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Nanoscale determination of antiviral drug acyclovir engaging bifunctionality of single walled carbon nanotubes – nafion film

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ABSTRACT

An elementary and exemplary approach is proposed for the accurate monitoring of antiviral drug acyclovir (ACV) utilizing glassy carbon electrode (GCE) fabricated with single-walled carbon nanotubes and nafion composite film employing square wave voltammetry for the first time. The developed sensor exhibits effective and sustained electron mediating behavior displaying higher peak currents at lower potential than those obtained at bare GCE. At optimal experimental conditions, oxidation current showed a wide linear response for ACV in the concentration range from 10 nM to 30 μM. The proposed sensor exhibited pronounced analytical performance for the determination of ACV with limit of detection corresponding to 1.8 nM and high sensitivity of 15.4 μA μM^{-1}. The modified sensor showcased high recognition selectivity, fair reproducibility and long term stability of signal response in the physiological environment. The developed prototype was successfully implemented to quantify ACV in several commercially available pharmaceuticals. The versatile method described herein was efficaciously applied further in detecting ACV in real human urine sample of patient undergoing pharmacological treatment with ACV. The results explicitly demonstrate the applicability of the developed sensor in quality control, pharmacokinetic studies and clinical analysis.

1. Introduction

Over the past two decades, the rapid emergence of viral diseases has posed a consequential threat to all human and veterinary health [1]. In recent years, viral diseases have evolved to be the paramount cause of death among all infectious diseases worldwide. Acyclovir (ACV) which is a synthetic purine based nucleoside analogue, plays a pivotal role in the therapy of viral diseases and possesses the best safety profile of all antivirals licensed so far. ACV is an emerging drug for the clinical treatment against herpes simplex virus, hepatitis B virus, varicella zoster virus and epstein-barr virus [2]. Phosphorylation of ACV by herpes virus specific thymidine kinase to monophosphate and sequential phosphorylation by other kinase enzymes ultimately converts it to ACV triphosphate which is highly selective towards inhibition of herpes virus DNA replication [3]. ACV is marketed in the form of ophthalmic ointments, dermal cream, injections, capsules and oral tablets which have been extensively and effectively used for the treatment of herpes virus infections. ACV provides remarkable therapeutic benefit in the therapy of viral diseases like cold sores, keratitis, corneal blindness, encephalitis and virally infected central nervous system [4]. The drug has its potential in the prophylaxis of cytomegalovirus infections in immunologically compromised patients [5]. The oral or intravenous administration of ACV is the drug of preference for the treatment of diseases like shingles and chickenpox [6]. Several adverse outcomes like neurotoxicity, nephrotoxicity, urticaria, phlebophlogosis, diarrhoea, cephalalgia, emesis and swoon are associated with higher intake of ACV and its abuse [7–9]. Since, ACV commenced a new era in antiviral therapy because of its high selectivity and low cytotoxicity therefore, sensitive determination of ACV in biological fluids and pharmaceuticals seems to be imperative.

In view of the prominence of ACV in clinical applications, several analytical methods such as spectrophotometry [10,11], high performance liquid chromatography [12,13], radioimmunoassay [14], liquid chromatography [15], flow injection electrochemiluminescence [16], high performance capillary electrophoresis [17], liquid chromatography-tandem mass spectrometry [18], fluorimetric method [19], micellar electrokinetic chromatography [20] and enzyme-linked immunosorbent assay [21] have been utilized for its determination alone and in mixture with other analytes. Binding characteristics of molecularly imprinted polymers for ACV determination has been studied earlier using solid phase extraction and high performance liquid chromatography [22]. Nevertheless,

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most of the above mentioned methods have several inconveniences which include lengthy and tedious sample preparation procedures, long analysis time and need of trained technicians. Chromatographic methods suffer from consumption of large volumes of high purity organic solvents and requirement of expensive sophisticated instruments. These methods also suffer from lack of selectivity and sensitivity which makes them unsuitable for routine analysis. Electrochemical methods on the contrary encompassing the advantages such as instrumental simplicity, rapid response time, minimal reagent consumption, moderate cost and portability have garnered profuse attention in modern analytical chemistry for the detection of biologically significant analytes [23–25]. Literature survey reveals that electrochemical techniques have been explored earlier for the detection of ACV involving diverse chemically modified sensors [26–35]. Some of the sensors exhibited narrow linear range while others had lower sensitivity and poor selectivity. However, no attempt has been made so far to determine ACV in patient samples undergoing pharmacological treatment with ACV. Hence, the aim of this work is to introduce an efficacious composite to develop a selective and sensitive interface for the electrochemical quantification of ACV in real matrix samples.

In recent years, much attention has been focused on the preparation of a variety of nanomaterials with highly controllable size, shape, surface charge and physicochemical characteristics. The alluring properties of nanomaterial exhibit signal amplification due to which they are prodigiously used for the fabrication of a wide range of electrochemical sensors [36,37]. Single walled carbon nanotubes (SWNTs) displaying quantum dots and wires at very low temperatures enhances the electrode conductivity and facilitates the electron transfer between myriad electroactive species and the underlying electrode [38,39]. Nafion which is a sulfonated tetrafluorethylene copolymer possessing pronounced antifouling capacity, chemical inertness, strong adsorption ability, thermal stability and biocompatibility renders it to be extensively employed as an electrode modifier [40]. A homogenous dispersion of SWNTs in nafion solution engenders hydrophobic side chains and polar head groups which permits a variety of manipulations, including modification of electrode surfaces and preparation of biosensors, where association of nafion does not impair the electrocatalytic properties of SWNTs [41,42]. In the present investigation, a composite film of SWNTs and nafion on glassy carbon electrode (GCE) have been utilized for the determination of ACV employing square wave voltammetry (SWV) which is an expeditious electroanalytical technique with well-established advantages, including good discrimination against background current and low detection limits. The developed biosensor with adequate selectivity was conveniently applied for the assay of ACV in pharmaceutical dosage forms. The performance of the proposed sensor was further evaluated by the detection of ACV for the first time in patient urine samples being dosed with the analyte. The experimental results proclaim that decreased over potentials coupled with increased current values instigates the potential application of the developed biosensor for manifold analytical purposes.

2. Experimental

2.1. Chemicals and reagents

ACV and nafion were obtained from Sigma Aldrich (India) and used as received. The SWNTs of purity >99.5% was purchased from Sigma Aldrich (India) as well. All other reagents were of analytical grade and used without further purification. ACV containing tablets manufactured by different pharmaceutical companies were purchased from the local market of Mumbai, India. The human urine sample of patients undergoing treatment with ACV was collected from Alwin pathology laboratory, Mumbai, India. Phosphate buffer solutions (PBS) of 1 M were prepared by mixing the stock solutions of Na_2HPO_4 and NaH_2PO_4, according to the method of Christian and Purdy [43]. Double distilled water was used throughout the experiments.

2.2. Instrumentation

All electrochemical measurements were performed with electrochemical analyzer of Metrohm (Autolab PGSTAT 302N, Netherlands) which was run on a computer using GPES 4.9 software. A conventional three electrode single compartment cell equipped with GCE as the working electrode substrate having 3 mm diameter, a Pt wire as the auxiliary electrode and an Ag/AgCl (3.0 M KCl) as the reference electrode was utilized. The electrochemical cell utilized had an inner diameter of 24.4 mm with 51.0 mm height. The pH measurements were done with a digital pH/mV Eutech pH meter, Singapore. The surface morphology of the electrodes was characterized by recording field emission gun scanning electron microscopy (FEG-SEM) using Tescan MIRA 3 model. All the potentials reported are versus Ag/AgCl reference electrode at an ambient temperature of $28 \pm 2\,^{\circ}C$.

2.3. Procedure

A stock solution of ACV was prepared by dissolving the required amount of the compound in doubly distilled water and then stored at low temperature. The solutions for the voltammetric experiments were prepared by adding required amount of the stock to 3.0 mL of PBS of desired pH and the total volume was made to 6.0 mL with double distilled water. The solutions were deaerated by bubbling high-purity nitrogen for 12–15 min before recording the cyclic voltammograms to prevent the oxygen interference. The optimized parameters used for SWV were initial E: 500 mV, final E: 1100 mV, square wave amplitude (E_{sw}): 25 mV, potential step (E): 4 mV, square wave frequency (f): 15 Hz. Urine samples from healthy human volunteers and patients suffering from chickenpox were obtained from the pathology lab and used after fifty times dilution to reduce the matrix complexity.

2.4. Fabrication of glassy carbon electrode

Initially, the surface of the GCE was carefully polished with alumina powder using micro-cloth pad followed by rinsing with double distilled water until a mirror like surface was obtained. A 0.5 mg/mL suspension was prepared by dispersing 0.5 mg SWNTs in 1.0 mL nafion (0.1% in ethanol) using ultrasonic bath. The cleaned GCE was uniformly coated with 10 μL of the suspension and air dried at room temperature. For comparison, SWNTs modified GCE was prepared by drop casting 10 μL dispersion of 0.5 mg SWNTs in 1.0 mL of N, N-dimethylformamide. The working electrode surface with a well-coated layer of SWNTs and nafion composite was then ready for use, denoted as SWNT/Naf/GCE. A comparison of the surface morphology of bare and modified electrode was studied by recording FEG-SEM images as shown in Fig. 1 which evidently indicates the deposition of SWNTs at the surface of bare GCE.

3. Results and discussion

3.1. Electrochemical characterization of the electrodes

Cyclic voltammetry was employed in order to assess the electrochemical properties of the four different electrodes, namely bare GCE, GCE modified with Nafion, GCE coated with SWNTs and SWNT/Naf/GCE. Fig. 2A showcases the comparison of the voltammetric response obtained at the four different electrodes in 0.1 M