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Electrochemical mechanism and sensitive assay of antiretroviral drug Abacavir in biological sample using multiwalled carbon nanotube modified pyrolytic graphite electrode

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ABSTRACT

Multiwalled carbon nanotube modified edge plane pyrolytic graphite electrode (MWCNT/EPPGE) was developed and implemented for the determination of antiretroviral drug Abacavir using differential pulse adsorptive stripping voltammetry (DP AdSV). Cyclic voltammogram showed two oxidation peaks located at +0.91 V and +1.10 V on (MWCNT/EPPGE). Dependency of peak potential and peak current on pH was investigated as details. The oxidation potentials affected by the pH indicating protons are involved in the electrochemical oxidation of Abacavir. Mass transport to the electrode surface was found as a combination of diffusion and adsorption which was concluded by studying the potential scan rate dependency of both peak currents. The oxidation mechanism was proposed using the obtained data and discussed. A large enhancement in both of the peak currents was observed with applying an accumulation step presenting the effect of adsorption. Peak currents showed a linear relation upon the Abacavir concentration within a range of 1×10^{-7}–2×10^{-5} M with ($r = 0.999$) for both peak responses. The method was fully validated related with selectivity, sensitivity, precision and accuracy studies. Abacavir was also determined with the proposed method in its pharmaceutical dosage forms and human serum samples. No interferences from the excipients of the dosage form or endogenous substances from biological material were found.

1. Introduction

Abacavir, systematic IUPAC name {(1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-en-1-yl}methanol, is a synthetic carbocyclic nucleoside, inhibitor of the HIV type 1 replication [1]. The compound belongs to the class of antiretroviral drugs metabolizing into carbovir triphosphate, the active intracellular agent, which lacks a 3′-OH group thus resulting in chain termination. Further, Abacavir blocks reverse transcriptase protein and, as a result, it belongs to a class of drugs called reverse transcriptase inhibitors [2–7]. Abacavir slows down HIV desease by blocking reverse transcriptase protein which is required for regeneration of HIV viruses. The drug may also reduce the damage to the immune system [8]. As known, Abacavir is rapidly absorbed after oral administration and it is primarily metabolised by the liver. The dominant pathways of metabolism are by alcohol dehydrogenase and by glucuronidation to produce the 5′-carboxylic acid and 5′-glucuronide respectively. The metabolites are excreted in the urine and approximately 2% of the administered dose is renally excreted as an unchanged compound [9]. These features lead to intense interest in analytical methods to determine drug levels in patient samples.

Very few analytical methods have been established for the determination of Abacavir in pharmaceuticals or human serum, mainly using liquid chromatography either with UV or tandem mass detection [10–14]. The liquid chromatographic methods are influenced by endogenous substances, and require expensive instrumentation. On the other hand, a spectroscopic method involving the diazotization of the drug with nitrous acid followed by coupling with phloroglucinol and resorcinol to obtain purple and red-violet coloured chromogens was developed [15]. Concerning electrochemical methods, one of them was introduced by Ozkan and Uslu using the reduction peak of Abacavir at dropping mercury electrode [16]. The oxidation peak of Abacavir on glassy carbon was applied to determine the drug by the same researchers [17]. The oxidation process was reported to be diffusion controlled and the adenine moiety in the molecule assumed to be involved in the oxidation process. Goncalves reported that both adsorption and diffusion play role in the oxidation of adenine at edge-plane

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pyrolytic graphite electrode resulted in some level of accumulation time and consequently lower limits of detection [18].

In addition, an enhancement in the adsorption of organic compounds is expected on carbon nanotube (CNT) surfaces as discussed by Xing [19]. Neither CNT surface area and porosity, nor diameter and hydrophobic interactions, cannot completely explain the interaction between organic chemicals and CNTs alone. The enhanced adsorption is attributed to all former mentioned mechanisms and other mechanisms include π–π interactions (between bulk π systems on CNT surfaces and organic molecules with C=C double bonds or benzene rings), hydrogen bonds (because of the functional groups on CNT surfaces), and electrostatic interactions (because of the charged CNT surface) [19].

The aim of the current study was to develop a multiwalled carbon nanotube modified graphite edge plane electrode (MWCNT/EPPGE) as an implement for determination of Abacavir to increase the adsorption portion in the oxidation mechanism of the compound. Hence, fully validated, simple, rapid, selective and sensitive procedures for the determination of Abacavir in pharmaceutical dosage forms and spiked human serum samples was achieved.

2. Experimental

2.1. Equipment

A modular electrochemical analyzer AUTOLAB equipped with PGSTAT 302 controlled by GPES software (EcoChemie, Utrecht, The Netherlands) was used for all voltammetric measurements. The conventional three-electrode configuration either with edge plane pyrolytic graphite electrode (BAS: ⌀: 3 mm, diameter) (EPPGE) or multiwalled carbon nanotube modified edge plane pyrolytic graphite electrode (MWCNT/EPPGE) was employed as a working electrode throughout the work. The Ag|AgCl (BAS; 3 M KCl), platinum wire counter electrode served as the reference and auxiliary electrodes, respectively. All electrochemical experiments were carried out in one-compartment voltammetric cells (10–20 mL) at conditioned room temperature (23 ± 1 °C). In voltammetric measurements, a magnetic stirrer was employed during the electrochemical accumulation step. All pH measurements were carried out using a pH meter Model 538 (WTW, Austria) with a combined electrode (glass electrode–reference electrode) with an accuracy of pH ± 0.05. Differential puls voltammetry (DPV) conditions were given as follows; step potential: 8 mV; modulation amplitude: 50 mV; modulation time: 0.07 s; interval time: 0.4 s. Optimum differential puls adsorptive stripping voltammetry (DP AdSV) conditions were; accumulation potential (E_{acc}): −0.5 V and accumulation time (t_{acc}): 180 s. Large area graphite electrode (geometric area 2.2 cm^2) was used for electrolysis. Moreover, the measurement of controlled potential coulumetry was performed in a two-compartment H-type cell for 10^{-5} M electrolyzed solution 10 mL under −1.2 V.

2.2. Reagents and chemicals

Abacavir and its pharmaceutical dosage form (Ziagen®) were kindly provided by Glaxo Smith Kline Pharm. Ind. (Istanbul, Turkey). All chemicals for preparation of buffers and supporting electrolytes were reagent grade (Merck or Sigma). Stock solutions of Abacavir (1×10^{-2} M) were prepared in bi-distilled water and kept in the dark in a refrigerator. Britton–Robinson buffer (0.04 M, pH 2.0–9.0) and 0.1 M H_2SO_4 were prepared in doubly distilled water. Standard solutions were prepared by dilution of the stock solution with supporting electrolyte. Solid MWCNT (purified to more than 95% C with an average diameter of 10 nm and an average length of 1.5 mm) were obtained from DropSens Inc.

2.3. Pharmaceutical dosage form assay procedure

Five tablets of Ziagen® (each tablet contains 300 mg Abacavir) were accurately weighed and finely powdered. A weighed portion of pharmaceutical powder equivalent to 10^{-2} M of Abacavir was transferred into a 50 mL calibrated flask and completed to the volume with bi-distilled water. The content of the flask was sonicated for 10 min to effect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte in order to obtain a final solution. The amount of Abacavir per tablet was calculated using the linear regression equation obtained from the calibration curve of pure Abacavir.

2.4. Analysis of spiked serum samples

Drug-free human blood from healthy volunteers (after obtaining their written consent) was obtained and centrifuged (5000 rpm) for 30 min at room temperature and separated serum samples were stored frozen until assay. To remove the serum protein more effectively 5.4 mL acetonitrile was added to a mixture of 3.6 mL of serum sample and 1 mL of 10^{-2} M Abacavir resulting a final concentration of 10^{-3} M of Abacavir in serum. The blank solution was prepared with initially mixing 0.25 mL of distilled water, 1.35 mL acetonitrile and 0.9 mL serum sample. Blank and stock solution of Abacavir were transported to ultrasonic bath and agitated for 10 min and subsequently centrifuged for 10 min at 5000 rpm to remove serum protein residues, then the supernatant was taken carefully. Appropriate volumes of the supernatant were transferred into the volumetric flask and diluted up to the volume with the selected supporting electrolyte. The concentration of Abacavir was varied in the range between 3×10^{-6} and 5×10^{-5} M in human serum samples. Quantifications were carried out by means of the calibration curve method from the related calibration equations.

2.5. Preparation of MWCNT modified EPPGE

MWCNT 1.0 mg, was added to 2.0 mL Dimethylformamide (DMF) and carried to ultrasonic bath and agitated for 2 h to obtain a homogeneous suspension. Resulted suspension was stable for one week. EPPGE surface was cleaned mechanically with alumina slurry on a polishing pad, rinsed with distilled water and dried in air. 5 μL of the suspension was dropped on the electrode surface and dried at 60 °C for 15 min in oven. Before applying for the measurement, the electrode was placed in room temperature to cool down.

3. Results and discussion

3.1. Electrochemical behaviour of Abacavir

The electrochemical study of 5×10^{-4} M Abacavir solution in 0.1 M H_2SO_4, at a EPPG and MWCNT/EPPG electrodes has been performed using cyclic voltammetry (Fig. 1). The voltammograms exhibit an irreversible feature with two anodic peaks, one at +0.91 V (peak 1) and the second one at +1.1 V (peak 2) on bare electrode. Depending on the pH of the medium, small wave can be observed in CV curves. In the second and further scans this wave is dissapered. A high enhancement in both peak currents was achieved and the peak potentials were shifted to +0.96 V and +1.15 V on the MWCNT/EPPG modified electrode. No electrochemical response was observed in blank solution. The current enhancement with MWCNT/EPPGE could be attributed to several reasons including the hydrophobic surface of organic compounds which resulted