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Anodic voltammetry of abacavir and its determination in pharmaceuticals and biological fluids

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Abstract

Abacavir has an antiretroviral activity against HIV and is oxidizable at the glassy carbon electrode. Cyclic voltammetry studies showed one well-defined oxidation wave or splitted two waves depending on pH. The oxidation was irreversible and exhibited diffusion controlled process depending on pH. The mechanism of the oxidation process was discussed. According to the linear relation between the peak current and the concentration, differential pulse voltammetric (DPV) and square wave voltammetric (SWV) methods for its quantitative determination in pharmaceutical dosage forms and biological fluids were developed. These two voltammetric techniques for the determination of abacavir in Britton–Robinson buffer at pH 2.0, which allows quantitation over the 8×10^{-7} to 2×10^{-4} M range in supporting electrolyte for both methods were proposed. The linear response was obtained in Britton–Robinson buffer in the ranges of 1×10^{-5} to 1×10^{-4} M for spiked urine sample at pH 2.0 and 2×10^{-5} to 2×10^{-4} M for spiked serum samples at pH 3.0 for both techniques. The repeatability and reproducibility of the methods for all media (such as supporting electrolyte, serum and urine samples) were determined. Precision and accuracy were also checked in all media. The standard addition method was used in biological media. No electroactive interferences from the endogenous substances were found in the biological fluids.

Keywords: Abacavir; Biological fluids; Determination; Voltammetry; Tablets

1. Introduction

Abacavir {((1*S*,*cis*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-enyl) methanol} is a nucleoside reverse transcriptase inhibitor with antiretroviral activity against HIV (Scheme 1). It is a carbocyclic synthetic nucleoside analog. Intracellularly, abacavir is converted by cellular enzymes to the active metabolite, carbovir triphosphate. Carbovir triphosphate is an analog of deoxyguanosine-5′ triphosphate. Carbovir triphosphate inhibits the activity of HIV-1 reverse transcriptase both by competing with the natural substrate deoxyguanosine-5′ triphosphate and its incorporation into viral DNA [1,2].

The drug is safe and well tolerated in both children and adults. Abacavir is rapidly absorbed following oral administration with a bioavailability of about 80%. It is about 50% bound to plasma proteins. The elimination half-life is about 1.5 h following a single dose [1,2]. The adult dose is the equivalent of 300 mg of abacavir twice daily. Abacavir is metabolized into the pharmacological inactive 5′-glucuronide and the 5′-carboxylate.

Abacavir has been studied and determined by very few procedures: liquid chromatography with UV detection [3–6] and liquid chromatography with tandem mass spectrometry [7]. The reported methods were influenced by interference of endogenous substances and potential loss of drugs in the re-extraction procedure and involving lengthy, tedious and time-consuming plasma sample preparation and extraction processes and requiring a sophisticated and expensive instrumentation.

The development of a new method capable of determining drug amount in pharmaceutical dosage forms is important. Electroanalytical techniques have been used for the determination of a wide range of drug compounds with the advantages that there are, in most, instances no need for derivatization and that these techniques are less sensitive to

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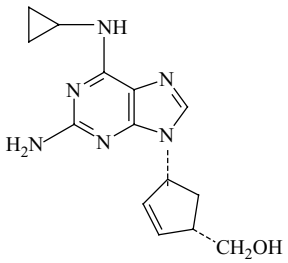

Scheme 1. Structure of abacavir.

matrix effects than other analytical techniques. Additionally application of electrochemistry includes the determination of electrode mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity [8–14].

Despite the analytical importance of the electrochemical behavior and oxidation mechanism of abacavir, the literature has no report on electrode processes of the drug. Furthermore, there appears to be no analytical methods for the determination of abacavir, either in bulk form or pharmaceutical dosage forms that have been reported up-to-date.

The goal of this work was the development of new voltammetric methods for the direct determination of abacavir in pharmaceutical dosage forms, raw materials, spiked human serum and urine samples without any time-consuming extraction or evaporation steps prior to drug assay. This paper describes fully validated, simple, rapid, selective and sensitive procedures for the determination of abacavir employing DPV and SWV at the glassy carbon disc electrode. This work was also aimed to study the voltammetric behavior and oxidation mechanism of abacavir using cyclic, linear sweep, DPV and SWV techniques.

2. Experimental

2.1. Equipment

Voltammetric measurements were performed using a Bioanalytical Systems (BAS 100 W, USA) electrochemical analyzer. A glassy carbon working electrode (BAS; ø: 3 mm, diameter), an Ag/AgCl reference electrode (BAS; 3 M KCl) and a platinum wire counter electrode and a standard one-compartment three-electrode cell of 10 mL capacity were used in all experiments. Before each measurement the glassy carbon electrode was polished manually with aqueous slurry of alumina powder (ø: 0.01 μm) on a damp smooth polishing cloth (BAS velvet polishing pad). All measurements were realized at room temperature. The pH was measured using a pH meter Model 538 (WTW, Austria) using a combined electrode (glass electrode–reference electrode) with an accuracy of ±0.05 pH.

Operating conditions for SWV were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step, 4 mV; and for DPV were: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate, 20 mV s^{-1}.

2.2. Reagents

Abacavir and its pharmaceutical dosage form (Ziagen®) were kindly provided by Glaxo Smith Kline Pharm. Ind. (Istanbul, Turkey). Model compound valacyclovir and gancyclovir were also kindly provided by Glaxo Smith Kline Pharm Ind. (Istanbul, Turkey) and Roche Pharm. Ind. (Istanbul, Turkey), respectively. Adenine and Guanine were also used as model compounds and they were supplied from Sigma. All chemicals for preparation of buffers and supporting electrolytes were reagent grade (Merck or Sigma).

Stock solutions of abacavir (1×10^{-3} M) were prepared in bi-distilled water and kept in the dark in a refrigerator.

All other stock solutions were also prepared in bi-distilled water and kept in the dark in a refrigerator. Four different supporting electrolytes, namely sulphuric acid (0.1 and 0.5 M), phosphate buffer (0.2 M, pH 2.0–11.0), acetate buffer (0.2 M, pH 3.5–5.7) and Britton–Robinson buffer (0.04 M, pH 2–12.0) were prepared in doubly distilled water. Standard solutions were prepared by dilution of the stock solution with selected supporting electrolyte to give solutions containing abacavir in the concentration range of 8×10^{-7} to 2×10^{-4} M. The calibration curve for DPV and SWV analysis was constructed by plotting the peak current against the abacavir concentration.The ruggedness and precision were checked at different days, within day ($n = 5$), and between days ($n = 5$) for three different concentrations. Relative standard deviations were calculated to check the ruggedness and precision of the method [15,16].

The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (Bias%). One example of relative error is the accuracy, which describes the deviation from the expected results.

All solutions were protected from light and were used within 24 h to avoid decomposition. However, current–potential curves of sample solutions recorded 72 h after preparation did not show any appreciable change in assay values.

2.3. Pharmaceutical dosage form assay procedure

Ten tablets of Ziagen® (each tablet contains 300 mg abacavir) were accurately weighed and finely powdered by pestle in a mortar. A weighed portion of this powder equivalent to 10^{-3} M of abacavir was transferred into a 100 mL calibrated flask and completed to the volume with bi-distilled water. The content of the flask were 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. Recovery studies

To study the accuracy and reproducibility of the proposed techniques, recovery experiments were carried out using the standard addition method. In order to know whether the excipients show any interference with the analysis, known amounts of pure abacavir were added to the pre-analyzed tablet formulation and the mixtures were analyzed by the proposed method. After five repeated experiments, the recovery results were calculated using the calibration equation.

2.5. Analysis of spiked serum samples

Serum samples, obtained from healthy individuals (after obtaining their written consent) were stored frozen until assay. After gentle, thawing, an aliquot volume of sample was fortified with abacavir dissolved in bi-distilled water to achieve final concentration of 1×10^{-3} M and treated 0.7 mL of acetonitrile as serum denaturating and precipitating agent, then the volume was completed to 2 mL with the same serum sample. The tubes were vortexed for 10 min and then centrifuged for 5 min at 5000 × *g* for removing of protein residues. The supernatant was taken carefully. The concentration of abacavir was varied in the range of 2×10^{-5} to 2×10^{-4} M in human serum samples. These solutions were analyzed in the voltammetric cell containing Britton–Robinson buffer at pH 3.0. The amount of abacavir in spiked human serum samples for the recovery studies was calculated from the related calibration equation.

2.6. Analysis of spiked urine samples

The urine samples taken from healthy individuals immediately before the experiments. An aliquot volume of urine sample was fortified with abacavir dissolved in bi-distilled water to achieve final concentration of 1×10^{-3} M and treated with 0.7 mL of acetonitrile as endogenous substance precipitating agent, and then the volume was completed to 2 mL with the same urine sample. The tubes were vortexed for 10 min, and then centrifuged for 5 min at 5000 × *g* for getting rid of residues. The supernatant was taken carefully.

The concentration of abacavir was varied in the range of 1×10^{-5} to 1×10^{-4} M in human urine samples. These solutions were analyzed in the voltammetric cell containing Britton–Robinson buffer at pH 2.0.

The amount of abacavir in spiked human urine samples for the recovery studies was calculated from the related calibration equation.

3. Results and discussion

No previous electrochemical data were available concerning the electrode behavior of abacavir. Therefore, several measurements with different electrochemical techniques (cyclic, linear sweep, differential pulse and square wave voltammetry) were performed using various supporting electrolytes and buffers in order to obtain such information.

Abacavir was electrochemically oxidized in a broad pH range (1.5–12.0) using a glassy carbon disc electrode.

The cyclic voltammetric behavior of abacavir yielded one well-defined wave in strongly acidic solution such as 0.1 M sulphuric acid and Britton–Robinson buffer at pH 2.0 (Fig. 1a and b). As the pH increased, this one wave was splitted into two waves (Fig. 1c and d). Cyclic voltammetric measurements showed an irreversible nature of the oxidation process (Fig. 1). The scanning was started at −0.30 V in the positive direction at pH 2.0, the anodic oxidation of abacavir did not occur until about +1.0 V. By reversing at +1.80 V no reduction wave or peak corresponding to the anodic wave was observed on the cathodic branch. It was observed that at the second and higher cycles the abacavir wave decreased (Fig. 2). This phenomenon may be partly attributed to the consumption of adsorbed abacavir on the electrode surface.

Due to the poorly resolved signal obtained by cyclic voltammetry with a decrease in pH above 8.0, the effect of pH on peak potential and peak intensity were studied using DPV and SWV techniques. The peak potential of the oxidation process moved to less positive potential and oxidation peak splitted by raising the pH. Both obtained graphs were found similar. For this reason, only DPV graph for the first peak was given as Fig. 3a and b. The plot of the first peak potential versus pH showed three linear segments between 1.5 and 11.0. At pH 12.0 first peak disappeared.

The linear segments can be expressed by the following equations in all supporting electrolytes using by DPV technique.

$$E_p(\text{mV}) = 1249.2 - 75.10\,\text{pH};$$
$$r : 0.935 \text{ (between pH 1.5 and 3.5)}$$

$$E_p(\text{mV}) = 1014.2 - 0.998\,\text{pH};$$
$$r : 0.267 \text{ (between pH 4.0 and 9.0)}$$

$$E_p(\text{mV}) = 1261.3 - 28.00\,\text{pH};$$
$$r : 0.997 \text{ (between pH 9.0 and 11.0)}$$

As it can be seen from the second equation, and Fig. 3a the peak potential nearly becomes pH independent (between pH 4.0 and 9.0).

One of the intersection points of the curves is close to the p*K*a value of adenine molecule present in abacavir molecule which is $pKa = 3.8$ [17]. Abacavir has another intersection point at pH 9.0. It can be explained by changes in protonation of the acid–base functions in the molecule.

The influence of pH on the abacavir current at glassy carbon disc electrode was also studied. The ip versus pH plot (Fig. 3b) shows that peak current is maximum in the acidic media. The experimental results showed that shapes of the curves were better in Britton–Robinson buffer at pH 2.0

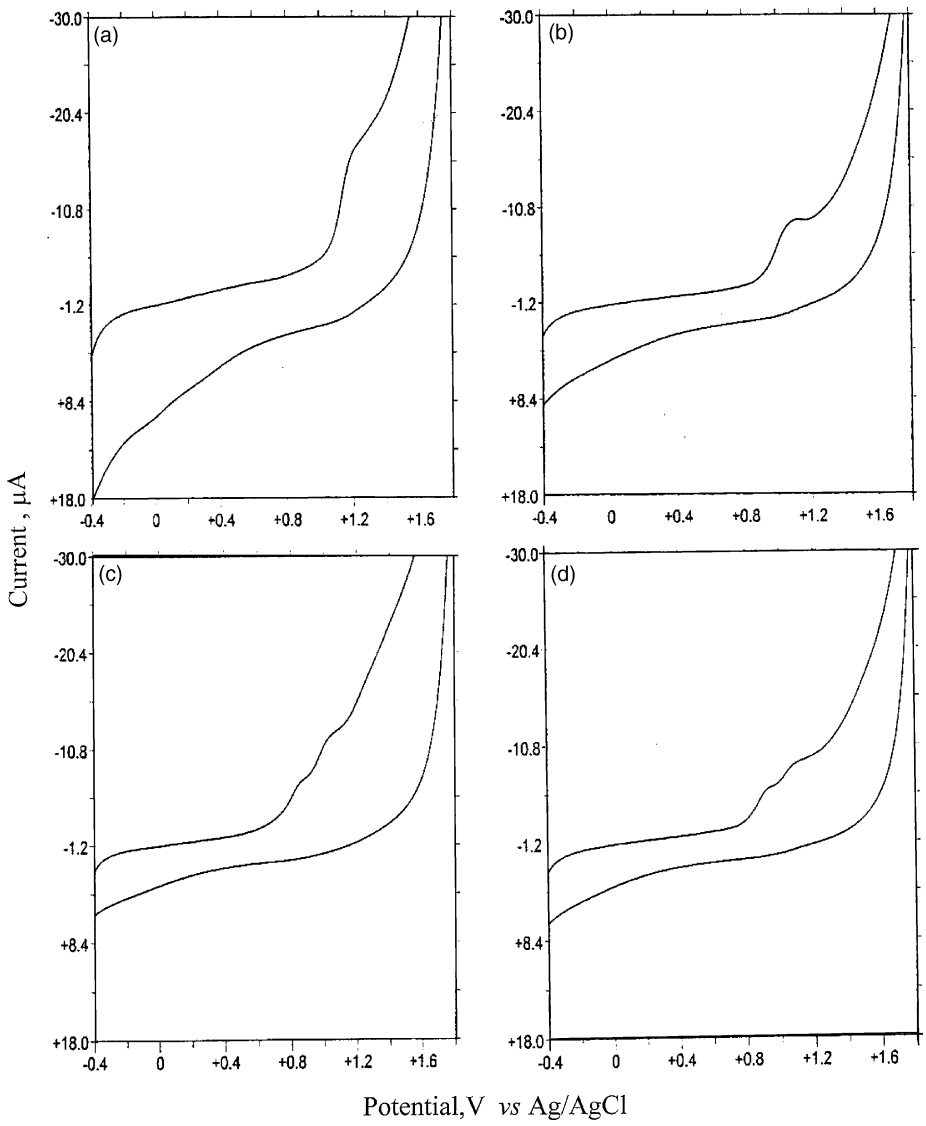

Fig. 1. Cyclic voltammograms of 1×10^{-4} M abacavir in 0.1 M H_2SO_4 (a); Britton–Robinson buffer at pH 3.00 (b); at pH 5.00 (c); at pH 10.00 (d). Scan rate 100 mV s^{-1}.

and 3.0 than in sulphuric acid. A Britton–Robinson buffer at pH 2.0 was chosen with respect to sharp response and better peak shape for the calibration curve for pharmaceutical dosage form and urine samples. pH 3.0 was chosen for the same reasons, instead of pH 2.0 for human serum samples. At pH 3.0 lower peak potential value was obtained than at pH 2.0. However, possible small interferences were obtained at pH 2.0 from the serum samples. For this reason Britton–Robinson buffer at pH 3.0 was chosen for the calibration and study from the serum samples. 0.04 M concentration of the buffer was selected to obtain an adequate buffering capacity.

The effect of the potential scan rate between 5 and 750 mV s^{-1} on the peak current and potential of abacavir were evaluated. A 170 mV positive shift in the peak potential confirmed the irreversibility of the oxidation process. Scan rate studies were carried out to assess whether the processes at the glassy carbon electrode was under diffusion or adsorption control. When the scan rate was varied from 5 to 750 mV s^{-1} in 2×10^{-4} M solution of abacavir, a linear dependence of the peak intensity ip (μA) upon the square root of the scan rate $\nu^{1/2}$ (mV s^{-1}) was found, demonstrating a diffusional behavior. The equation is noted below in Britton–Robinson buffer at pH 2.0:

$$\text{ip}\,(\mu\text{A}) = 0.48\nu^{1/2}(\text{mV}\,\text{s}^{-1}) - 0.24,$$

$$r = 0.998\,(n = 9)$$

A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.59, very close to the theoretical value of 0.5, which is expressed for an ideal reaction the diffusion controlled electrode process [18]. The

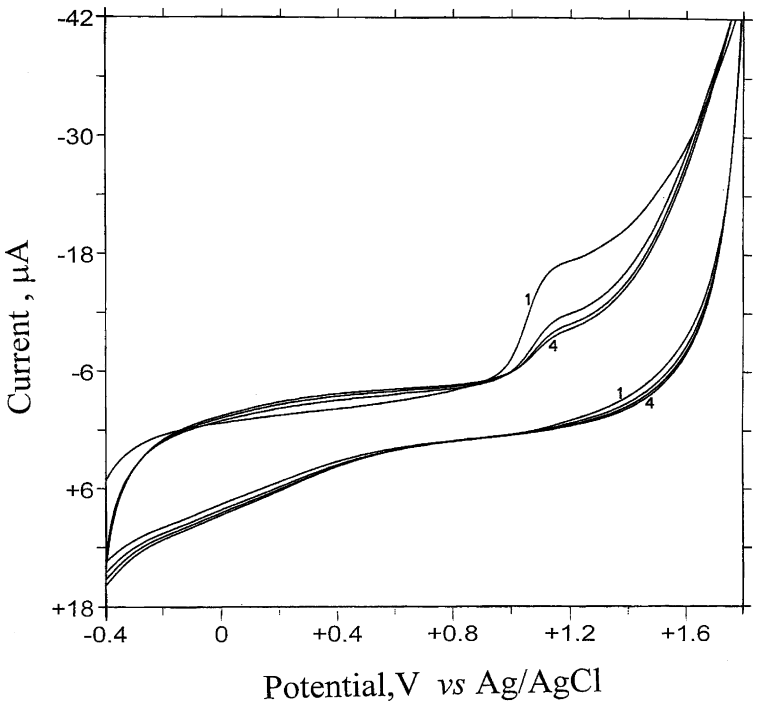

Fig. 2. Multisweep cyclic voltammograms of 2×10^{-4} M abacavir solutions in Britton–Robinson buffer at pH 2.00. Scan rate 100 mV s^{-1}. The numbers indicate the number of scans.

equation obtained is:

$$\log ip\,(\mu A) = 0.59 \log \nu\,(mV\,s^{-1}) - 0.53,$$
$$r = 0.996\,(n = 9)$$

The Tafel plots (log *i* versus E) was obtained with a scan rate of 5 mV s^{-1} beginning from a steady-state potential in Britton–Robinson buffer at pH 2.0. The αn value of anodic reaction from the slope of the linear part of the tafel plot was found to be as 0.15. The exchange current density (i_o) is 1.08×10^{-8} A cm^{-2} for this system. These values together with the absence of cathodic waves in cyclic voltammetry (Fig. 1) indicated the irreversibility of the oxidation reaction.

Considering the above results and bearing in mind the electrochemical behavior of adenine, the parent base of abacavir at glassy carbon electrode [19–22], we may assume that the oxidation process is located on the adenine moiety in the molecule. As reported for other adenine compounds [19–22], cyclic voltammograms of abacavir obtained with 100 mV s^{-1} presents an irreversible oxidation process at all pH values (Fig. 1) attributed to the oxidation of the adenine moiety of this compound. The anodic oxidative behavior of abacavir was compared to adenine oxidation, which was performed by cyclic voltammetry at the glassy carbon electrode, as a function of pH in order to identify the oxidation process of abacavir. Our results revealed a good agreement with the redox mechanism postulated for similar compounds in the literature [19–22] and our obtained results from adenine molecule. The oxidation of adenine itself has been shown to follow a two-step mechanism involving the total loss of four electrons [23]. The first two electrons loss is rate-determining step [23,24]. Our obtained results revealed a good agreement with the redox mechanism of adenine. The slopes of 75.10 mV pH^{-1} show that two protons take part in the rate-determining step.

From the experimental data it can be assumed that oxidation of N9 adenine derivatives take place by a mechanism similar to that for adenine itself. Adenine oxidation is irreversible, and occurs in two steps [25–27]. These two steps could be clearly identified for abacavir using by differential pulse voltammetry. The first peak of at about +1.00 V corresponds to adenine oxidation and the second obtained at more positive potentials to the oxidation of adenine dimers. These results confirmed our obtained results. The electroactive adenine oxidation products 2,8-dihydroxyadenine derivatives are oxidized rapidly giving a molecule with a quinone-diimine structure [20]. The electrochemical oxidation of abacavir occurs in consecutive steps, with the formation of dimers.

Nevertheless, the electrochemistry of adenine and N9 substituted adenine derivatives shows some differences such as small shifted potentials, overlapped peaks etc. Taking into account all the studies performed, we suggest that the oxidation process may be occurring on the adenine moiety in abacavir molecule. The adsorption of adenine could be controlled by the choice of buffer pH [28,29]. These researchers investigated the effect of pH on the peak potentials and peak currents wide pH range (between pH 2 and 12). Below pH 4 the current was found as diffusion-controlled, which indicated absence of adsorption [28]. This conclusion was also

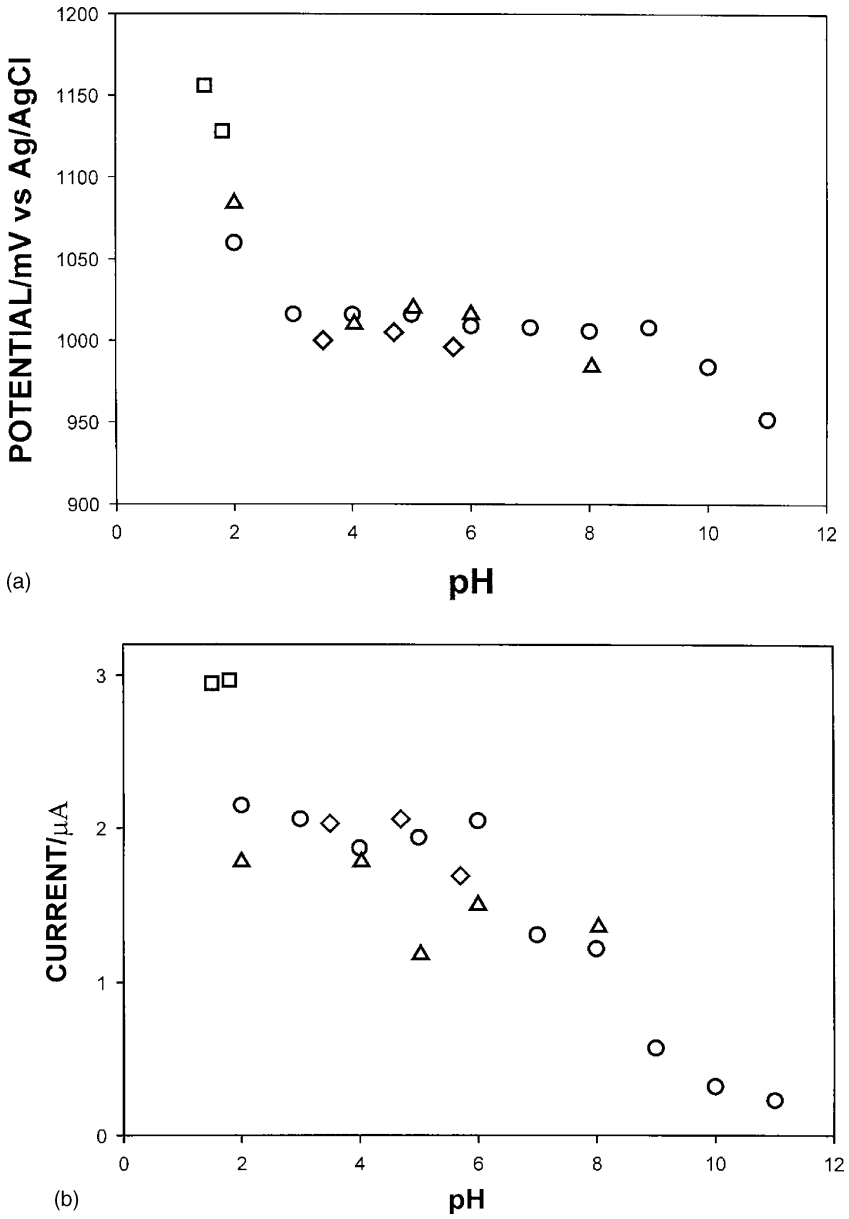

Fig. 3. Effects of pH on abacavir anodic peak potential (a) and peak current (b); abacavir concentration 1×10^{-4} M. 0.1 and 0.5 M H_2SO_4 (□); 0.04 M Britton–Robinson (○); 0.2 M acetate (◇); and 0.2 M phosphate (△) buffers.

confirmed by the observation that calibration graphs were linear at pH < 4 [29]. These literatures also confirmed, that is why we choose the Britton–Robinson buffer at pH 2 and 3 for the analytical evaluation. Abacavir also adsorbs on the electrode surface after pH 4.0, additions to the other reasons for the investigation and determination of analytical purposes of abacavir more acidic pH (2.0 or 3.0) was selected.

3.1. Analytical applications and validation of the proposed method

Various electrolytes, such as sulphuric acid, perchloric acid, Britton–Robinson, acetate and phosphate buffer were examined. The best results with respect to signal enhancement and peak shape accompanied by sharper response was obtained with Britton–Robinson buffer at pH 2.0 and 3.0. These supporting electrolytes were chosen for the subsequent experiments. In order to develop a voltammetric procedure for determination of the drug, we selected the DPV and SWV techniques, since the peaks were sharper and better defined at lower concentration of abacavir than those obtained by cyclic and linear sweep voltammetry with a lower background current, resulting in improved resolution. DPV and SWV are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection limits [8,9]. Two calibration graphs from the standard solution of

Table 1
Regression data of the calibration lines for quantitative determination of abacavir by DPV and SWV in supporting electrolyte, human serum and urine samples

	DPV			SWV		
	Supporting electrolyte	Serum	Urine	Supporting electrolyte	Serum	Urine
Measured potential (V)	1.07	1.05	1.04	1.10	1.07	1.08
Linearity range (M)	8×10^{-7} to 2×10^{-4}	2×10^{-5} to 2×10^{-4}	1×10^{-5} to 1×10^{-4}	8×10^{-7} to 2×10^{-4}	2×10^{-5} to 2×10^{-4}	1×10^{-5} to 1×10^{-4}
Slope ($\mu A\,M^{-1}$)	1.58×10^{4}	1.08×10^{4}	1.58×10^{4}	1.94×10^{4}	1.24×10^{4}	2.41×10^{4}
Intercept (μA)	0.255	0.169	0.269	0.405	0.297	0.212
Correlation coefficient	0.996	0.999	0.996	0.996	0.999	0.999
S.E. of slope	4.74×10^{2}	2.72×10^{2}	6.99×10^{2}	5.59×10^{2}	2.36×10^{2}	5.00×10^{2}
S.E. of intercept	0.034	0.028	0.042	0.040	0.024	0.030
LOD (M)	2.20×10^{-7}	2.69×10^{-6}	1.26×10^{-6}	1.18×10^{-7}	1.82×10^{-6}	1.14×10^{-6}
LOQ (M)	7.34×10^{-7}	8.96×10^{-6}	4.21×10^{-6}	3.93×10^{-7}	6.07×10^{-6}	3.80×10^{-6}
Repeatability of peak current (R.S.D.%)	1.02	0.245	0.324	0.456	0.988	0.34
Repeatability of peak potential (R.S.D.%)	0.358	0.57	0.192	0.516	0.18	0.215
Reproducibility of peak current (R.S.D.%)	1.93	0.771	1.50	1.76	1.23	0.84
Reproducibility of peak potential (R.S.D.%)	0.484	0.69	0.314	0.545	0.29	0.468

abacavir according to the procedures described above were constructed by using DPV and SWV. A linear relation in the concentration range between 8×10^{-7} and 2×10^{-4} M was found, indicating that the response was diffusion controlled in this range. Above this concentration (3×10^{-4} M) a loss of linearity was probably due to the adsorption of abacavir on the electrode surface. The characteristics of the calibration plots are summarized in Table 1.

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated on the peak current using the following equations:

$$\mathrm{LOD} = 3\,s/m; \qquad \mathrm{LOQ} = 10\,s/m$$

where s is the standard deviation of the peak currents (three runs) and m is the slope of the calibration curve. The LOD and LOQ values were also shown is Table 1.

Repeating four experiments on 4×10^{-5} M abacavir for both techniques tested the repeatability and reproducibility of peak potential and peak currents. The results were shown also in Table 1.

Repetition of sample analysis after 72 h period did not show any significant change in results of analyses.

3.2. Determination of abacavir in tablets

On the basis of above results, both DPV and SWV methods were applied to the direct determination of abacavir in tablet dosage forms, using the related calibration straight lines without any sample extraction or filtration and after an adequate dilutions. The results show that the proposed methods were successfully applied for the assay of abacavir in its pharmaceutical dosage forms (Table 2).

The accuracy of the method was determined by its recovery during spiked experiments. Recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulation of abacavir. According to the results, excipients presented in tablet do not interfere with the analysis (Table 2). There is no official method in any pharmacopoeias (e.g. USP, BP or EP) or literature method related to pharmaceutical dosage forms of abacavir. To prove the absence of interferences by excipients, recovery studies were carried out. The results demonstrate the validity of the proposed method for the determination of abacavir in tablets. These results reveal that both methods had adequate precision and accuracy and consequently can be applied to the determination of abacavir in pharmaceuticals without any interference from the excipients.

Table 2
Assay results from Abacavir tablets (Ziagen®) and mean recoveries in spiked tablets

	DPV	SWV
Labeled claim (mg)	300	300
Amount found (mg)[a]	303.6	295.2
R.S.D. (%)	0.98	1.14
95% confidence limit	3.69	4.20
Bias (%)	−1.2	1.6
Added (mg)	20.00	20.00
Found (mg)[a]	19.87	19.97
Recovery (%)	99.37	99.83
R.S.D. (%) of recovery	1.25	1.32
Bias (%)	0.65	0.15

[a] Each value is the mean of five experiments.

3.3. Determination of abacavir in spiked biological samples

Acetonitrile and methanol were tried as a serum and urine precipitating agents. Also, different amount of acetonitrile were tried. The best results were obtained using 0.7 mL acetonitrile. The measurements of abacavir in serum and urine samples were performed as described in Section 2. The applicability of the proposed methods to the human serum and urine samples, the calibration equations were obtained in spiked biological samples. Calibration equation parameters and necessary validation data were shown in Table 1. Obtained recovery results of spiked biological samples were given in Table 3. Analysis of drugs from serum or urine samples usually requires extensive time-consuming sample preparation, use of expensive organic solvents and other chemicals. In this study, the serum proteins and endogenous substances in serum and urine samples are precipitated by the addition of acetonitrile, which is centrifuged at $5000 \times g$, and the supernatant was taken and diluted with the supporting electrolyte and directly analyzed. Typical DPV and SWV curves of abacavir examined in serum samples are shown in Fig. 4a and b; in urine samples are shown in Fig. 5a and b. Using both proposed techniques, no sample pre-treatment was required, other than precipitation and dilution steps. The recovery results of abacavir (Table 3) in biological samples were calculated from the related linear regression equations, which are given in Table 1. As can be seen in Figs. 4 and 5, no oxidation compounds and no extra noise peaks present in biological material peak occurred in the potential range where the analytical peak appeared. Stability of serum and urine samples kept in refrigerator (+4 °C) was tested by making five consecutive analyses of

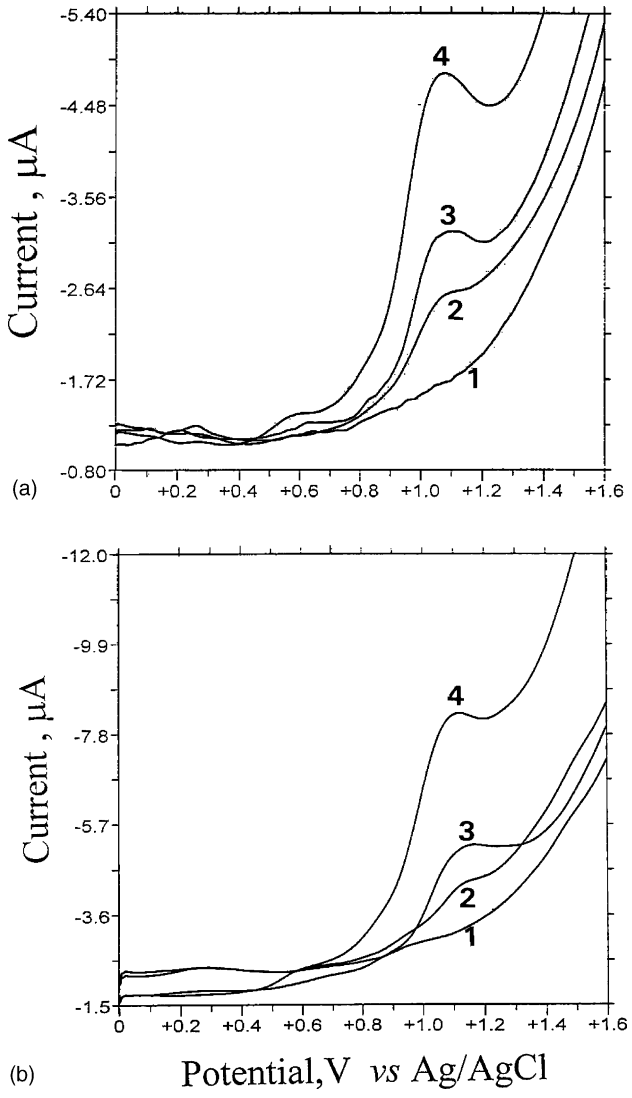

Fig. 4. Differential pulse (a) and square wave (b); voltammograms obtained for the determination in spiked serum (1) blank; (2) 2×10^{-5} M; (3) 6×10^{-5} M; (4) 1×10^{-4} M abacavir extract in 0.04 M Britton–Robinson buffer at pH 3.0.

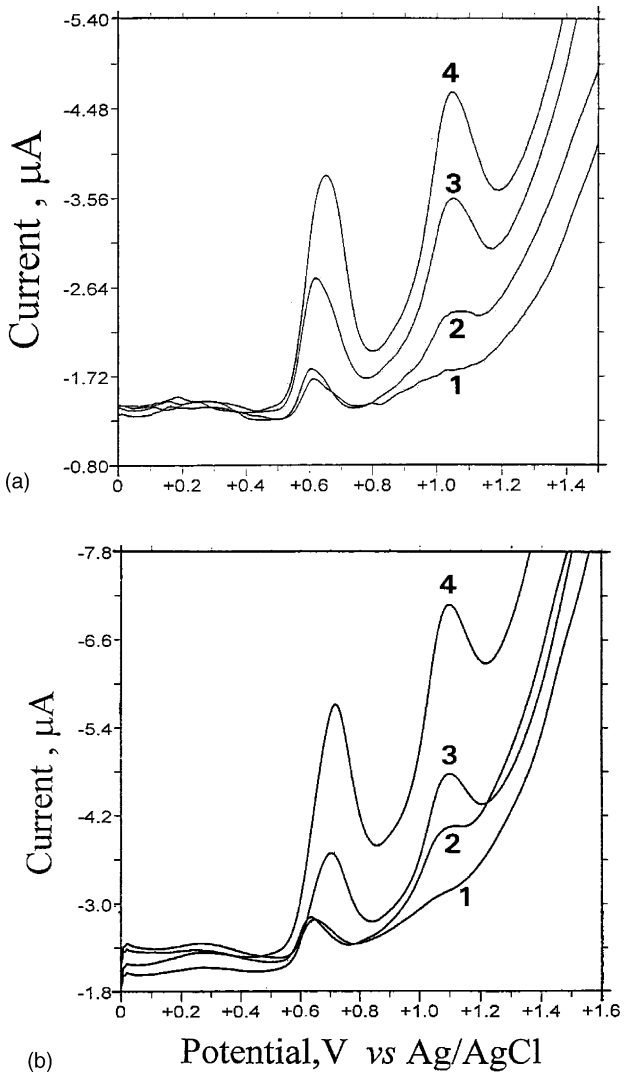

Fig. 5. Differential pulse (a) and square wave (b); voltammograms obtained for the determination in spiked urine (1) blank; (2) 1×10^{-5} M; (3) 4×10^{-5} M ; (4) 8×10^{-5} M abacavir extract in 0.04 M Britton–Robinson buffer at pH 2.0.

Table 3
Application of the DPV and SWV methods to the determination of abacavir in spiked human serum and urine samples

Technique	Medium	Abacavir added (M)	n	Abacavir found (M)	Average recovery (%)	R.S.D. (%)	Bias (%)
DPV	Serum	4×10^{-5}	4	3.97×10^{-5}	99.19	1.02	0.75
DPV	Urine	4×10^{-5}	4	3.98×10^{-5}	99.44	1.26	0.5
SWV	Serum	4×10^{-5}	4	4.03×10^{-5}	100.86	1.32	−0.75
SWV	Urine	4×10^{-5}	4	3.99×10^{-5}	99.63	0.97	0.25

the sample over a period of approximately 5 h. There were no significant changes in the peak currents and potentials between the first and last measurements.

4. Conclusion

The electrochemical behavior of abacavir on glassy carbon electrode was established and studied for the first time. Abacavir is irreversibly oxidized at high positive potentials.

Two voltammetric techniques have been developed for the determination of abacavir in pharmaceutical formulations and biological samples. The results obtained show that the above-described methods are useful not only for abacavir determination in conventional electrolytes, but also in more complex matrices such as dosage forms, human serum and urine samples. The principal advantages of DPV and SWV techniques over the other techniques are that they may be applied directly to the analysis of pharmaceutical dosage forms and biological samples without the need for separation or complex sample preparation, since there was no interference from the excipients and endogenous substances. These methods are rapid, requiring less than 5 min to run sample.

This paper is not intended to be a study of the pharmacodynamic properties of abacavir, since only healthy volunteers were used for the sample collection and results may be of no significance. Its only show that the possibility of monitoring this drug makes the method useful for pharmacokinetic and pharmacodynamic purposes.

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