). In this work, Met-functionalized MWCNT (Met-MWCNT) was synthesized and used to act as an appropriate substrate for the indirect electrodeposition of Ag NPs on glassy carbon electrode. Met showed excellent coordination with Ag$^+$ to form Met–Ag$^+$–Met complexes through a bond between the nitrogen atoms on Met with Ag$^+$. Ag NPs were prepared through electrochemical reduction of Ag$^+$ at an optimum fixed potential. The prepared electrochemical sensor was characterized by cyclic voltammetry (CV), field emission scanning electron microscopy (FESEM) and differential pulse voltammetry (DPV). In the end, the developed sensor was effectively used for the determination of ENT in different blood samples of five normal human beings.

2. Experimental section

2.1. Materials

Potassium nitrate (>98%), silver nitrate (>99%) suprapur from Merck (www.merck.ae) were employed as received. MWCNTs (>95%, O.D.: 10–15 nm, I.D.: 2–6 nm, length: 0.1–10 μm) were purchased from Sigma-Aldrich (www.sigmaaldrich.com). The carboxylated MWCNT (denoted as MWCNT-COOH) was prepared from pristine MWCNT according to the literature reported in ever before [29]. SOCl$_2$, HCl, H_2SO_4, HNO$_3$, H_2O_2 (30 wt.%, aq), deionized water, NaH (80%), anhydrous dimethylformamide (DMF) and CaH$_2$ were obtained from Sigma-Aldrich (www.sigmaaldrich.com). Metformin hydrochloride was prepared from Sigma-Aldrich (www.sigmaaldrich.com) and treated before use as follow: 1.65 g of Met (10 mmol) and 0.40 g of NaOH (10 mmol) were added to 100 mL of ethanol and the resulting suspension was mixed for 5 h. Then, the resulted suspension was filtered and ethanol was removed with rotary evaporation leading to free Met in 99% yield. The obtained free Met was used in the whole of next experiments. Phosphate buffer was prepared by mixing the stock solutions of 0.1 M NaH$_2$PO$_4$ and 0.1 M Na$_2$HPO$_4$, and then adjusting the pH with H$_3$PO$_4$ or NaOH. The ENT solution was prepared by 0.1 M phosphate buffer (pH 7.0). The stock solution was stable when stored in a refrigerator at 4 °C for 1 month. All other chemicals were of analytical grade and were used as received without any purification process.

2.2. Apparatus and instrumentations

Cyclic voltammetry measurements were carried out on a Metrohm (797 VA Computrace, Switzerland, www.metrohm.com) controlled by personal computer. An Ag|AgCl|KCl (3 M) as reference electrode and a platinum wire as auxiliary electrode were used. Autolab electrochemistry instruments (Autolab, Eco Chemie, Netherlands, www.ecochemie.nl) was used for amperometry measurements. A glassy carbon electrode (GCE), Azar electrode (www.azarelectrode.com), Iran, with a geometrical area of 0.0314 cm^2, bare or modified, was used as working electrode. FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany, www.bruker.com). All experiments were performed at room temperature (25 ± 2 °C). X-ray diffraction (XRD, Rigaku Corporation, Tokyo, Japan, www.rigaku.com) patterns were obtained at room temperature on a Riga kuD/Max-2550 powder diffractometer with a scanning rate of 5° min^{-1}, and recorded in the 2θ range of 10–70 °C. The structure and the morphology of the samples were characterized using a FESEM, (Hitachi S4160 instrument, Tokyo, Japan, www.hitachi.com) and a Hitachi HT-7100 (150 kV) transmission electron microscope (TEM). A digital pH-Meter (780 pH Meter, Metrohm, www.metrohm.com) with precision of ±0.001 was used to read the pH value of the buffer solutions. Electrolyte solutions were de-oxygenated by purging pure nitrogen (99.99%) for 10 min prior to electrochemical experiments. All measurements were carried out under a nitrogen atmosphere.

2.3. MWCNT functionalization

Met functionalized MWCNT was prepared by previously reported method [30]. In general, MWCNT-COOH is activated with thionyl chloride to obtain an acyl chloride-MWCNT derivative (MWCNT-COCl). Subsequently, MWCNT-COCl was added to 1 mL solution of free Met + DMF and NaH (80%) and then stirred for 1 h and kept at 120 °C for 3 days. After that, Met-MWCNT was filtered, thoroughly washed with CH$_2$Cl$_2$ and deionized water to remove the free Met in the solution, followed by drying in a vacuum oven at 25 °C for 24 h.

2.4. Preparation of the modified electrodes

A glassy carbon electrode (GCE) was polished with 0.05 μm alumina slurry to a mirror-like, followed by rinsing thoroughly with deionized water. Then 6 μL of Met-MWCNT ethanol solution (1 mg/mL) was casted on the surface of a cleaned GCE and dried in air, and then the Met-MWCNT/GCE was obtained. Next, the electrode was
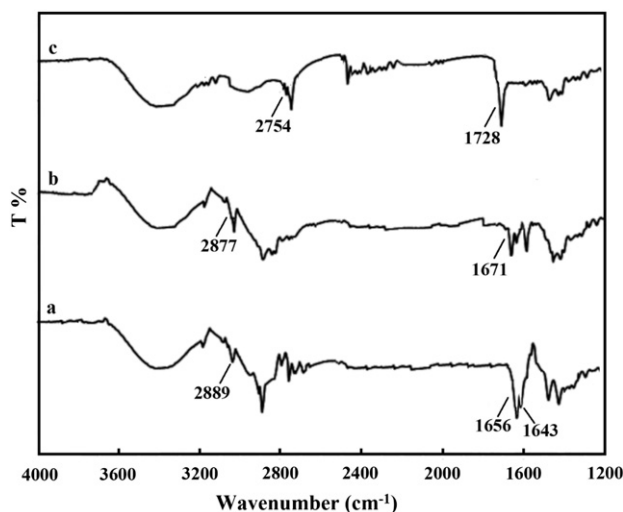

Fig. 1. FT-IR spectra of Ag@Met-MWCNT (curve a), Met-MWCNT (curve b) and MWCNT-COOH (curve c).