Nano molar detection of acyclovir, an antiviral drug at nanoclay modified carbon paste electrode

Nagaraj P. Shetti[a,*], Deepti S. Nayak[a], Shweta J. Malode[a], Raviraj M. Kulkarni[b]

[a] Electrochemistry and Materials Group, Department of Chemistry, K. L. E. Institute of Technology, Hubballi-580030, Visvesvaraya Technological University, Belagavi, Karnataka, India
[b] Department of Chemistry, K. L. S. Gogte Institute of Technology (Autonomous), Visvesvaraya Technological University, Belagavi 590008, Karnataka, India

ARTICLE INFO

ABSTRACT

A nano level voltammetric sensing method has been developed for determination of acyclovir (ACV) at nano clay modified carbon paste sensor by employing cyclic voltammetry (CV) and square wave voltammetry (SWV) techniques in pH 5.0. The electro-oxidation current of ACV was enhanced two times greater by the modification of the sensor. The modifier nano clay was characterized by utilizing X-ray diffraction (XRD) and scanning electronic microscope (SEM). The influence of parameters like scan rate, pH, accumulation time, amount of the modifier and concentration on the peak current of the drug were studied. The effect of ACV concentration variation was studied using SWV technique and got lowest detection limit compared to the earlier reported techniques. The fabricated sensor was employed for the determination of acyclovir in pharmaceutical and biological samples.

1. Introduction

Purines and its derivatives symbolize an essential classification of compounds effectively contemplated as potential therapeutics against viral diseases. The drug of choice for the treatment of herpes zoster and cold sores is acyclovir (ACV) which has the preeminent safety progile of all antiviral (Scheme 1). It is a synthetically prepared acyclic purine nucleoside, structurally analogous to guanine, which standout amongst the best antiviral medication against herpes simplex viruses (HSV), hepatitis B virus (HBV) and varicella zoster viruses (VZV), Epstein-barr virus, cytomegalovirus and human herpes virus 6 [1,2]. The unsuitable dosage of ACV induces adverse reactions in the body, such as diarrhea, emesia, neurotoxicity, phlebophlogosis, urticaria, swoon and cephalalgia [3–6]. Since ACV is a guanine analogue, constitute the crucial segments of important biological compounds, for example, polynucleic acids. The knowledge of electrochemical behavior of these compounds is of biological interest. Thus it is very important to inspect the electrochemistry of acyclovir. The circumstance of irrational utilization of drug and urgency in study of pharmacokinetics still exist. Therefore monitoring of ACV concentration in blood serum or in urine is very essential.

Many analytical methods based on different principles, such as spectrophotometric methods [7–10], radioimmunoassay (RIA) [11,12], high-performance capillary electrophoresis (HPCE) [13], flow injection-chemiluminescence [14], thin layer chromatography (TLC) [15], micellar electrokinetic chromatography [16], and high performance liquid chromatography (HPLC) with different detectors [17–26] have been reported for ACV analysis. However, these methods are well-demonstrated and broadly accepted, but they frequently agonized from high cost equipment, laborious sample pretreatment, specialized expertise and are time-consuming which make them unsuitable for routine analysis. While, on another hand, the electrochemical techniques by utilizing different working electrodes, has been recognized as the best contender for the detection of any drug because of their accuracy, easiness, minimal effort of instrumentation and quick response. In this regard, some electroanalytical methods have been accounted for the determination of ACV, utilizing different voltammetric techniques and sensors. The fabricated sensors which are already reported to detect ACV are, multi-wall carbon nanotubes (MWCNTs)-dihexadecyl hydrogen phosphate (DHP) film-coated glassy carbon electrode (GCE) [27], gold electrode modified with 2-mercapto-benzothiazole-[5, 10, 15, 20-tetrakis-(3-methoxy-4-hydroxyphenyl) porphyrinato] copper(II) [28], fullerene-C_{60}-modified glassy carbon electrode [29], ultra trace graphite and glassy carbon electrode [30], copper nanoparticles-modified carbon paste electrode [31], polyvinylpyrrolidone modified carbon paste electrode [32], bilayer of multi-walled carbon nanotube/tiron-doped polypyrrole modified GCE [33], polymer film modified GCE [34].
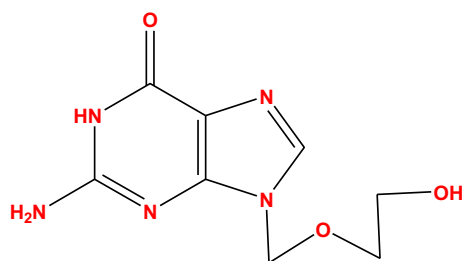
Scheme 1. Chemical structure of acyclovir (ACV).

In current science and technology one of the crucial uniqueness is to survey and to discover the new efficient materials as electrode surface modifiers to determine various types of analytes selectively. The materials which have high surface area, more porosity, low electrical conductivity, nontoxic, high stability with low cost, attracts researchers. In this regard, as electrode surface modifiers, clay, its composite materials captured copious interest of researchers and a considerable amount of research was executed in the previous decades, due to its attractive surface characteristics, like high surface area, more porosity, low electrical conductivity, nontoxic, high stability, adsorption properties, ion-exchange capabilities with low cost [35–37]. In general, new types of sensor devices are the outcome of the synthesis and preparation of innovative modifiers. The fabrication of the working electrode with nanoparticles enhances peak current, sensitivity, reproducibility [38–42].

In the present work we utilized montmorillonite nanoclay. It is a member of smectite group of clays having innate inorganic layered nanostructure [43–45]. Several substances like, organic molecules, heavy metals etc. showed high affinity towards nanoclay particles [45]. Great enactment of nanoclays in carbon paste electrodes (CPE) has been proposed and was used for different electrochemical applications [46–48].

From the literature survey, we found that there is a gap in ACV determination in trace quantity. Therefore in the current protocol, an incredible modifier i.e. nano clay was chosen and was characterized by SEM, and XRD. The nano clay was utilized in tailoring of modified carbon paste sensor and was applied for ACV determination and analysis. The fabricated electrode displayed an enhanced sensing ability towards ACV detection compared to CPE. The determination of ACV in pharmaceutical formulation as well as in human urine samples were performed and good percentage of relative standard deviation (RSD) values indicate the supremacy with regard to reproducibility and repeatability of the nanoclay modified sensor.

2. Experimental

2.1. Instrumentation and chemicals

The crystal structure and particle size of nano clay particles were determined by scanning electron microscope (SEM) (Jeol JSM-6360) and X-ray diffraction (XRD) analysis (Phillips PW1729, Cu kα). Voltammetric measurements were carried out in an electrochemical analyzer (CHI Company, D630, USA) with three electrode system, namely nano clay modified carbon paste electrode (NC/CPE) as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl (3.0 M KCl) as the reference electrode, respectively. Before each measurement, the modified and unmodified CPE surfaces were regenerated by renewing and polishing them on filter paper and washed with double distilled water. The pH measurements were performed using Elico pH meter (Elico Ltd., India).

The analyte acyclovir (purity ≥ 99%), the modifier: nano clay (particle size < 20 μm), were purchased from Sigma Aldrich and used as received without any further purification. A stock solution of ACV (0.01 mM) was prepared by dissolving an appropriate amount of pure

powdered sample in double distilled water at room temperature. The interferents utilized namely oxalic acid, citric acid, ascorbic acid, lactose, sucrose, dextrose, glucose, gum acacia, glysine, urea were purchased from Sigma-Aldrich. Phosphate buffer saline (PBS) solution (I = 0.2 M) of different pH ranging from 3.0 to 11.2 was used as supporting electrolyte by using appropriate amount of sodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) and trisodium phosphate (Na_3PO_4), which are also purchased from Sigma-Aldrich [49]. Mineral oil (Paraffin oil) was procured from Fluka. Double distilled water was used in this work. All other solvents and chemicals used were of analytical reagent grade.

2.2. Preparation of modified electrode

The CPE modified with nanoclay was prepared by homogeneous mixing of an appropriate amount of graphite powder: nanoclay particles: mineral oil in an agate mortar [50,51]. The homogeneous blended carbon matrix was packed firmly in a polytetrafluoroethylene tube (PTFE), and electrical contact was made with cupper wire at the back. The surface is best smoothed by rubbing the electrode several times and slowly polishing on a mildly abrasive surface. Surface renewal can be done by replacing the used paste and again smoothing it, providing a fresh surface unaffected by the electrode history. Activation of the working electrode surface was done by recording cyclic voltammograms (CV) in PBS of pH = 5.0 between 0.0 V to 1.2 V until a steady voltammograms was obtained. After every measurement, the paste was carefully removed prior packing a new paste. Likewise, the bare CPE was prepared with no addition of nanoclay.

The dynamic surface area of the sensing platform was calculated by using Randles - Sevcik equation; area was investigated utilizing cyclic voltammetric technique, 1.0 mM $K_3Fe(CN)_6$ as a test solution and 0.1 M KCl as supporting electrolyte, at different sweep rates in [52]. At T = 298 K and for a reversible process the equation is as follows:

$$I_p = (2.69 \times 10^5) \, n^{3/2} \, A \, D_0^{1/2} \, \nu^{1/2} \, C_0^* \tag{1}$$

The area of the electrode surface is signified as A, the diffusion coefficient as D_0, i.e. $7.6 \times 10^{-6} \, cm^2 \, s^{-1}$, sweep rate as ν, and C_0^* is the concentration of $K_3Fe(CN)_6$. From the slope of the plot of I_p vs. $\nu^{1/2}$, the surface area of the bare electrode was found to be 0.042 cm^2 and for the modified electrode surface area was increased up to 2–3 times.

2.3. Analysis of pharmaceutical dosage forms

By utilizing a mortar and pastel, the ACV tablets i.e. Acivir (Cipla Co. India) was finely ground, and related weight with the stock solution was dissolved and diluted up to 100 ml with 5.0 pH in volumetric flask. Proper dissolution was attained by sonication for 10 min. The analysis was carried out by SWV technique. The precision of the proposed technique was tested by recovery examines. The accuracy of the proposed method and the interference from the interferents used in dosage forms were evaluated using standard addition method.

2.4. Analysis of human urine samples

Urine samples were obtained from two healthy volunteers and at room temperature (25 ± 0.1 °C) was centrifuged for 5 min at 7000 rpm. The obtained samples undergo two-fold dilution, using phosphate buffer of pH 5.0 and the test solution was prepared by spiking the filtrate with the known amount of ACV (0.01 mM).

3. Results and discussion

3.1. Characterization of nano clay particles

Surface characterization plays a very important role in our under-