ELECTROCHEMICAL STUDIES OF GANCICLOVIR AS THE ADSORBED CATALYST ON MERCURY ELECTRODE

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Received March 28, 2009
Accepted August 27, 2009
Published online December 10, 2009

Dedicated to the memory of Professor Jaroslav Heyrovský on the occasion of the 50th anniversary of the Nobel Prize for polarography.

Although ganciclovir (gan) as a purine analogue is a compound of biological interest (antiviral drug), it has been rarely electrochemically studied. In this paper surface catalytic electrode mechanism based on the hydrogen evolution reaction is analyzed under conditions of square-wave voltammetry and differential capacity curves of double layer measurements. The electrode mechanism is assumed to involve a preceding chemical reaction in which the adsorbed catalyst (gan_{ads}) is protonated at the electrode surface, i.e., $gan_{ads} + H^+_{aq} \rightarrow ganH^+_{ads}$. The protonated form of the catalyst ($ganH^+_{ads}$) is irreversibly reduced at potential about –1.35 V vs Ag|AgCl, yielding the initial form of the catalyst and atomic hydrogen, i.e., $ganH^+_{ads} + e \rightarrow gan_{ads} + H_{aq}$. Changes of zero charge potential and surface tension point to the adsorption of ganciclovir molecule directed with guanine group to the mercury surface and suggests that ganciclovir molecules are not placed flat on the mercury surface. The effect of adsorption on mercury electrode was studied in detail in respect to analytical usefulness of the obtained results. A new catalytic method for voltammetric determination of ganciclovir was developed. The detection and quantification limits were 1.3×10^{-7} and 4.3×10^{-7} mol l^{-1} for square-wave voltammetry, and 1.4×10^{-7} and 4.7×10^{-7} mol l^{-1} for linear-sweep voltammetry.

Keywords: Ganciclovir; Adsorption effect; Square-wave voltammetry; Electrocatalysis; Hydrogen transfer; Electrochemistry.

Purine and pyrimidine derivatives play an important role in many biological processes. They occur in nature mostly as constituents of larger molecules, e.g. in nucleosides and nucleotides[1]. The antiviral drug ganciclovir, 9-{[(1,3-dihydroxypropan-2-yl)oxy]metyl}guanine (gan) (Fig. 1), is a syn-

doi:10.1135/cccc2009045

thetic nucleoside, an analogue of guanine. It is a potent drug that has been shown by a number of clinical studies to be effective against cyromegalovirus in immunocompromised patients[2]. In AIDS patients, ganciclovir prolongs the time of progression of cytomegalovirus retinitis. It is also effective against AIDS-related gastrointestinal and pulmonary infection in immunocompromised patients with transplanted organs.

Although ganciclovir is a compound of biological interest, it has been rarely electrochemically studied. Most of the reported research of ganciclovir relies on the use of chromatographic techniques[3–7]. We found only two papers on electrochemical behavior of ganciclovir[8,9]. Visor and co-workers[8] studied substituted purines, including antiviral drugs (ganciclovir) at a glassy carbon electrode by differential pulse voltammetry and noticed that polar substituent groups strongly influence the anodic peak potential. Ozkan et al.[9] performed electrochemical studies of ganciclovir at a glassy carbon electrode and its direct determination in spiked serum and pharmaceutics by square-wave voltammetry (SWV) and differential pulse voltammetry (DPV). To our knowledge, no information about the reduction signal of ganciclovir has appeared in the literature. Guanine is not reduced at mercury electrode[10–12] and ganciclovir, as a guanine derivative, is not likely to be reduced at the mercury.

Ozkan et al. recorded anodic current of ganciclovir, in our voltammetric studies we recorded cathodic signal of the compound in acid medium. Similarly to famotidine and metformin, whose electrode mechanism we described previously[13,14], ganciclovir contains in its structure the guanidine group –N=C(NH–)–NH–. It seems that the presence of the group in the structure of ganciclovir gives a "new" possibility of its electroactivity at the mercury electrode. Hence, we assumed that ganciclovir can also act as adsorbed catalysts for hydrogen evolution reaction. The catalytic reduction of hydrogen ion is associated with the large activation energy required for hydrogen ion reduction at most electrodes, especially at mercury electrodes,

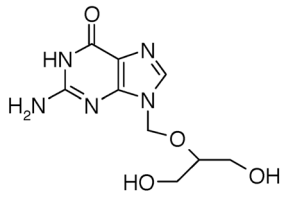

FIG. 1
The chemical structure of ganciclovir

and those less difficult reduction of the adducts formed by hydrogen ion with heterocyclic nitrogen or sulfur[10]. The structure of ganciclovir and the presence of guanidine group make this kind of electrochemical behavior possible. The proposed mechanism also seems to be in accordance with the schemes of catalytic reactions for the group of catalysts containing functional groups $-NH_2$ or =NH [15]. Furthermore, a detailed study of adsorption can also supply a lot of information on the electrochemical process occurring on the electrode.

The purpose of the work were the electrochemical studies of the including guanidine group drug at the hanging controlled growth mercury drop electrode (CGMDE) as an adsorbed electrocatalyst in catalytic hydrogen evolution. Also important was to evaluate a new voltammetric method of ganciclovir analysis.

EXPERIMENTAL

Apparatus

The experiments were performed on a microAutolab/GPES (General Purpose Electrochemical System, Version 4.8) and Autolab/GPES (Version 4.9) (Eco Chemie, Utrecht, Netherlands). The CGMDE (Entech, Cracow, Poland) were used (electrode areas were 1.02×10^{-2} cm^2 and 1.06×10^{-2} cm^2, respectively). All potentials were versus the Ag|AgCl (3 M KCl) reference electrode. The reference electrode was connected to the electrolytic cell via a bridge with the solution to be investigated. The counter-electrode was a platinum wire. The measurements were carried at 293 ± 0.1 K in thermostatic cells.

In the square-wave (SW) and linear-sweep (LS) voltammetric experiments optimal operating conditions were as follows: scan rate 80 mV s^{-1} and step potential –2 mV for LSV, and pulse amplitude E_{sw} = 60 mV, frequency f = 80 Hz and step potential ΔE = –5 mV for SWV.

In addition, automatic pipettes, pH-meter type HI 221 (Hanna Instruments, Poland) and electronic scale type MC 1 (Sartorius, Germany) were used.

Reagents and Solutions

Ganciclovir was purched from Roche Polska, Poland. A fresh stock solution of 10^{-3} M ganciclovir was prepared daily by dissolving 12.8 mg of the compound in 50 ml water. 0.2 M acetate buffers (pH 3.6–5.5), 0.04 M Britton–Robinson (BR) buffers (pH 1.44–8.11) and 0.1 M phosphate–citrate buffer (pH 2.35) were used as supporting electrolytes.

All solutions were prepared with triply distilled water.

Working Voltammetric Procedure

The general procedure used to obtain cathodic voltammograms was as follows: 10 ml of the supporting electrolyte (5 ml of buffer and 5 ml of water) was placed in a voltammetric cell and the solution was purged with argon for 10 min. When an initial blank was recorded, the required volumes of ganciclovir were added with a micropipette. After formation of

a new mercury drop, the solution was deoxygenated for 20 s followed by an equilibration time of 15 s, and a negative-going potential scan was applied from 0 to –1.75 V. To obtain well-shaped voltammetric peak for measurements, the blank was subtracted from the recorded ganciclovir peak current.

Parameters of Adsorption Measurements

The double layer capacity (C_d) was measured using the ac impedance method. The reproducibility of differential capacity measurements was ±0.5%. For the whole polarization range, the capacity dispersion was tested at frequencies between 200 and 1 000 Hz. To obtain proper equilibrium values of differential capacity, the linear dependence of capacity on square element of frequency was extrapolated to zero frequency. This procedure assumes that the impedance of the double layer is equivalent to a series of capacity–resistance combinations and that the rate of adsorption is diffusion-controlled[16].

The potentials of zero charge (E_z) were determined by use of a streaming electrode[17–19] with an accuracy of ±0.1 mV. The surface tension at the potential of zero charge (γ_z) was measured using the method of the highest pressure inside the mercury drop presented by Schiffrin[19]. The accuracy of the determined values of surface tension was ±0.2 nN m^{-1}.

The values of charge (σ_m) and surface tension (γ) obtained by integration of the differential capacity curves were used for the calculation of Parsons auxiliary function ξ, $\xi = \gamma \sigma_m E$ and interfacial pressure $\Phi = \Delta\xi = \xi^0 - \xi$ [20,21], where the superscript 0 refers to the supporting electrolyte containing the accelerating substance. In accordance with the Gibbs adsorbance isotherm, the relative surface excesses (Γ') of ganciclovir were determined at constant charge using the equation

$$\Gamma' = \left(\frac{1}{RT}\right)\left(\frac{\mathrm{d}\Phi}{\mathrm{d}\ln c_a}\right)_\sigma \qquad (1)$$

where c_a is the adsorbate concentration, R is the gas constant and T is the temperature in K.

RESULTS AND DISCUSSION

Preliminary Studies

As a preliminary study, we investigated the electrochemical behavior of ganciclovir over a wide pH range (1.44–8.11) at HMDE in buffered aqueous media using SWV. Among the studied electrolytes were BR, acetate, phosphate–citrate buffers. The best results regarding the shape and sensitivity of the peak were recorded in phosphate–citrate buffers at pH 2.35 (Fig. 2). For pH 1.44 of the buffer and ganciclovir concentration of the order 10^{-6} mol l^{-1}, the right arm of the peak overlapped the background.

The pH dependence of the peak current was investigated for the ganciclovir concentration 8×10^{-6} mol l^{-1} for different equilibration times which can be treated as the accumulation period without stirring at an accumulation potential of 0 V. For each pH the peak current recorded for 5 s was

lower than that obtained after 10 s; from accumulation time ca. 20 s, the dependences showed a trend to saturation (plateau). The differences in the peak currents can indicate the influence of adsorption process on the electrode mechanism. This was the main reason for investigation of the adsorption effect in detail.

Adsorption Measurements

Figure 3 presents the differential capacity curves of a double layer on the interface Hg/phosphate–citrate buffer and Hg/phosphate–citrate buffer–ganciclovir. A comparison of the course of differential capacity curves obtained for the solutions containing ganciclovir with the curve for supporting electrolyte allows to distinguish two potential regions. In the first region at $E > -800$ mV in the presence of ganciclovir an increase in differential capacity of the supporting electrolyte occurs. The differential capacities on the interface Hg/phosphate–citrate buffer–ganciclovir increase with increasing ganciclovir concentration. At the highest investigated concentrations of ganciclovir a slight hump appears on the differential capacity curves. In the region of more negative potentials (from –800 mV) a bigger decrease in the differential capacity appears with increasing ganciclovir concentration on the differential capacity curves in proportion to the sup-

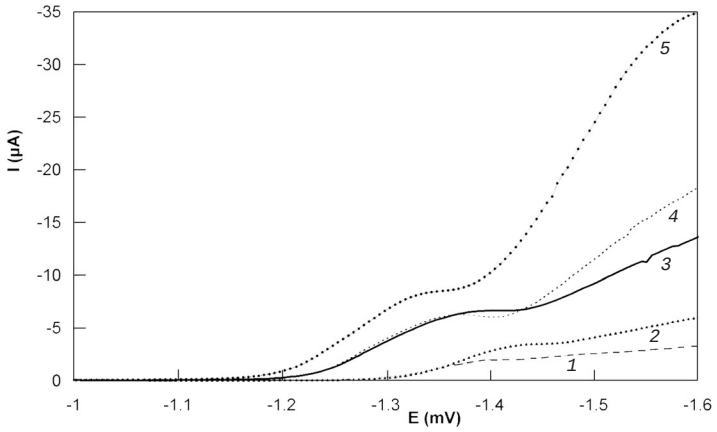

FIG. 2

Square-wave voltammograms of 1×10^{-5} M ganciclovir recorded in 0.02 M BR, 0.01 M acetate (Ac), 0.05 M phosphate–citrate (PC) buffers at different pH: BR 3.3 (*1*), BR 1.84 (*2*), PC 2.35 (*3*), PC 1.44 (*4*), Ac 3.6 (*5*). Other experimental conditions: $f = 25$ Hz, $E_{SW} = 20$ mV, $\Delta E = -4$ mV

porting electrolyte. It should be emphasized that the desorption peaks of ganciclovir do not occur. The desorption peaks overlap, presumably with the decomposition peaks of the supporting electrolyte, which is characteristic of the adsorption of high-molecular-weight compounds.

The values of potential of zero charge E_z and the surface tension values at potential of zero charge γ_z on the interfaces Hg/phosphate–citrate buffer and Hg/phosphate–citrate buffer in the presence of ganciclovir are presented in Table I.

TABLE I

Potential of zero charge (E_z) vs Ag|AgCl electrode and surface tension (γ_z) for E_z of double layer interface Hg/phosphate–citrate buffer (pH 2.35) and Hg/phosphate–citrate buffer (pH 2.35)–ganciclovir systems

c_{gan} μmol l^{-1}	$-E_z$ mV	γ_z mN m^{-1}	c_{gan} μmol l^{-1}	$-E_z$ mV	γ_z mN m^{-1}
0.00	425.6	423.79	50	424.5	422.05
5	425.3	423.70	80	424.1	421.17
8	425.1	423.65	100	423.8	420.30
10	425.0	423.35	300	420.3	415.94
30	424.9	423.30	500	417.0	411.58

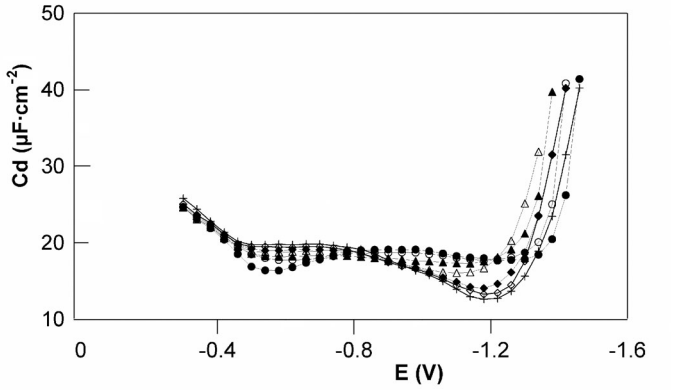

FIG. 3

Differential capacity curves of double layer interface Hg/phosphate–citrate buffer (pH 2.35) (●) and Hg/phosphate–citrate buffer (pH 2.35) in the presence of different concentrations of ganciclovir (mol l^{-1}): 5×10^{-6} (○), 1×10^{-5} (▲), 3×10^{-5} (△), 5×10^{-5} (◆), 8×10^{-5} (◇), 5×10^{-4} (+)

As follows from the Table I, together with an increase in ganciclovir concentration, the values of potential of zero charge E_z are shifted towards more positive potentials. These changes increase with increasing adsorbate concentration. Such situation indicates the adsorption of ganciclovir directed by guanine group to the mercury surface and suggests that ganciclovir molecules are not placed in parallel with the mercury surface, which makes difficult the interaction between the ring π-electrons and mercury[22]. At higher ganciclovir concentrations such interaction becomes probably weaker as a result of the orientation change of ganciclovir molecules. From the values γ_z presented in Table I it results that for ganciclovir concentrations lower than 5×10^{-5} mol l^{-1}, an increase in the ganciclovir concentration causes a slight decrease in surface tension. For higher ganciclovir concentrations, the decrease in γ_z values is considerably higher. The decrease in γ_z values with increasing of ganciclovir concentration suggests an increase in the adsorption of the investigated substance on mercury.

Figure 4 presents the dependence $\Gamma' = f(\sigma_m)$ for the chosen ganciclovir concentrations. The Γ' values increase with increasing adsorbate concentration and electrode charge. It should be noted that the $\Gamma' = f(\sigma_m)$ dependences are linear in the whole range of investigated ganciclovir concentrations. For lower adsorbate concentrations, the slope of straight lines is smaller than for higher concentrations.

According to Uslu et al.[9], who studied the electrochemical behavior of ganciclovir at glassy carbon electrode in buffer (pH 2–10), the adsorption of

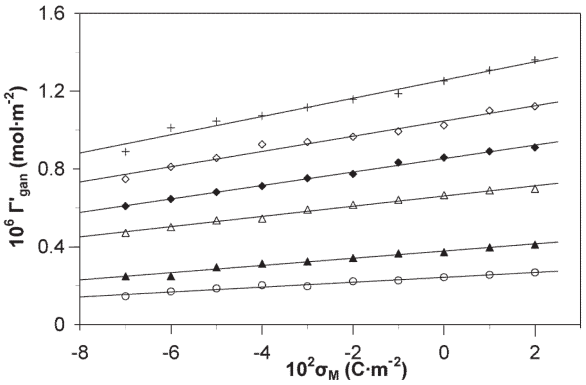

FIG. 4
Relative surface excesses (Γ′) of ganciclovir as a function of the electrode charge in the bulk for phosphate–citrate buffer (pH 2.35). Ganciclovir concentrations (× 10^{-5} mol l^{-1}) indicated by each line: 0.5 (○), 1 (▲), 3 (△), 5 (◆), 8 (◇), 10 (+)

ganciclovir at the glassy carbon electrode/buffer interface seems to occur in a fashion that the long side chain of ganciclovir is attached to the surface of the carbon electrode.

Nature of Electrode Process

The potential of the cathodic peak is shifted in BR (pH 1.8–6.0) with increasing pH to less positive potentials with dE_p/dpH [V/pH] = –0.10 pH – 1.16 (R^2 = 0.97) and the signal disappears above pH 7. This indicates participation of proton transfer in the electrode process[9]. Ganciclovir, similarly to famotidine or metformin whose electrode mechanisms on mercury electrode were analyzed earlier[13,14], contains in its structure the guanidine group. As our adsorption studies show, ganciclovir molecules seem to be attached to the surface of the electrode through the guanidine group. This places the group in the range of a possible electron transfer and suggests that for ganciclovir the same CE electrode mechanism is possible which as that observed for famotidine and metformin. So the ganciclovir electrode mechanism involves preceding chemical reaction in which the adsorbed catalyst (gan_{ads}) is undergoing protonation and the protonated form of the catalyst ($ganH^+_{ads}$) is irreversibly reduced yielding the initial form of the catalyst and hydrogen.

$$ganH^+_{ads} + H^+_{aq} \rightleftarrows ganH^+_{ads}$$

$$ganH^+_{ads} + e \rightarrow gan_{ads} + H_{aq}$$

In this scheme the protonated form of the compound acts as adsorbed at the mercury surface catalysts and catalyses effectively hydrogen evolution. The concentration of protons is assumed to be constant in the course of the voltammetric experiment due to the buffered solution, whereas the current is controlled by variation of the surface concentration of both unprotonated and protonated forms of the catalyst.

Quantitative Studies

In order to develop a voltammetric method for ganciclovir determination using the hydrogen evolution current at potential ca. –1.35 V, we selected the SWV technique as one of the most selective and sensitive. For comparison, LSV was also applied. The influence of amplitude and frequency on the SWV peak current was studied. For further experiments, the amplitude

60 mV and frequency 80 Hz were chosen. The influence of the buffer concentration in the range 0.002–0.1 mol l^{-1} at constant pH (2.35) was found. The buffer concentration 0.05 mol l^{-1} was used as optimal. The effect of scan rate in the LSV technique on the peak current was also observed; the scan rate 100 mV s^{-1} was applied in further experiments. The applicability of the SWV and LSV as the analytical methods for ganciclovir determination was tested as a function of its concentration in the range 0.1–10 μmol l^{-1} for SWV and 1–100 μmol l^{-1} for LSV. Good correlation between the peak current and ganciclovir concentration was obtained in SWV and LSV in the range 0.8–9 μmol l^{-1} (Fig. 5) and 1–10 μmol l^{-1}, respectively. The plot leveled off at higher concentrations, as expected for a process that is limited by adsorption of the compound.

Validation of the procedure for the quantitative assay of ganciclovir was examined by evaluation of the limit of detection (LOD), limit of quantification (LOQ), repeatability, recovery and precision. Limits of detection and quantification were computed, respectively, as 3 SB/*m* and 10 SB/*m*, where SB is the standard deviation of the peak current for the blank and *m* is slope of the analytical curve[23].

According to parameters listed in Table II, SWV was found to be the most sensitive technique. Moreover, the shape of the voltammetric peak at low concentrations of the analyte is much better defined compared with linear sweep voltammetry. The repeatability (1 day) of the SW voltammetric pro-

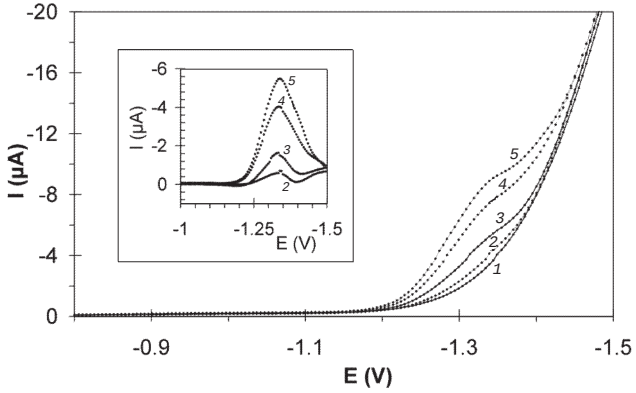

FIG. 5

Square-wave voltammograms of ganciclovir recorded in 0.05 M phosphate–citrate buffer (pH 2.35). Ganciclovir concentrations (mol l^{-1}): 0 (*1*), 1×10^{-6} (*2*), 2×10^{-6} (*3*), 4×10^{-6} (*4*), 6×10^{-6} (*5*). The inset shows ganciclovir voltammograms after subtraction of the blank current. Other experimental conditions: f = 80 Hz, E_{SW} = 60 mV, ΔE = –4 mV

cedure was assessed on the basis of six measurements at a single ganciclovir concentration. In the concentration range 0.8–9 μmol l^{-1} the RSD of the net SW peak current changed from 2.1 to 7.7%.

Precision and recovery of the method were investigated by determination of ganciclovir at three different concentrations in the linear range. The results are presented in Table III.

TABLE II
Determination of ganciclovir in 0.05 M citrate–phosphate buffer (pH 2.35) by square-wave and linear-sweep voltametries

Parameter	SWV	LSV
Linear concentration range, μmol l^{-1}	0.8–0.9	1–10
Slope of calibration graph, μA mol^{-1} l	1×10^6	5.09×10^5
RSD of slope	0.016	0.017
Intercept, μA	–0.55	–0.163
RSD of intercept	0.13	0.15
Correlation coefficient	0.999	0.993
Number of measurements	6	6
LOD, μmol l^{-1}	0.13	0.14
LOQ, μmol l^{-1}	0.43	0.47

TABLE III
Recovery and precision obtained by square-wave voltametry

Added μmol l^{-1}	Found $\bar{x} \pm t_{0.95}\bar{s}$ μmol l^{-1}	Precision RSD	Recovery[a] %
0.80	0.73 ± 0.02	0.024	91
1.00	1.02 ± 0.02	0.021	102
5.00	5.07 ± 0.28	0.053	101

[a] Recovery = 100% + [(found – added)/added] × 100%.

CONCLUSION

A new square-wave voltammetric method for ganciclovir determination based on hydrogen evolution catalyzed by adsorbed ganciclovir at CGMDE was developed. Ganciclovir is adsorbed on mercury electrode (CGMDE) from acid phosphate–citrate buffers. The decrease of the surface tension values at potential of zero charge with the increase of ganciclovir concentration testifies to the increase of the investigated substance adsorption on mercury. The changes of zero charge potential values point also to the adsorption of ganciclovir molecule direct with guanine group to the mercury surface. It suggests that ganciclovir molecules are not placed flat on the mercury electrode. This arrangement enables electron transfer of protonated form of ganciclovir which is the base of its electrochemical activity on the mercury. It also proves the participation of the guanidine group in the in the recorded voltammetric signal.

The advantage of the present electrochemical study is based on the fact that a compound inactive at mercury electrode acts as electrocatalyst and can be determined by voltammetry.

This work was supported by the University of Lodz (Grant No. 505/663).

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