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# Quantitative determination of acyclovir based on electrochemical response

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## ABSTRACT

The electrochemical reduction behaviour of Acyclovir has been exploited to develop sensitive reliable and accurate cyclic voltammetric and differential pulse voltammetric methods for its quantification. To maximize the sensitivity and to minimize the detection limit a β-cyclodextrin midified carbon paste electrode has been employed which imparts a selective procedure for the determination of carbonyl group containing compounds. The proposed method is linear over the concentration range of $1.0 \times 10^{-9}$ to $0.2 \times 10^{-8}$M with a detection limit of $1.8 \times 10^{-9}$M. The developed method has been applied for the determination of Acyclovir in pharmaceutical formulations, human urine samples and human serum samples successfully.



## KEYWORDS

Acyclovir;
β-cyclodextrin;
Cyclic voltammetry;
Differential pulse voltammetry;
Pharmaceutical formulations;
Urine samples;
Serum samples.

## INTRODUCTION

Acyclovir (ACL), a synthetic purine nucleoside derivative is the most commonly used antiviral drug for diseases like herpes zoster, herpes simplex and chickenpox. It can be given orally or intravenously or topically. About 15% of the drug is bound to plasma proteins. It is excreted from the urine almost in the unchanged form. Acyclovir undergoes phosphorylation with the help of viral thimidinekinase enzyme to form acyclovir triphosphate and this metabolite selectively inhibits the DNA synthesis of the virus and thus prevents its growth. Its structure is given in Figure l

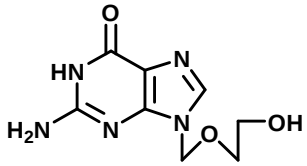


**Figure 1 : Structure of acyclovir**

Acyclovir has been determined by HPLC[1-6], LC[7-9], near IR spectroscopy[10] and spectrophotometric[11-13] methods. On the other hand voltammetric methods have been shown to be excellent for the determination of drugs in different samples. Concerning electrochemical methods voltammetric behaviour with nanostructured film electrode has been used for the determination of

acyclovir[15]. Bersier et al.[16] reviewed the electrochemical behavior of CDs and CD-inclusion complexes.

The aim of the present work is to develop a differential pulse voltammetric method and to apply it for determination of acyclovir in pharmaceutical formulations, human serum samples and human urine samples. In this work bare carbon paste electrode (CPE) and β-cyclodextrin modified carbon paste electrodes (CDMCPE) were used as working electrodes.

## EXPERIMENTAL

### Apparatus

Voltammograms were recorded with Metrohm 757 VA computrace (Herisau, Switzerland). A model Metrohm 632-pH meter was used to carry out the pH measurements.

### Chemicals and reagents

The modifier β-cyclodextrin was purchased from Fluka. Paraffin oil and Graphite powder from Aldrich. ACL is purchased from Sigma. Stock solutions are prepared in methanol and kept at dark place. The supporting electrolyte (Britton-Robinson buffer) is prepared using 0.04 M orthophosphoric acid ($H_3PO_4$), acetic acid ($CH_3COOH$) and boric acid ($H_3BO3$); pH is adjusted by the addition of 0.2 M sodium hydroxide (NaOH) solution. Standard solutions are prepared on dilution of the stock solution with triple distilled deionized water. All the chemicals used for the preparation of solutions and supporting electrolytes are of reagent grade.

### Process of analysis

An appropriate amount of analyte and supporting electrolyte (BRB) are taken in to 50 mL electrolytic cell and purged with oxygen - free nitrogen for 10 min, the voltammograms are recorded. After the addition of each aliquot of the standard solution voltammograms were recorded. The required accumulation potential of-0.16 V is then applied to the unmodified CPE and modified CPE with a stirring speed of 2000rpm. The stirring is stopped and after 10s of rest the voltammograms are recorded by scanning the potential towards the negative direction. All the measurements are performed at room temperature 21±1 °C.

## RESULTS AND DISCUSSION

### Cyclic vottammetry studies

Figure 2 represents the cyclic voltammogram for 3.2x $10^{-7}$ M Acyclovir in BR buffer solution of pH 3.0 at bare carbon paste electrode (CPE) and β-cyclodextrin modified carbon paste electrode (CDMCPE) with accumulation times of 300s and 150s respectively. On scanning towards negative potential, the compound yields one peak which is due to the reduction of the carbonyl-group and no peak is observed on the anodic branch indicating that the reduction of Acyclovir is irreversible. For the reduction peaks observed, the voltammetric currents at CDMCPE are higher in comparison with the bare CPE, indicating that the CDMCPE has better efficiency for accumulating drugs.

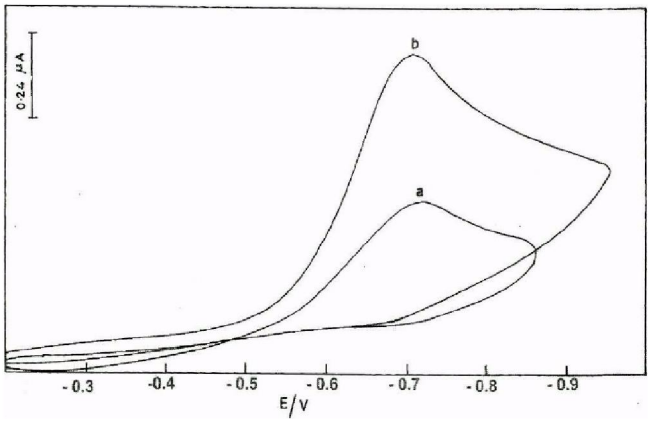


**Figure 2 : Typical CV of 3.2 x $10^{-7}$ M DNZ at (a) bare CPE (b) CDCMPE**

### Differential pulse adsorptive stripping voltammetry

Figure 3 illustrates DPAdSV for 1.2x$10^{-9}$ M Donepezil with a bare CPE and CDMCPE. The higher stripping peak is observed at CDMCPE in comparison with bare CPE. The systematic studies of various experimental and instrumental parameters that affect the adsorptive stripping voltammogram response are carried out to establish the optimum conditions.

### Effect of pH

The effect of pH on the peak current ($i_p$) and peak potential (Ep) at 1.2x$10^{-9}$ M Acyclovir is evaluated. The peak current increases gradually with the increase of pH respect of the solution until it reaches the maximum value at pH 3.0; but for pH values higher than 3,

peak current decreases. Hence BR buffer of pH 3.0 is selected for further studies.

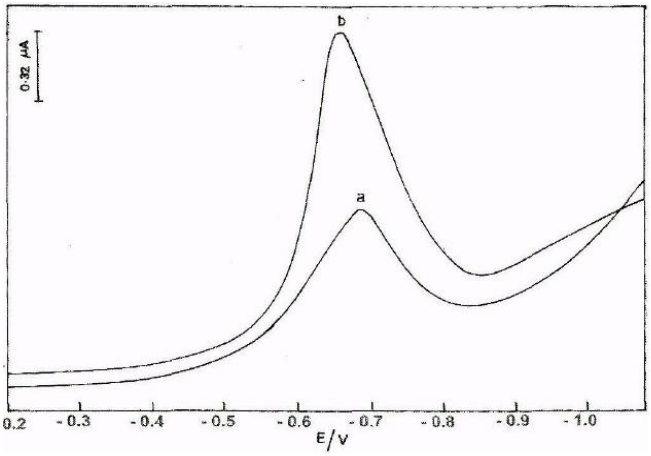


**Figure 3 : Typical DPAdSV of 1.2 x $10^{-9}$ M ACL at (a) bare CPE (b) CDCMPE**

## Effect of other parameters

The other experimental parameters those that directly affect the voltammetric response such as scan rate, stirring rate, pulse amplitude and rest period are optimized. All the experimental conditions are given in TABLE 1.

**TABLE 1 : Chosen experimental conditions**

| Variable | Chosen Value |
|---|---|
| pH | 3.0 |
| Buffer volume (ml) | 10 |
| Temperature ($^oC$) | 21 |
| Purge time (min) | 10 |
| Accumulation potential (V) | -0.4 |
| Accumulation time (s) | 150 |
| Rest time (s) | 10 |
| Stirring rate (rpm) | 2000 |
| Scan rate ($mVs^{-1}$) | 10 |
| Pulse amplitude (mV) | 50 |

## Determination of ACL in pharmaceutical formulations by DPAdSV

The standard addition procedure is employed to the direct determination of Acyclovir in pharmaceutical formulations by using CDMCPE and the recoveries are satisfactory. This means that the proposed technique can be applicable to the analysis of the above mentioned formulations containing Acyclovir with great success.

**TABLE 2 : Experimental data of acyclovir**

| Acvclovir | CPE | CDMCPE |
|---|---|---|
| Linearity range (M) | 2.0x $10^{-8}$to 2.2x $10^{-7}$ | l.0x $10^{-9}$to 0.2 x $10^{-8}$ |
| Calibration curve equation | Y(μA)=0.8373X+ 0.00913 | Y = 0.8333 X + 0.01381 |
| Correlation coefficient | 0.9998 | 0.9997 |
| L.O.D(M) | 1.9x $10^{-8}$ | 1.8x $10^{-9}$ |
| L.O.Q(M) | 0.633 x $10^{-7}$ | 0.6x $10^{-8}$ |
| Repeatability of peak currents %RSD] | 5.29 | 5.48 |
| Repeatability of Peak potentias %RSD) | 0.56 | 0.62 |
| Reproducibility of peak currents %RSD) | 5.20 | 5.44 |
| Reproducibility of potentials %RSD) | 0.52 | 0.57 |
| "Numbers of assays | 12 | 12 |

**TABLE 3 : Determination of ACL in pharmaceutical formulations by DPAdSV**

| Name of the drug | Amount Spiked (μ.g / L) | Average amount found* (μ.g / L) | Recovery percentage (%) | ± S.D | RSD |
|---|---|---|---|---|---|
| Acyclovir | 8 | 7.94 | 99.21 | 0.07 | 0.881 |
| | 10 | 9.97 | 99.73 | 0.05 | 0.462 |
| | 12 | 11.91 | 99.27 | 0.06 | 0.505 |

***Each value is an average of three determinations**

## Determination of ACL in spiked human serum samples by DPAdSV

Human serum samples are obtained from healthy individuals and are stored frozen until assay. An aliquot volume of serum sample is spiked with Acyclovir solution individually. 2x$10^{-3}$ M concentrated drug solutions are diluted to 1 ml volume with acetonitrile in a 2.5 ml centrifuge tube, this is vortexed for 10 min and centrifuged for 5 min at 2000 rpm for removing of protein residues. The separated supernatant of the sample was taken carefully. From this sample required volume of supernatant solution is transferred into the electrolytic cell containing BR buffer of pH 3.0. The voltammograms are recorded by the method differential pulse adsorptive stripping voltammetry and the

data are shown in the TABLE 4.

**TABLE 4 : Determination of ACL in spiked human serum samples**

| Name of the drug | Amount Spiked (μ.g / L) | Average amount found* (μ.g / L) | Recovery Percentage (%) | ± S.D | RSD |
|---|---|---|---|---|---|
| Acyclovir | 8 | 7.923 | 99.03 | 0.066 | 0.839 |
| | 10 | 9.97 | 99.7 | 0.035 | 0.347 |
| | 12 | 11.906 | 99.21 | 0.065 | 0.545 |

***Each value is an average of three determinations.**

## Determination of ACL in human urine samples by DPAdSV

Blank urine samples are collected (for 24 hrs) from healthy male voluntaries. This is added to the 50ml voltammetric cell containing BR buffer of pH 3.0. Firstly blank urine sample is introduced and voltammograms are recorded, and then spiked with donepezil. Each time, the differential pulse adsorptive stripping voltammetric signals are recorded and the data are given in the TABLE 5.

**TABLE 5 : Determination of ACL in spiked human urine samples by DPAdSV**

| Name of the drug | Amount Spiked (μ.g / L) | Average amount found* (μ.g / L) | Recovery percentage (%) | ±S.D | RSD |
|---|---|---|---|---|---|
| Acylcovir | 6 | 5.93 | 98.83 | 0.036 | 1.607 |
| | 8 | 7.917 | 98.96 | 0.087 | 1.103 |
| | 10 | 9.957 | 99.57 | 0.032 | 0.032 |

***Each value is an average of three determinations.**

## CONCLUSION

The present method certainly is used as an alternative to the colorimetric, spectrophotometric and chromatographic methods. Electrochemical technique is easy to handle, cheaper and time saving. The determination in pharmaceutical formulations and spiked urine and blood serum samples without any preliminary treatment by DPAdSV with a CDMCPE is a suitable method. Further due to CDMCPE stability, accuracy and low cost, it offers a good possibility as a substitute for the previous approaches used in routine analysis.

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