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Modification of glassy carbon electrode with a bilayer of multiwalled carbon nanotube/tiron-doped polypyrrole: Application to sensitive voltammetric determination of acyclovir

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ABSTRACT

A novel voltammetric sensor based on glassy carbon electrode (GCE) modified with a thin film of multi-walled carbon nanotubes (MWCNTs) coated with an electropolymerized layer of tiron-doped polypyrrole was developed and the resulting electrode was applied for the determination of acyclovir (ACV). The surface morphology and property of the modified electrode were characterized by field emission scanning electron microscopy and electrochemical impedance spectroscopy techniques. The electrochemical performance of the modified electrode was investigated by means of linear sweep voltammetry (LSV). The effect of several experimental variables, such as pH of the supporting electrolyte, drop size of the cast MWCNTssuspension, number of electropolymerization cycles and accumulation time was optimized by monitoring the LSV response of the modified electrode toward ACV. The best response was observed at pH 7.0 after accumulation at open circuit for 160 s. Under the optimized conditions, a significant electrochemical improvement was observed toward the electrooxidation of ACV on the modified electrode surface relative to the bare GCE, resulting in a wide linear dynamic range (0.03–10.0 μM) and a low detection limit (10.0 nM) for ACV. Besides high sensitivity, the sensor represented high stability and good reproducibility for ACV analysis, and provided satisfactory results for the determination of this compound in pharmaceutical and clinical preparations.

1. Introduction

Acyclovir, (2-amino-9-[(2-hydroxyethoxy) methyl]-6,9-dihydro-3H-purin-6-one), Scheme 1, is a synthetic deoxyguanosine analog. It is the prototype antiviral agent that is activated by viral thymidine kinase, converting it to ACV monophosphate, which is finally converted to ACV triphosphate by other enzymes. The triphosphate derivative can competitively prevent viral DNA polymerase and compete with the natural deoxyguanosine triphosphate, for incorporation into viral DNA. Acyclovir triphosphate also prevents DNA synthesis by acting as a chain terminator [1]. ACV is widely used in treatment of herpes simplex, herpes zoster infections, primary genital herpes, herpetic encephalitis, and varicella zoster virus infections in immunosuppressed patients. It is also helpful in inhibiting HSV infections in renal allograft receptors [2] and its anti-hepatitis B virus activity has been demonstrated [3].

ACV may lead to nephrotoxicity (crystallization of ACV within renal tubules, enhancement of serum creatinine, transient), and neurotoxicity (coma, hallucinations, lethargy, seizures, tremors) [1]. Therefore, quantitative determination of ACV seems to be very important. Several analytical methods have been proposed for the analysis of ACV in biological fluids, including spectrophotometric and spectrofluorimetric methods [4], near infrared spectroscopy [5], liquid chromatography [6], high-performance liquid chromatography (HPLC) [7], radioimmunoassay (RIA) [8] and liquid chromatography/tandem mass spectrometry (LC/MS–MS) [9]. These methods are generally time consuming and need expensive equipment and tedious operations, such as optimization of chromatographic conditions, pretreatment of samples for HPLC analysis, and handling the radioactive wastes of RIA. Whereas, electrochemical methods including LSV, cyclic voltammetry, differential pulse voltammetry (DPV), square wave voltammetry (SWV), electrochemiluminescence, polarography and amperometry [2,10–14] provide advantages such as high sensitivity, fast response, simplicity, low cost, and more environmentally friendly measurements [15]. Generally, the bare electrodes suffer from slow electron transfer reactions which lead to low sensitivity of the electrochemical methods. It has been demonstrated that sensitivity and selectivity of the electrochemical measurements can be greatly improved by chemical modification of the surface of bare electrodes. The enhancement of the performance of the modified electrodes may be due to electrocatalysis of slow electron transfer reactions, increasing the effective surface area, increasing

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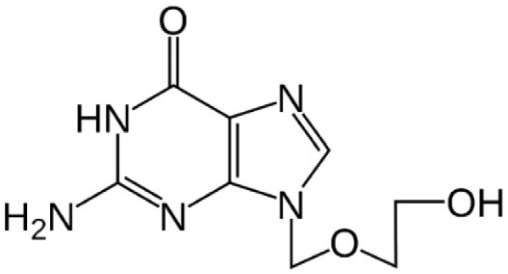

Scheme 1. Molecular structure of acyclovir.

analyte mass transport, accumulation of analyte at the electrode surface or any combination of these factors [16–22].

Since their introduction in 1991 [23], carbon nanotubes (CNTs) have attracted great interest in electrochemical fields [24–26] due to their unique features such as high chemical, thermal and mechanical stabilities, excellent electrical conductivity and high surface area [27]. CNTs also provide electrocatalytic activity resulting from their particular structure that leads to enhancement of the kinetics of electron transfer reactions [26,28–30]. Therefore, CNTs have been considered as electrode modifier to ease the determination of biomolecules, pharmacological substances and inorganic ions, and to provide electrochemical sensors and biosensors with higher sensitivities and lower detection limits [31–36].

Conducting polymers (CPs) are specified by an expanded π-conjugation along the polymer backbone, which promotes an intrinsic conductivity [37]. They represent the electrical properties of metals, while possessing the specifications of organic polymers, such as light weight, flexibility, resistance to corrosion and ease of fabrication [38]. Due to their unique electronic, chemical and biochemical properties, CPs have been widely used in the construction of electrochemical sensors and biosensors [39–41]. Polypyrrole (PPY), with valuable properties such as low cost, high stability, high electrical conductivity, reversibility between its conducting and insulating states, facile synthesis, efficient polymerization, easy formation from aqueous solutions and controllable thickness, is one of the most interesting CPs [42–44]. Electropolymerization is an efficient procedure for preparation of PPY film using an anodic potential in a solution containing pyrrole and an anionic dopant [45]. Selection of the anionic dopant is critical for the preparation of an adherent and conductive film, and influences the deposition yield, physical characteristics, morphology and electrical neutrality of the electropolymerized PPY film [46]. Aromatic sulfonate dopants provide polymer films with very good electronic conductivity [47,48].

Composites of PPY and CNT (PPY/CNT) combine the advantages of both constituents, including electrocatalytic activity, high stability and reproducibility, strong adherence to the electrode surface, large number of active sites, high electrical conductivity, and nanoporosity resulting in large surface area. These characteristics lead to excellent performance of the electrodes modified with PPY/CNT for sensorial applications [49–51]. Furthermore, CNTs operate as a backbone for a homogeneous deposition of PPY [52]. There are two ways for the preparation of PPY/CNT composite: electrodeposition of PPY on a precasted CNT film [40,41] or co-deposition of the polymer and CNTs on the electrode surface [53,54].

In this study, a thin layer of MWCNTs was casted on the surface of GCE, which was then coated with an electrochemically polymerized PPY doped with tiron. The resulting electrode was used for a highly sensitive determination of ACV in pharmaceutical formulations and plasma. The surface morphology of the modified electrode was investigated by field emission scanning electron microscopy (FESEM). The electrode exhibited a considerable increase in the oxidation peak current of ACV compared to the bare GCE, due to increased microscopic surface area and effective accumulation of ACV on the porous thin film of the modified electrode.

2. Experimental

2.1. Materials and reagents

ACV was kindly prepared by Bakhtar Bioshimi. Pyrrole, dimethylformamide (DMF) and tiron were purchased from Merck. MWCNTs (purity > 95%) were obtained from Nanostructured & Amorphous Materials (USA). All other chemicals were of analytical reagent grade from Merck. Pyrrole was purified by distillation and then kept in a dark vial in a refrigerator before use to avoid degradation. Stock solutions of ACV (100 μM) were freshly prepared daily in aqueous solution and kept in the dark at 4 °C before measurements to avoid any decomposition. Double distilled water was used throughout. Buffer solutions (0.1 M) of different pHs were prepared by mixing appropriate amounts of phosphoric acid (pH: 2.0, 3.0, 6.0, 7.0, 8.0, 9.0 and 10) or acetic acid (pH: 4.0 and 5.0) with sodium hydroxide solution. ACV tablets (200 mg, Bakhtar Bioshimi Co., Tehran, Iran) and ampoules (500 mg, Mylan S.A.S., France) were purchased from the local pharmacies. Fresh frozen plasma samples were obtained from Iranian Blood Transfusion Organization.

2.2. Apparatus

All electrochemical measurements were performed with a Metrohm Computrace Voltammetric Analyzer (model 797 VA) instrument. A three-electrode system was used, including a glassy carbon working electrode (unmodified or modified) with a geometric surface area of 2 mm, a KCl saturated Ag/AgCl reference electrode and a platinum wire auxiliary electrode. The pH measurements were done with a digital pH/mV/Ion meter (Metrohm, pH Lab 827). Electrochemical impedance spectroscopy (EIS) measurements were performed with a Potentiostat/Galvanostat EG&G model 273A (Princeton Applied Research, USA) equipped with a Frequency Response Detector model 1025 (Power Suite software), which was used with a frequency between 100 MHz and 10 kHz and a 5 mV rms sinusoidal modulation (effective value of a varying AC amplitude) in 0.1 M KCl solution containing 1 mM of both $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ (1:1 mixture) at the $E_{1/2}$ of the $[Fe(CN)_6]^{3-/4-}$ (0.13 V vs. Ag/AgCl). Microscopic images were obtained with a FESEM Mira 3-XMU. In order to obtain more distinct images, the electrode surface was first made conductive for current via coating with an extremely thin layer of the sputtered gold.

2.3. Preparation of the modified electrode

The bare GCE was first polished to a mirror-like surface with 0.05 μm alumina slurry on a polishing cloth, followed by rinsing with water and drying at room temperature. An appropriate amount of pure MWCNTs was functionalized by treatment with concentrated nitric acid for 24 h in order to obtain more edge sites and better dispersion of the nanotubes. The MWCNT suspension was prepared by dispersing 1 mg of the functionalized MWCNTs (CNT-COOH) in 1 mL of DMF solvent under ultrasonic agitation for 30 min, giving a black dispersion that is quite stable for 3–4 months. A desired volume of the dispersed nanotubes was casted on the electrode surface with a microsyringe and then the electrode was placed in oven at 50 °C for 10 min to evaporate the DMF solvent. This electrode is recognized as CNT/GCE. Subsequently, a PPY film was electrodeposited on the CNT/GCE surface from an aqueous solution containing 5 mM pyrrole and 3 mM tiron as the dopant anion by potential cycling between 0.0 and +0.75 V (vs. Ag/AgCl) at a scan rate of 50 mV s^{-1} for a total of 15 cycles. This electrode is denoted as PPY/CNT/GCE. The resulting electrode was washed with water and then subjected to ten voltammetric cycles between −0.20 and +1.30 V (scan rate 100 mV s^{-1}) in phosphate buffer solution (pH 7.0) to overoxidize the PPY film and obtain a reproducible background current. Overoxidation results in the formation of some carbonyl groups in β-position of the pyrrole rings in the polymer film [55]. This