Voltammetric Study of Aciclovir Using Controlled Grow Mercury Drop Electrode

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The electrochemical properties of aciclovir (Acy) were studied by square wave voltammetric method (SWV) over pH range 1.5–8.0 using a controlled growth mercury drop electrode (CGMDE). In the acid medium the cathodic peak current was observed. Surface catalytic electrode mechanism based on the hydrogen evolution reaction was analyzed. The dependence of the peak current at about –1.3 V vs Ag/AgCl electrode on pH, buffer concentration, nature of the buffer, amplitude, frequency and scan rate was investigated. The best results were obtained in solution of nitric acid at pH 1.9. This electroanalytical procedure enabled to determine aciclovir in the concentration range 2 × 10⁻⁷–2 × 10⁻⁶ mol L⁻¹. Repeatability, precision and accuracy of the developed method were checked. The detection and quantification limits were found to be 7 × 10⁻⁸ and 2 × 10⁻⁷ mol L⁻¹ respectively.

Badano elektrochemiczne własności acyklowiru (Acy) metodą voltamperometryi z falą prostokątną (SWV) używając elektrody rtęciowej o kontrolowanym wzroście kropli (CGMDE) w zakresie pH 1,5–8. W środowisku kwaśnym zaobserwowano katodowy prąd piku. Analizowano mechanizm elektrodowy związany z katalityczną redukcją wodoru w obecności acyklowiru. Zbadano zależność prądu piku przy potencjale około –1,3 V od rodzaju i stężenia buforu, amplitudy, częstotliwości, szybkości przykłania potencjalu. Najlepsze wyniki uzyskano w kwasie azotowym(V) o pH 1,9. Oznaczono acyklowir w zakresie stężeń 0,2 × 10⁻⁶–2 × 10⁻⁶ mol L⁻¹. Sprawdzono powtarzalność, precyzję i dokładność opracowanej metody. Stwierdzono, że granica detekcji wynosi 7 × 10⁻⁸ mol L⁻¹, zaś granica ilościowego oznaczenia 2 × 10⁻⁷ mol L⁻¹.

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Aciclovir (2-amino-9-[[2-hydroxyethoxy)methyl]-3,9-dihydro-6H-purin-6-one) (Fig. 1A), a synthetic purine nucleoside derived from guanine, demonstrates strong and selective activity against herpes simplex and varicella zoster virus. It plays a key role in the therapy of the virus diseases. The quantitative determination of aciclovir become very important and has been widely studied. Analytical methods for its determination are based on HPLC [1–5] liquid chromatography/electrospray mass spectrometry [6], capillary electrophoresis [7–9], Raman spectroscopy [10] and NIR spectroscopy [11].

There is no much evidence about electrochemical studies related to aciclovir except Alvarez-Lueje [12] and Wang [13] studies on electrochemical oxidation of aciclovir at glassy carbon electrode. Recently [14] the electrochemical behaviors of aciclovir on the MWNTs–DHP film-coated glassy carbon electrode were investigated. In spite of the well-known utility of voltammetric methods for determination of drug traces at a hanging mercury drop electrode, the voltammetric properties of aciclovir at mercury electrode are yet unknown.

The aim of the work was to study SW voltammetric behavior of aciclovir at the controlled growth mercury drop electrode. SWV is one of the most advanced and most sensitive electroanalytical techniques. It combines advantages of the pulse voltammetric techniques capable to discriminate against the capacitative current and the cyclic voltammetry that provides an insight into the mechanism of the electrode reaction. Besides, SWV is one of the fastest voltammetric techniques enabling inspection of the rapid electron exchange in the case of surface electrode reactions. In addition, this technique is particularly well suited for quantitative determination of the redox couples immobilized on the electrode surface [15]. We have demonstrated, that even though aciclovir is electrochemically inactive on the mercury electrode, it is adsorbed at the CGMDE exhibiting effective catalytic activity toward hydrogen evolution reaction, which is a basis for its quantitative voltammetric determination.

EXPERIMENTAL

Instrumentation

The experiments were performed on the µAutolab/GPES (General Purpose Electrochemical System – version 4.8, Eco Chemie). A controlled growth mercury drop electrode (Entech s.c., Cracow, Poland) was used. All potentials were referred vs the Ag/AgCl, (3 mol L⁻¹ KCl) reference electrode. The counter electrode was a platinum wire. Operating conditions were: pulse amplitude E_sw = 80 mV, frequency f = 25 Hz, and step potential dE = 5 mV.
Reagents and solutions

Aciclovir was kindly provided by Nobel Inc. (Istanbul, Turkey). A fresh stock solution of $1 \times 10^{-3}$ mol L$^{-1}$ aciclovir was prepared daily by dissolving of 11.6 mg of the compound in 50 mL water with addition of 0.2 mL 0.2 mol L$^{-1}$ NaOH. The 0.2 mol L$^{-1}$ acetate buffers (pH 3.6–5.5), 0.04 mol L$^{-1}$ Britton–Robinson buffers (pH 1.9–8.0), 0.2 mol L$^{-1}$ citrate buffers (pH 1.4–4), 0.01 mol L$^{-1}$ citrate-phosphate buffers (pH 5.0–8.0) and solutions of nitric acid(V) were used as supporting electrolytes. All chemicals were of analytical grade (POCh SA Gliwice, Poland, or Merck). All solutions were prepared with distilled water purified by deionization system.

Working voltammetric procedure

The general procedure used to obtain SW voltammograms was as follows: 10 mL of the supporting electrolyte was placed in the voltammetric cell and the solution was purged with argon for 10 min. When an initial blank was recorded, the required volumes of aciclovir were added by means of a micropipette. After forming a new mercury drop, the solution was de-oxygenated for 20 s and a rest time of 30 s was applied without stirring. The square wave voltammograms were recorded from 0 V to –1.75 V. To receive well-shaped voltammetric peak for measurements, the blank was subtracted from the recorded aciclovir peak current.

RESULTS AND DISCUSSION

Aciclovir exhibits a special behavior at the mercury electrode. Although the compound is not reduced itself, during the voltammetric measurements the peak current appears in the potential range characteristic for catalytic hydrogen reduction [16]. Similarly to metformin (N,N-dimethylimidodicarbonimidicdiamide hydrochloride) (Fig. 1B) and famotidine [3-(((2-((aminoiminomethyl)amino)-4-thiazolyl)-methyl)-thio)-N’-(aminosulfonyl) propanimidamide] (Fig. 1C) aciclovir includes in its structure a guanidine group (–NH–C(NH–)=N–), which allows to deduce that the electrode mechanism of aciclovir at CGMDE is the same as for famotidine [15] and metformin [17]. Each of the mentioned compounds as the adsorbed catalyst (Cat$_{(ads)}$) undergoes protonation at the electrode surface, the protonated form of the catalyst is irreversibly reduced yielding the initial form of the catalyst and atomic hydrogen, i.e.,

\[
\text{Cat}^{(ads)}_{(ads)} + \text{H}^+_{(aq)} \rightarrow \text{CatH}^+_{(ads)}
\]

\[
\text{CatH}^+_{(ads)} + e \rightarrow \text{Cat}^{(ads)}_{(ads)} + \text{H}^-_{(aq)}.
\]

It is in accordance with the schemes of catalytic reactions for the group catalysts containing functional groups such as –NH$_2$, or =NH [18].
The electrochemical behavior of aciclovir was studied over a wide pH range (1.5–8.0) using square wave voltammetry. Among the studied electrolytes were Britton–Robinson, acetate, citrate, citrate-phosphate buffers and nitric acid(V) solution. The best shaped peak at about –1.3V and the most sensitive current were obtained in nitric acid(V) (pH 1.9).

The influence of the frequency, amplitude and step potential on the aciclovir SWV peak current ($c_{Acy} = 1 \times 10^{-6} \text{ mol L}^{-1}$) was also studied.

**Quantitative studies**

The applicability of the SWV as an analytical method for the determination of aciclovir was tested as a function of its concentration in the range $2 \times 10^{-7}–2 \times 10^{-6} \text{ mol L}^{-1}$ (Fig. 2). The analytical characteristic is summarized in Table 1. The limits of detection (LOD) and quantification (LOQ) of the procedure were calculated from the calibration curves as $k\text{SD}/b$ were $k = 3$ for LOD and 10 for LOQ, SD is the standard deviation of the intercept, and $b$ is the slope of the calibration line [19].
Table 1. Quantitative determination of aciclovir in nitric acid(V) at pH 1.9 by square wave voltammetry (SWV)

<table>
<thead>
<tr>
<th></th>
<th>SWV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear concentration range, mol L$^{-1}$</td>
<td>$0.2 \times 10^{-4}$--$2 \times 10^{-4}$</td>
</tr>
<tr>
<td>Slope of calibration graph, μA M$^{-1}$</td>
<td>3.43</td>
</tr>
<tr>
<td>RSD % of slope</td>
<td>2.70</td>
</tr>
<tr>
<td>Intercept, μA</td>
<td>0.59</td>
</tr>
<tr>
<td>RSD % of intercept</td>
<td>1.40</td>
</tr>
<tr>
<td>Correlation coefficient, r</td>
<td>0.987</td>
</tr>
<tr>
<td>Number of measurements</td>
<td>6</td>
</tr>
<tr>
<td>LOD, mol L$^{-1}$</td>
<td>$0.07 \times 10^{-4}$</td>
</tr>
<tr>
<td>LOQ, mol L$^{-1}$</td>
<td>$0.2 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Figure 2. SWV voltammograms of aciclovir ($4 \times 10^{-7}$, $6 \times 10^{-7}$, $8 \times 10^{-7}$ mol L$^{-1}$) in nitric acid(V) before (A) and after (B) subtract the background, pH = 1.9. (Continuation on the next page)
The repeatability of the procedure was assessed on the basis of 6 measurements by SWV at the same aciclovir concentration. Repeatability of the catalytic peak current at various aciclovir concentration is shown in Table 2.

Table 2. Repeatability of the aciclovir SW peak currents at various aciclovir concentrations

<table>
<thead>
<tr>
<th>Concentration of aciclovir ( \times 10^{-6} \text{ mol L}^{-1} )</th>
<th>Peak current (an average of 6 measurements) ( \mu \text{A} )</th>
<th>RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>1.74</td>
<td>9.0</td>
</tr>
<tr>
<td>0.6</td>
<td>2.60</td>
<td>5.5</td>
</tr>
<tr>
<td>0.8</td>
<td>3.38</td>
<td>3.2</td>
</tr>
<tr>
<td>1.0</td>
<td>4.76</td>
<td>2.8</td>
</tr>
<tr>
<td>1.5</td>
<td>5.59</td>
<td>5.4</td>
</tr>
<tr>
<td>2.0</td>
<td>7.26</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Precision and recovery of the method were investigated by determination of aciclovir at different concentrations in the linear range. Results are presented in Table 3.
Table 3. Recovery and precision obtained by SWV

<table>
<thead>
<tr>
<th>Added × 10^(-4) mol L⁻¹</th>
<th>Found X ± 1.05 × 10^(-4) mol L⁻¹</th>
<th>Precision RSD</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0.33 ± 0.05</td>
<td>0.14</td>
<td>83.4</td>
</tr>
<tr>
<td>0.6</td>
<td>0.58 ± 0.04</td>
<td>0.07</td>
<td>99.6</td>
</tr>
<tr>
<td>0.8</td>
<td>0.81 ± 0.03</td>
<td>0.04</td>
<td>101.2</td>
</tr>
<tr>
<td>1.5</td>
<td>1.45 ± 0.09</td>
<td>0.06</td>
<td>99.97</td>
</tr>
<tr>
<td>2.0</td>
<td>1.93 ± 0.08</td>
<td>0.04</td>
<td>96.5</td>
</tr>
</tbody>
</table>

* Recovery = 100 % + [ (Found – Added) / Added] × 100%

CONCLUSION

A new square wave voltammetric method for aciclovir determination based on hydrogen evolution catalyzed by adsorbed aciclovir at the CGMDE was developed. The most important advantage of the presented studies arises from the fact that the compound inactive at mercury electrode acts as electrocatalyst and can be determined by voltammetric method. Similar behavior was observed in the case of metformin and famotidine, which contain a guanidine group. The drawbacks to the method are unsatisfying precision and recovery. After improvement of the analytical parameters in the further studies, the procedure could be used in the analysis of the drugs containing aciclovir. The proposed method is simple, fast, and more cost-effective than HPLC procedure. Additionally most of common reducible substances exhibit electrochemical activity at much more positive potentials than the response due to hydrogen evolution reaction and they will not hinder in aciclovir determination.

Acknowledgements

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REFERENCES


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