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Electrochemical Oxidation and Thermodynamic Parameters for an Anti-viral Drug Valacyclovir

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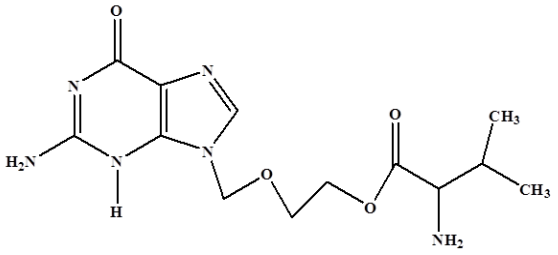
Abstract- The electro-oxidation of valacyclovir has been studied at a glassy carbon electrode in phosphate buffer media by using cyclic voltammetric technique. Effects of anodic peak potential (E_{pa}), anodic peak current (i_{pa}), pH and heterogeneous rate constant (k_o) have been discussed, single irreversible voltammogram was observed. The effects of scan rate, pH, concentration and temperature were evaluated. The electrode processes were shown to be diffusion controlled and irreversible involving adsorption effects. The electro-oxidation product of valacyclovir has been identified by MALDI2-((2-amine-6,8-dioxo-7,8-dihydro-3H-purin9(6H)-yl)methoxy)ethyl2-amino-3-methylbutanoate), involving 2-electron and 2-porton oxidation. Thermodynamic parameters such as activation energy E_a=27.51 kJmol^{-1}, enthalpy $\Delta H^{\#}$=25.03 kJmol^{-1}, entropy $\Delta S^{\#}$=-284.8 JK^{-1}mol^{-1}, Gibbs free energy $\Delta G^{\#}$=109.9 kJmol^{-1} and Arrhenius factor, logA=-2.08 and analytical parameters linearity range 5.0×10^{-3} to 7.5×10^{-5} M, LOD=1.44 µM, LOQ=4.83 µM and RSD=5.26% were calculated and presented.

Keywords- Valacyclovir, Voltammetric techniques, Electrochemical studies, Oxidation thermodynamic parameters

1. INTRODUCTION

Valacyclovir (VCH) is named as L-valine -2-[(2-amino-1, 6-dihydro-6-oxo-9-hipurin-9-yl) methoxy] ethyl ester is the L-valyl ester prodrug of the antiviral drug acyclovir that exhibits activity against herpes siplex virus types, (HSV-1) and (HSV-2) and vercellazoster virus [1]. The mechanism action of acyclovir involves the highly selective inhibition of virus DNA replication, via enhanced uptake in herpes virus-infected cells and phosphorylation by viral thymidine kinase. The substrate specificity of acyclovir triphosphate for viral, rather than cellular DNA polymerase contributes to the specificity of the drug [2-4]. But VCH has side effectsSkin rash (which may also occur after exposure to UV light e.g., sunbathing or using a sun bed) , Central nervous system effects with symptoms such as dizziness, confusion, headache, numbness, paralysis, agitation, hallucinations, Blood clotting disorder with symptoms such as bruising, bleeding (from gums), fever, fatigue Destruction of red blood cells creating anemia with symptoms such as bloody diarrhea, abdominal pain, fatigue, nausea, vomiting, confusion, swelling of hands and feet. Pain in the side (between ribs and hip) or kidney area of back and some people may feel sick [5]. These side effects may be probable of oxidative product and thermodynamic parameters of VCH. Literature survey revealed the dissolution studies [6,7], pharmacological data [8-9] and few methods are reported in literature for the estimation of valacyclovir in pharmaceutical dosage forms which includes spectrophotometry [10-13], HPLC [14] and RPHPLC methods [15]. However, literature reveals that, there is no report on thermodynamic parameters for valacyclovir.



Scheme 1. Chemical Structure of Valacyclovir

Investigation of the redox behavior of biologically occurring compounds by means of electrochemical techniques have the potential for providing valuable insights into the biological redox reaction of these molecules. Due to their high sensitivity, voltammetric methods have been successfully used to the redox behavior of various biological compounds [16-27]. Since the development of modern computer based electrochemical instrumentation, electro-analytical techniques, especially modern pulse technique, such as differential pulse voltammetry (DPV) have been used for the sensitive determination of a wide range of pharmaceuticals. The use of carbon based electrodes for electro-analysis has gained

popularity in recent years because of their applicability to the determination of substances that undergo oxidation reaction[28,29].

The purpose of the present study is to investigate the electro-oxidation mechanism, calculate thermodynamics parameters and determination of an antiviral drug VCH using voltammetric technique. Determination of VCH in real samples without any time-consuming extraction or evaporation steps prior to VCH assay. The glassy carbon electrode (GCE) has been widely used in electro analysis for various substrates for a long time because of its stability, wide potential window and fast electron transfer rate. The influences of some interfering species will also be investigated. In addition, an electrochemical behavior of VCH is investigated with cyclic voltammetry and differential pulse voltammetry (DPV).

2. EXPERIMENTAL

2.1. Materials and reagents

A stock solution of valacyclovir was obtained from Dr. Reddy's laboratories Ltd, Hyderabad, valacyclovir (5×10^{-4}M) was prepared in milli-pore water and stored in a refrigerator at 4 °C. In the present study, phosphate buffer solutions (pH range, 3-10) were used as an electrolyte. All the solutions were prepared in milli-pore water and K_2HPO_4, H_3PO_4, Na_2HPO_4, Na_3PO_4. (Sd. fine chem limited Mumbai**)** were used [30].

2.2. Instrumentation

The voltammetric experiments were performed with instruments, USA (model CHI1112C Version 9.03). A three electrode system consisting of a glassy carbon electrode (3 mm diameter) as the working electrode, an Ag/AgCl (3 M KCl) reference electrode and a platinum wire as the auxiliary electrode was used. In order to provide a reproducible active surface and to improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode was polished to a mirror finish with 0.3 micron alumina on a smooth polishing cloth and then rinsed with milli- pore water prior to each electrochemical measurement. The cleaning procedure of the electrode required less than 3 minutes. The solutions were purged with nitrogen gas. All measurements were carried out at room temperature 25 °C. DPV conditions maintained were: pulse amplitude 50 mV; pulse width 60 ms and scan rate 20 mV/s.

The area of the electrode was calculated using 1.0 mM, $K_3(Fe[CN]_6)$ as a probe at different scan rates [31] For a reversible process, the Randles- Sevcik formula has been used [32,33].

$$I_p=(2.69\times10^5)\, n^{3/2}\, A\, D_0^{1/2}\, \nu^{1/2}\, C_0^{*} \quad (1)$$

Where, n=number of electrons transferred i.e., 1, A=surface area of the electrode, D_0=diffusion coefficient, ν=sweep rate (0.1/Vs) and C_0^*=concentration of electro active species (1 mM). The surface area of the electrode was found to be 0.05 cm^2.

2.3. Analytical procedure

For good reproducible results, improved sensitivity and resolution of voltammetry peaks, the working electrode was polished carefully with 1 µm, 0.3 µm, 0.05 µm α-alumina on a smooth polishing cloth and then washed in a milli-pore water. In this method three electrode system consisting of a glassy carbon electrode (3 mm diameter) as the working electrode, an Ag/AgCl (3 M KCl) reference electrode and a platinum as counter electrode. Working solutions were prepared by diluting the stock solution as required with relevant buffer of required pH. For differential pulse voltammogram (DPV) the following parameters were maintained: sweep rate-20 mV/s, pulse amplitude-50 mV, pulse width-60 ms, pulse period-200 ms for analytical applications. All experiments were carried out at 25±1 °C.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of valacyclovir

Valacyclovir exhibited a pair of irreversible peaks with oxidation peak potentials at E_{pa}=1.190 and 1.443V and there is no reduction peak respectively in phosphate buffer of pH 4.2 [Fig.1] shows possibility of two electron transfer in the electro-oxidation process of Valacyclovir, with increase in the pH of the supporting electrolyte, the second oxidation peak becomes weaker and disappears at pH 6 and above.

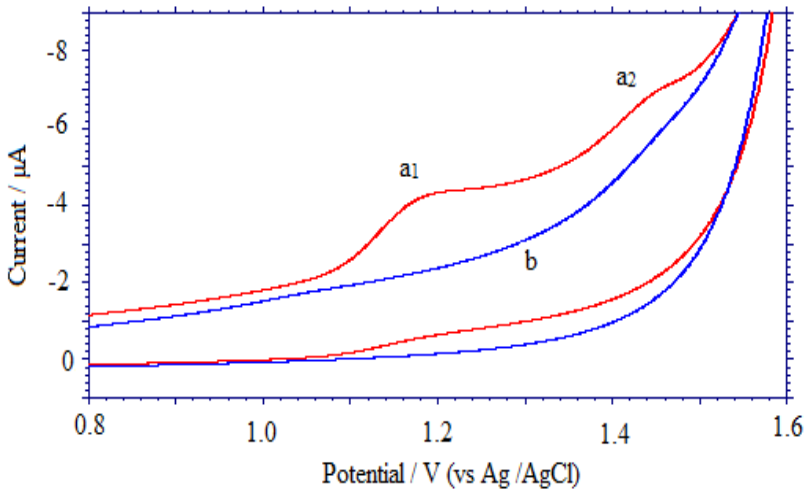


Fig. 1. Cyclic voltammogram obtained for [VCH]=5.0×10^{-5} M on GCE in phosphate buffer of pH 4.2 (a) VCH and (b) Blank at 100 mVs^{-1}

3.2. Effect of pH

The electrochemical behavior of a drug may depend on pH of the medium. Hence, the electrochemical behavior of VCH was investigated over a pH range 3-10. We carried out electrochemical oxidation VCH in different electrolytes viz., acetate buffer and phosphate buffer. Since, phosphate buffer gave good peak response (peak shape and peak current) it was selected for further studies. It was observed that the VCH was oxidized in pH range of 3-10. But, oxidation peak current was observed to decrease beyond pH 6.2. The I_p*versus* pH plot of VCH showed a maximum peak current at pH 6.2 with a scan rate 100 m V/s [Fig. 2(A)]. Further, the anodic peak potential was shifted towards more negative potential with increase in pH [Fig. 2].

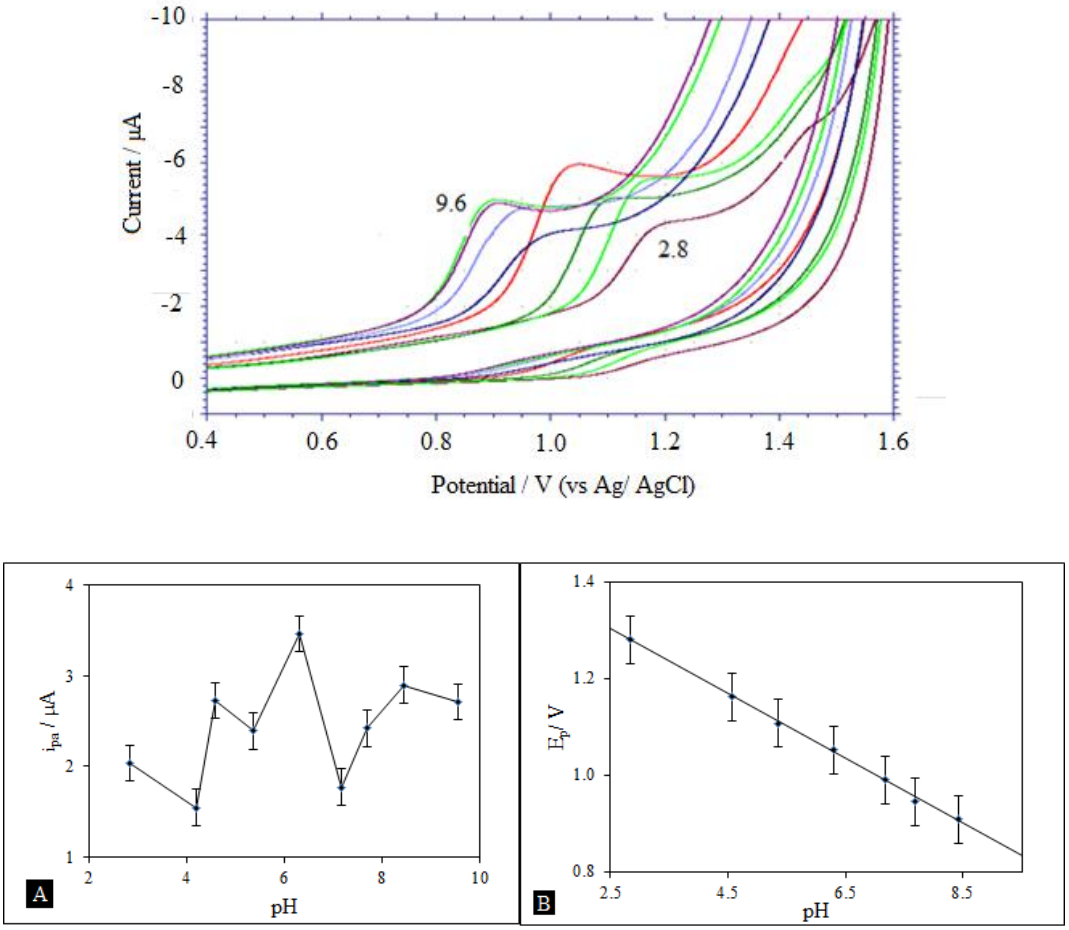


Fig. 2. Cyclic voltammogram of 5.0×10^{-5} Mof VCH in phosphate buffer of pH 2.8, 4.2, 4.6, 5.8, 6.3, 7.2, 7.7, 8.4 and 9.6 at scan rate 100 mv/s; **(A)** Plot of Current i_p(µA) *vs* pH; **(B)** Plot of peak potential E_p/ V *vs* pH

These results most likely indicated the participation of protons in the electrode process. Further, the shift in peak potential with increase in pH indicated that the pH of supporting

electrolyte exerted a significant influence on electro oxidation of VCH at glassy carbon electrode. A good linear relationship between E_{pa} and pH of the medium [Fig. 2(b)] at glassy carbon electrode. E_p=0.067pH+1.4691 The slope value of 67 mV/pH being close to the expected value of 59 mV/pH indicated that equal number of electrons and protons are involved in the electrochemical oxidation process [34-36].

3.3. Effect of scan rate

The effect of scan rate (υ) on the anodic oxidation of valacyclovir was studied at a concentration of 5×10^{-5} M in phosphate buffer media at pH 6.2 [Fig. 3], a linear relationship was observed between the oxidation peak current and square root of the scan rate with a significant correlation coefficient of 0.9929 indicating there by that electrode process is diffusion controlled in the scan rate range of 50-300 mV/s [Fig. 3(A)] [37].

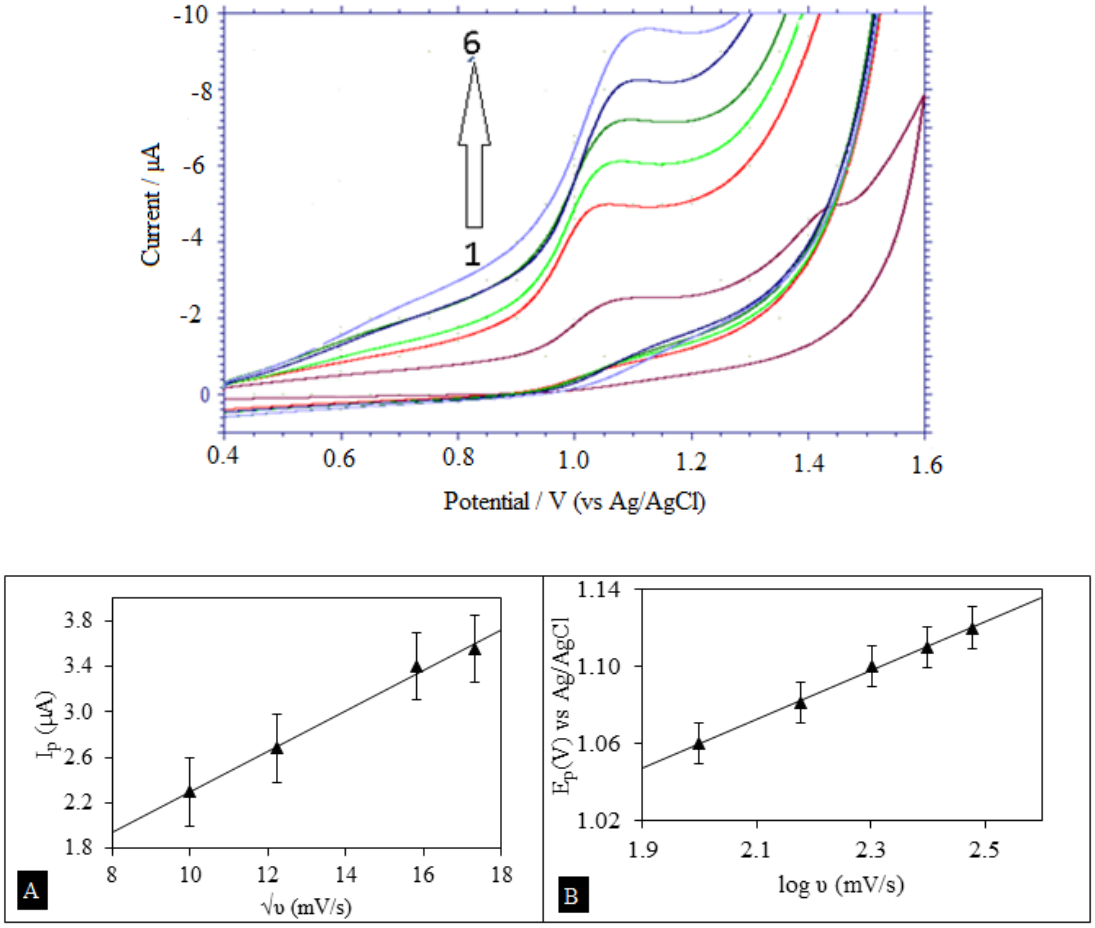


Fig. 3. Cyclic voltammograms of 5.0×10^{-5} M [VCH] in phosphate buffer of pH 6.31 at different scan rates (υ): 50 (1), 100 (2), 150 (3), 200 (4), 250 (5), and 300 mV/s(6); **(A)** plot of Current I_p µA *vs* square root of scan rate mV/s; **(B)** Peak potential E_p*vs* log scan rate

The E_{pa} of the oxidation peak was also dependent on scan rate. The plot of E_{pa} v/s log υ was linear having a correlation coefficient of 0.9976 [Fig. 3(B)] and this behavior was consistent with the nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step [38]. The relation between E_{pa} and log υ can be expressed by the equation E_{pa} (V)=0.1269logυ+0.806.

3.4. Electro-oxidation and mechanism

The electro-oxidation of VCH at a GCE was studied by cyclic voltammetry (CV) in phosphate buffer at pH 6.2. The cyclic voltammogram obtained for 5×10^{-5} M VCH solution at a scan rate 100 mVs^{-1} shows one anodic peak that occur at E_{pa}=1.052 V. On scanning in the negative direction, no reduction peak was observed, showing that the oxidation of VCH is an irreversible process. A decrease of the oxidation current occurs with the number of successive scans and is due to the adsorption of VCH oxidation products on the GCE surface [Fig. 4].

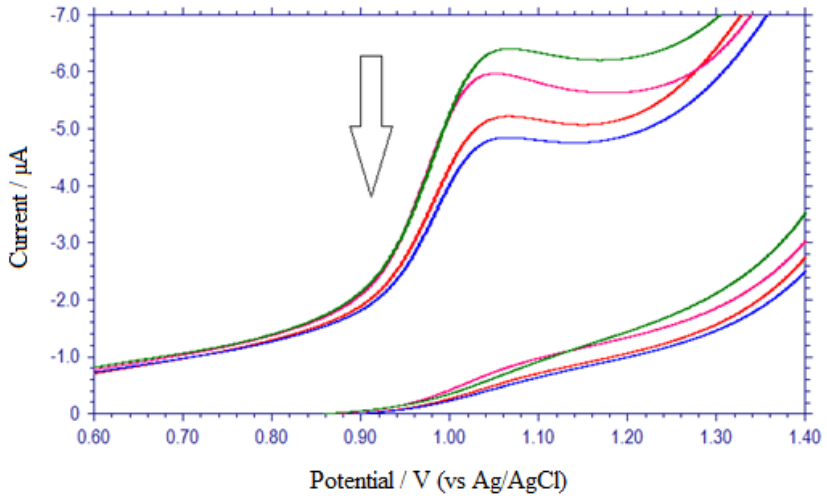
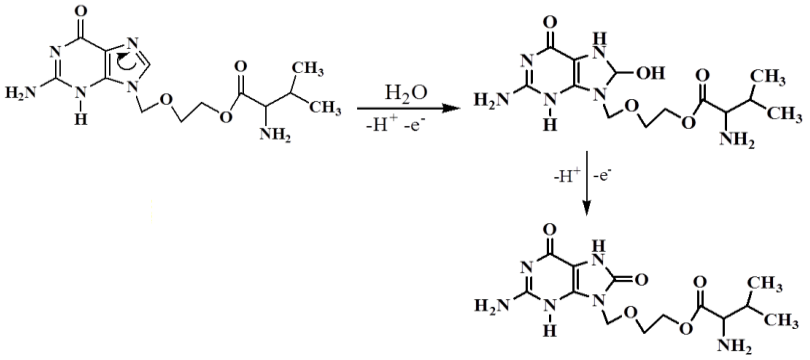


Fig. 4. Successive cyclic voltammograms obtained for 5.0×10^{-5} M valcyclovier on GCE at scan rate=100 mVs^{-1}

The electro-oxidative product of VCH was identified as 2-((2-amine-6,8-dioxo-7,8-dihydro-3H-purin9(6H)-yl)methoxy)ethyl2-amino-3-methylbutanoate) [39], which is confirmed by MALDI. The oxidized VCH solution was subjected for mass spectrometer matrix-assisted laser desorption ionization technique (MALDI). The matrix 50% of 2, 5- di hydroxy benzoic acid, acitonitrile and 0.1% trifluoroacetic acid mixed with oxidized solution. The mixture was subjected to MALDI analysis at the rate of 5µL/min with retention time 0.51–0.98 sec in the applied voltage of 30 kV with a glass micro syringe. The nitrogen gas was used as nebulizer. The MALDI analysis of reaction mixture [Fig. 5] indicated the presence of products with molecular ion peak, m/z at 140 (139.259±1m/z) was expected for

2-((2-amine-6,8-dioxo-7,8-dihydro-3H-purin9(6H)-yl)methoxy)ethyl2-amino-3 methylbutanoate).



Scheme 2. Mechanism path way for electro-oxidation of valacyclovir

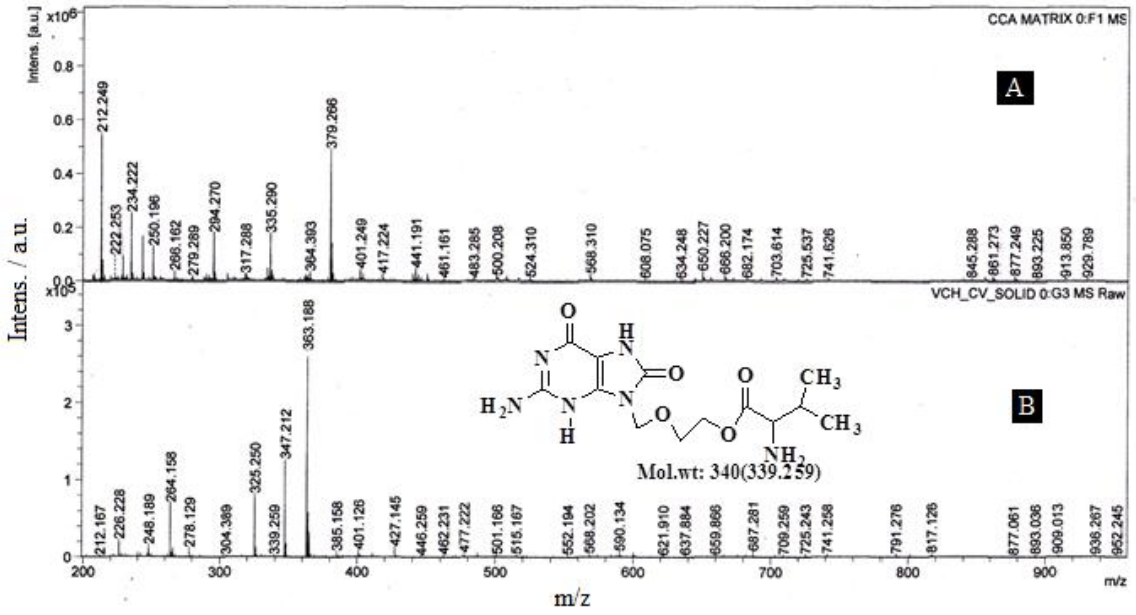


Fig. 5. MALDI spectrum of the product**,**2-((2-amine-6,8-dioxo-7,8-dihydro-3H-purin9 (6H)-yl) methoxy) ethyl2-amino-3-methylbutanoate) resulted due to the Electro- oxidation of valcyclovir hydrochloride (A) MALDI spectrum of only matrix; (B) MALDI spectrum of mixture of matrix and oxidized solution.

3.5. Effect of temperature

The electro-oxidation of VCH was carried out at different temperatures (293-318 K). Cyclic voltammograms of mixture of [VCH] (5×10^{-5} M) and phosphate buffer pH 6.2 were recorded at different temperatures. The anodic peak current increased linearly [Fig. 6] with correlation coefficient 0.9996. The heterogeneous rate constants (k_o) were calculated at different temperatures by using the Butler-Volmer equation [40].

$$i_0 = nFk_0C_0^{(1-\alpha)}C_R^{\alpha} \quad (2)$$

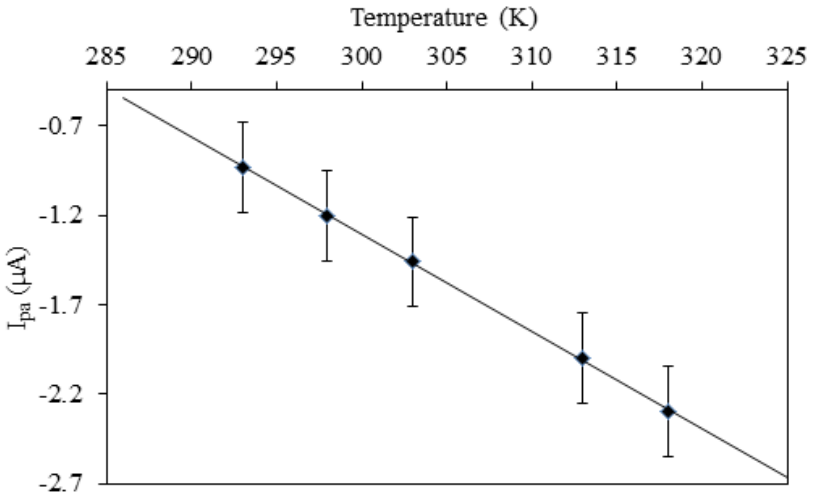


Fig. 6. Observed dependence of i_{pa} (µA) on temperature for 5.0×10^{-5} M valacyclovir at glassy carbon electrode

Table 1. Calculate rate constants at different temperatures for 5.0×10^{-5} M valacyclovir at scan rate 100 mVs^{-1}

Temperature(K)	**i_{pa}(µA)**	**$k_0\times10^{-7}$ cm s^{-1}**
293	-0.93	0.93
298	-1.20	1.25
303	-1.46	1.52
313	-2.00	2.08
318	-2.30	2.39

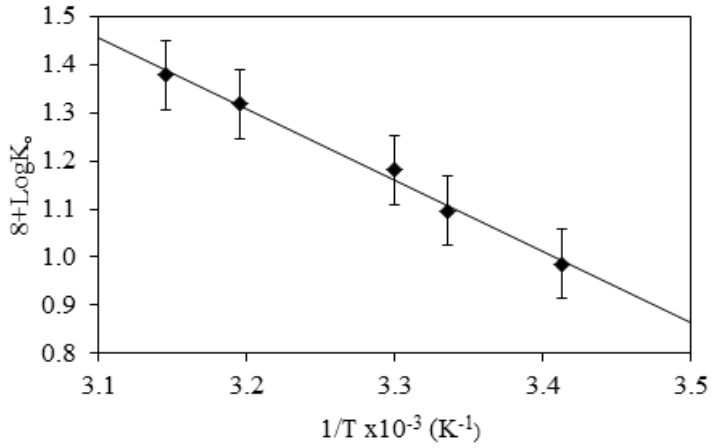


Fig. 7. Effect of temperature on electro-oxidation of valacyclovir in phosphate buffer at pH 7 with scan rate 100 mVs^{-1} (Arrhenius plot)

The calculated rate constants were tabulated in [Table 1] the energy of activation (E_a) was evaluated from the Arrhenius plot of log k_o versus 1/T [Fig. 7], which was linear with the slope=-1436.6, the other activation parameters were obtained from this E_a value and are tabulated in [Table 2]. The less value of $\Delta H^{\#}$ indicates the electro-oxidation of VCH might be taking place through physical adsorption. The more negative $\Delta S^{\#}$ value indicates the electro-oxidation of VCH might be taking place via the formation of an activated adsorbed complex [41] before the products are formed. Such adsorbed intermediate complex is more ordered than reactant molecules itself.

Table 2. Thermodynamic parameters for the electro-oxidation of 5.0×10^{-5} M valacyclovir at glassy carbon electrode

Activation Parameters	Values
E_a (kJmol^{-1})	27.51
$\Delta H^{\#}$ (kJmol^{-1})	25.03
$\Delta S^{\#}$(JK^{-1}mol^{-1})	-284.8
$\Delta G^{\#}$(kJmol^{-1})	109.9
logA	-2.08

3.6. Analytical applications

An analytical method was developed by involving differential pulse voltammetry (DPV) for the determination of the drug. The differential pulse voltammograms of different concentrations of VCH are shown in [Fig. 8]. Under the optimized experimental conditions, a linear relation between the peak current of VCH and concentration was noticed in the range of 5×10^{-3} to 7.5×10^{-5} M. In this concentration range, the response was found to be diffusion controlled. Validation of the optimized procedure for the quantitative assay of VCH was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and recovery. LOD and LOQ were calculated based on the peak current using the following equations shown below [42].

LOD=3 s/m; LOQ=10 s/m

Where s is the standard deviation of the peak current (five runs) and m is the slope of the calibration curve. The LOD and LOQ values were calculated, respectively. Low values of both LOD and LOQ values confirmed the sensitivity of the proposed method. The process of validation was studied by analyzing five replicates of VCH TheRSD value for assay was found to be tabulated in (Table 3) respectively indicating good reproducibility of the method. The comparisons of LOD values by various reported methods for VCH were tabulated in Table 4.

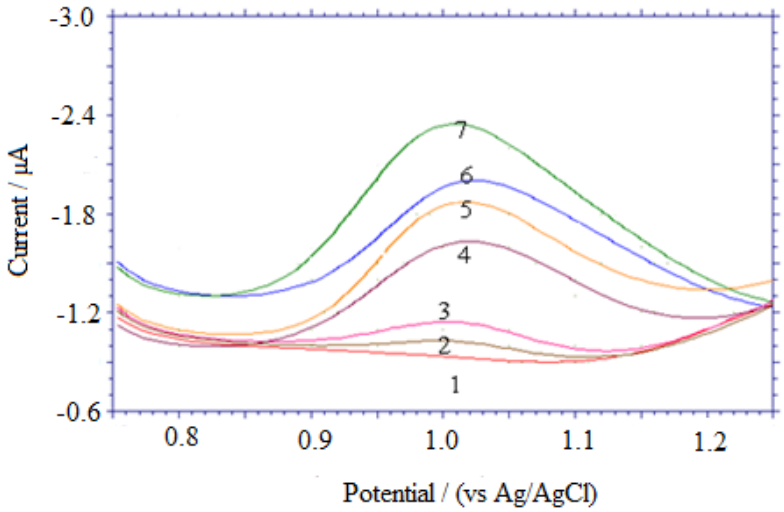


Fig. 8. DPV for the increasing concentrations of VCH in phosphate buffer of pH 6.2 pulse amplitude, 50 mV and pulse width, 20 ms. Blank (1); concentration: 7.5×10^{-7} (2), 2.5×10^{-6} (3), 5.0×10^{-6} (4), 7.5×10^{-6} (5), 2.5×10^{-5} (6), 5.0×10^{-5} (7).

Table 3. Characteristics of calibration plot for valacyclovir

Parameters	**DPV**
Linearity range (M)	7.5×10^{-5}–5.0×10^{-3}
LOD (µM)	1.44
LOQ (µM)	4.83
Inter-day assay RSD (%)	5.26
Intra-day assay RSD (%)	2.36

Table 4. Comparisons of LOD values by various reported methods for VCH.

Method	**LOD (µl)**	**References**
Spectroscopy	37.4	43
Chromatography	14.2	44
RP-HPLC	25.1	45
DPV/GCE	1.40	Present work

3.7. Effect of interference

To evaluate the effect of interference, 5×10^{-5} M VCH was used. The Table 5 shows that 1000 fold of citric acid, gum acacia, sucrose, and starch did not interfere with the voltammetry signal of VCH. The tolerance limit was less than ±5%. The tolerance limit is

defined as the maximum concentration of the interfering substance that caused error less than ±5% for determination of VCH.

Table 5. Influence of potential interferes on the voltammetry response of 5.0×10^{-5} M [VCH].

Interference	Concentration	Signal change (%)
Citric acid	0.01	2.33
Lactose	0.01	-1.53
Sucrose	0.01	-0.70
Dextrose	0.01	1.50
Glucose	0.01	2.30
Gum acacia	0.01	-2.90
Starch	0.01	0.88

3.8. Urine and recovery test

Urine analysis and recovery test for the determination of VCH in human urine sample differential pulse voltammetry technique was used. Drug free human urine samples were obtained from healthy volunteers who gave their informed consent, filtered through a filter paper, and stored frozen until the assay was carried out. By spiking the drug-free urine sample with known amount of drug, the recovery study was carried out. For the determination of spiked VCH in urine sample, calibration graph was used. Five urine samples were used for the detection, and obtained results are tabulated in Table 6. The recovery determination was in the range from 97.5 to 99.2% with RSD of 2.97%.

Table 6. Application of DPV for the determination of VCH in spiked human urine samples

Samples	Spiked ($\times10^{-5}$M)	Detected ($\times10^{-5}$ M)	Recovery
Urine sample -1	0.1	0.093	97.5%
Urine sample -2	0.2	0.194	98.9%
Urine sample -3	0.5	0.496	99.2%
Urine sample -4	0.8	0.799	99.8%

4. CONCLUSIONS

Electro - oxidation of valacyclovir at glassy carbon electrode, in phosphate buffer at pH 4.2 was performed and presented. The electro-oxidation of VCH was irreversible process and with two electrons and two protons transferred, leading to formation of oxidative product. The oxidative product was identified and confirmed by MALDI mass spectrometer.

Thermodynamic parameters were also calculated. DPV technique was used to calculate the limit of detection and quantification. Interference and real sample analysis were also carried out. The method is very simple and less expensive as compared to other methods and it can be adopted in quality control process.

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