Electrochemical Studies of the Neuraminidase Inhibitor Zanamivir and its Voltammetric Determination in Spiked Urine

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Abstract
Zanamivir is a member of a new class of antiviral agents that selectively inhibit the enzyme neuraminidase of influenza A H5N1 and H1N1 viruses. Although zanamivir is the compound of biological interest, so far it has not been a subject of any electrochemical studies. It was stated, that zanamivir can act as an electrocatalyst at HMDE. The electrode mechanism is connected with the hydrogen evolution reaction catalyzed by zanamivir as the guanidine compound. A new adsorptive catalytic method for its voltammetric (SW AdSV) determination was developed. The dependence of the peak current on pH, buffer concentration, nature of the buffer and instrumental parameters were studied. The best results were received in citrate-phosphate buffer at pH 2.2. This electroanalytical procedure enabled to determine zanamivir in the concentration range 4.8×10^{-7}–1.2×10^{-5} mol L^{-1} in supporting electrolyte and diluted urine. Precision, repeatability and accuracy of the method were checked in both media. The detection and quantification limits were found to be 1.5×10^{-7} and 4.8×10^{-7} mol L^{-1} respectively. A standard addition method was used to determine zanamivir in spiked urine.

Keywords: Catalytic mechanism, Neuraminidase inhibitor, Urine, Voltammetry, Zanamivir

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1. Introduction

New and recurring strains of influenza virus cause epidemics in the human population and result in many deaths each year. Recently, influenza A viruses subtype H5N1 also known as "bird flu" and H1N1 ("swine flu") are the most common cause of influenza in humans. In June 2009, World Health Organization declared that flu, due to a new strain of swine-origin H1N1, was responsible for the 2009 flu pandemic [1]. Treatment options are limited to the neuraminidase inhibitors oseltamivir and zanamivir which are available in oral and inhaled formulations, respectively. Peramivir is also a potent and selective neuraminidase inhibitor with demonstrated activity against influenza viruses, including H5N1, and is undergoing human clinical trials [2].

Zanamivir (Zan.) {(2*R*,3*R*,4*S*)-4-[(diaminomethylidene)amino]-3-acetamido-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid}, is a member of a new class of antiviral agents that selectively inhibit the enzyme neuraminidase of both influenza A and B viruses. Influenza virus contains two surface glycoproteins that are essential to viral replication and infectivity. The hemagglutinin recognizes sialic acid conjugates on the surface of host cell membranes, and through binding these residues anchors the virus to the cell surface. The neuraminidase is a hydrolytic enzyme that cleaves sialic acid from conjugates on the host cell at the end of the viral replication cycle to facilitate the spread of progeny virions [3]. In playing such a critical role in the life cycle of influenza virus, neuraminidase has been the focus of its new inhibitors design [4–10]. One of the earliest reported inhibitors of neuraminidase, which exhibits only moderate potency ($IC_{50}\approx10$ μM), is 2,3-didehydro-2-deoxy-*N*-acetylneuremic acid (Neu5Ac2en). Zanamivir, the 4-GuanNeu5Ac2en, is a simple analogue of Neu5Ac2en and was reported to be an extremely potent inhibitor ($IC_{50}\approx5$ nM) of both influenza A and B neuraminidase [11].

Although zanamivir is the compound of biological interest, so far it has not been a subject of many chemical studies. Analytical methods for quantitative determina-

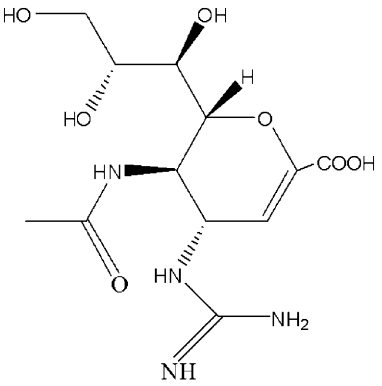

Fig. 1. Chemical structure of zanamivir.

tion of zanamivir are based on HPLC [12–14] or liquid chromatography – tandem mass spectrometry [15–16]. To the best my knowledge there are no published results of electrochemical studies. Zanamivir belongs to guanidinobenzoic acids (GANA) and the presence of the guanidine group in its structure gives possibility of the catalytic hydrogen evolution reaction at a hanging mercury drop electrode. The property has been noticed for other guanidine compounds like famotidine [17–18], metformine [19] or acyclovir [20] under conditions of square wave voltammetry and it was the base of adsorptive catalytic voltammetric determinations.

Among various catalytic electrode mechanisms, those based on hydrogen evolution are the most sensitive [21]. Although the reduction of hydrogen at the mercury electrode requires high overpotential, it can be effectively catalyzed in the presence of different substances immobilized on the electrode surface such as organic bases, microcrystals of platinum metals, platinum complexes with organic ligands or transition metal complexes [19]. Recently some of the compounds including the guanidine group have joined the substances mentioned above. The catalytic electrode reaction, based on the hydrogen evolution, has been proven to be of particular analytical utility for the determination of compounds of pharmacological relevance [22] or anticancer alkaloids [23] and is still applied [24].

The aim of the work was electrochemical studies of zanamivir with regard to catalytic hydrogen evolution and using the catalytic peak current of the neuraminidase inhibitor for its determination in urine.

2. Experimental

2.1. Instrumentation

The experiments were performed on a microAutolab/GPES (General Purpose Electrochemical System – Version 4.8), Eco Chemie. The controlled growth mercury drop electrode (Entech s.c, Cracow, Poland) was used. All potentials were referred against Ag/AgCl, (3 mol L^{-1} KCl) reference electrode. The counter electrode was a platinum wire. The measurements were carried out at 293 ± 0.1 K.

In the CV and SW voltammetric experiments the optimal operating conditions were: scan rate 100 mV s^{-1}, step potential −5 mV, equilibrium time 5 s for CV and pulse amplitude $E_{sw} = 40$ mV, frequency $f = 250$ Hz, and step potential $\Delta E = -9$ mV for SWV.

Additionally, the following equipment was also used: automatic pipettes, pH-meter type HI 221(Hanna Instruments, Poland), and electronic scales type MC 1 (Sartorius, Germany).

2.2. Reagents and Solutions

Zanamivir was obtained from Glaxo Smith Kline (U.K). A fresh stock solution of 1.2×10^{-3} mol L^{-1} zanamivir was prepared every two weeks by dissolving 20 mg of the compound in 50 mL deionized water. 0.2 mol L^{-1} citrate buffers (pH 1.5–2.5), 0.1 mol L^{-1} citrate-phosphate buffers (pH 2.2–4.8) and 0.04 mol L^{-1} Britton–Robinson buffers (pH 1.9–7.0) were used as a supporting electrolyte. All other chemicals were analytical grade (POCh SA Gliwice, Poland, Merck, Sigma-Aldrich). All solutions were prepared with deionized water.

2.3. Analysis Procedure

The general procedure used to obtain cathodic voltammograms was as follows: 5 mL of the supporting electrolyte and 5 mL of water 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 zanamivir were added by means of a micropipette. After forming a new mercury drop, the solution was deoxygenated for 20 s followed by a resting period of 15 s, and a negative-going potential scan was applied. To receive a well-shaped voltammetric peak for measurements, the blank was subtracted from the recorded zanamivir current and a baseline correction was applied.

2.4. Analysis in Diluted Urine Samples

5 μL of urine was placed in 5 mL voltammetric flash and completed to the volume with water. To investigate possible interference with urine, 80 μL of diluted urine was added into voltammetric cell containing 5 mL citrate – phosphate buffer (pH 2.2) and 5 mL of water. When an initial blank was recorded, the required volumes of zanamivir were added by means of a micropipette in the concentration range 0.24–12 μM. Voltammograms were recorded under the same conditions as for pure zanamivir. The recovery of the drug was calculated after six replicate experiments. Quantifications were performed by means of the calibration curve method.

2.5. Analysis in Spiked Urine Samples

5 μL of urine and 1 mL of zanamivir were placed in a 5 mL calibrated flask and filled to the volume with water. 80 μL of the spiked, diluted urine was added to the voltammetric cell containing the selected supporting electrolyte. Voltammograms were recorded under the same conditions as for pure zanamivir. The spiked urine was analyzed using the standard addition method and the recoveries obtained after six replicate experiments were calculated.

3. Results and Discussion

3.1. Electrochemical Behavior of Zanamivir at HMDE

Cyclic voltammogram of 1.6×10^{-4} mol L^{-1} Zan. recorded at 100 mV s^{-1} in a citrate-phosphate buffer at pH 2.2 is shown in Figure 2. The voltammogram recorded from 0 to −1.7 V consists of a single cathodic peak at negative

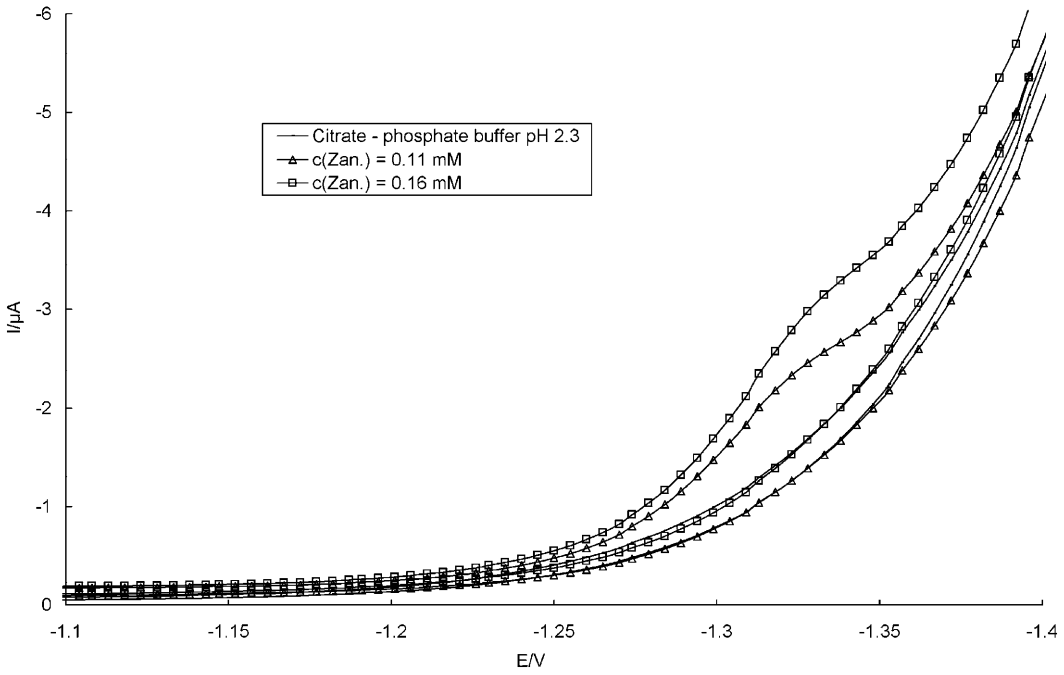

Fig. 2. Cyclic voltammograms of 1.1×10^{-4} and 1.6×10^{-4} mol L^{-1} zanamivir recorded in 0.05 mol L^{-1} citrate–phosphate buffer at pH 2.2 and scan rate 100 mV/s.

potential of −1.33 V vs. Ag/AgCl reference electrode. Since no anodic peak can be observed at any scan rate available by the instrumentation, the electrode process appears totally irreversible.

Such signal occurring at the negative end of the potential window at the HMDE is frequently related to the hydrogen evolution reaction [25–27] catalyzed by species adsorbed on the electrode surface. On the other side the position of the peak is not a criterion of the current character; some compounds can also reduce themselves at mercury electrode at low potentials [28].

Recently we have theoretically and experimentally studied in detail the catalytic hydrogen evolution reaction in the presence of an adsorbed catalyst by means of square-wave voltammetry [18]. The electrode mechanism involves preceding chemical reaction, in which the adsorbed catalyst (Cat.) is undergoing protonation (Equation 1) and the protonated form of the catalyst is irreversibly reduced yielding the initial form of the catalyst and atomic hydrogen (Equation 2)

$$Cat_{(ads)} + H^+_{(aq)} \underset{k_d}{\overset{k_p'}{\rightleftharpoons}} CatH^+_{(ads)} \quad (1)$$

$$CatH^+_{(ads)} + e^- \rightarrow Cat_{(ads)} + H_{(aq)} \quad (2)$$

k_p' and k_d are rate constants of the protonation and dissociation reactions, respectively and Zan. plays the role of a catalyst. As the experiment is performed in a buffered solution, the concentration of H^+ at the electrode surface is constant on the time scale of the experiment. Hence, the protonation reaction can be associated with a pseudo-first order rate constant defined as $k_p = k_p'c(H^+)$. The influence of pH on the recorded signal was studied in Britton–Robinson buffer in the pH range 1.9–7.0. It was noticed that from pH 2.2 the peak decreased with increasing of pH and disappeared at pH 4.2. From Figure 3, the best response was observed in medium pH approximately 2.2.

At a pH lower than 2.2 the net peak current cannot be precisely measured due to significant overlapping with the uncatalyzed hydrogen evolution current. However, at pH > 2.2, the magnitude of the response decreases as a consequence of the shift of the chemical equilibrium (I) toward the left-hand side, diminishing the amount of the electroactive catalyst Zan.$H^+_{(ads)}$ on the electrode surface.

From the results obtained in citrate–phosphate (pH 2.2–4.8) and citrate (pH 2.1–2.7) buffers the pH dependence on the reduction peak current, the citrate-phosphate buffer at pH 2.2 was selected for further investigations. The ionic strength influences the catalytic currents, so the influence of the buffer dilution (from 30% to 90% volume of buffer in the voltammetric cell) at constant pH on Zan. net peak current was checked. Parabolic changes in the dependence of I_p–%(v/v) buffer was found with the small maximum for the sample where $v_{(buf.)}:v_{(H2O)}$ was 4:6. In the next studies the samples contained 1:1 diluted citrate-phosphate buffer.

The analysis of the frequency influence on the recorded peak current provides further information on the type of electrode mechanism. In the previous theoretical analysis [18], it was stated that the overall catalytic effect depends on the dimensionless catalytic parameter defined as $\kappa_{cat} = k_p/f$. From the formulae, an increase of the frequency is expected to cause a decrease in the net peak current. On

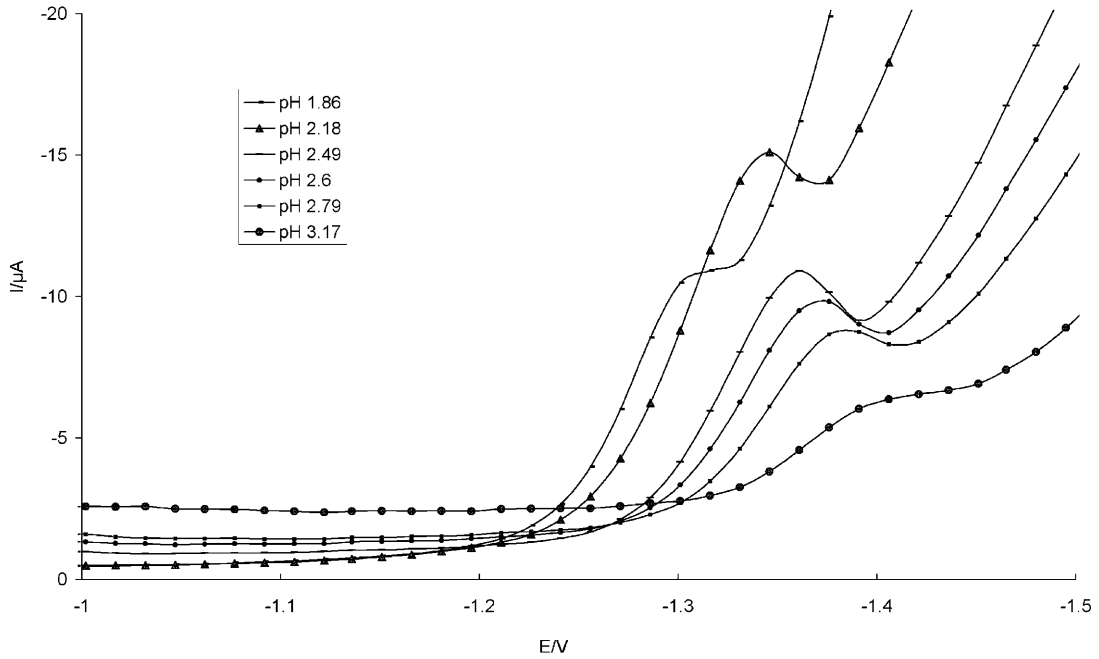

Fig. 3. SW AdS voltammograms of 3.6×10^{-5} mol L^{-1} zanamivir in Britton–Robinson buffers ($v_{buf}:v_{water}=1:1$). The parameters of the potential modulation were: frequency $f=250$ Hz, amplitude $E_{sw}=40$ mV, and step potential $\Delta E=-9$ mV.

the other hand, an increase of the frequency causes enhancement of the net peak current, which is a general property of all types of electrode mechanisms in SWV [25]. The overall dependence of the net peak current on the frequency is a compromise of the two opposite effects of the frequency. This dependence is illustrated by curve 1 in Figure 4.

Plotting the ratio, I_p/f versus the f, one observes a descending curve, which represents the influence of the catalytic parameter only (see curve 2 in Figure 4). This type of curve is typical for all catalytic mechanisms [29,30]. By numerical simulations of the voltammetric response [29], it has been established that the most typical feature of the current catalytic mechanism is the linear dependence between $\log(I_p/f)$ vs. $\log(\kappa_{cat})$, which holds under a large

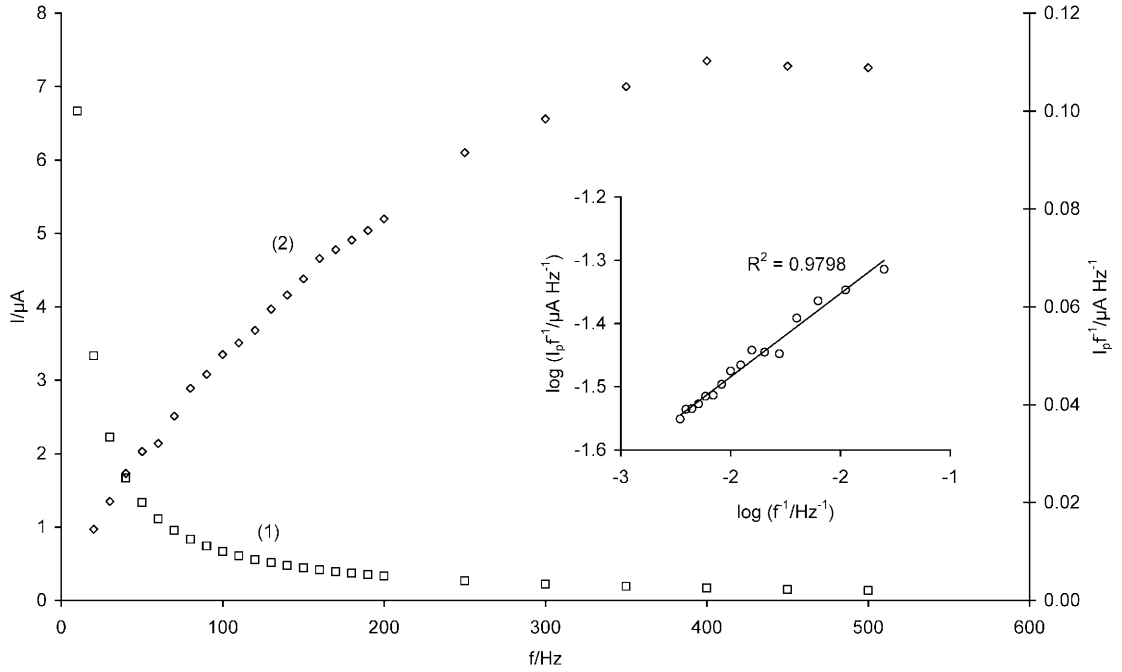

Fig. 4. The influence of the frequency (f) on the net peak current (I_p) (curve 1 left axis) and the ratio I_p/f (curve 2, right axis). The inset shows the dependence of $\log(I_p/f)$ on the $\log(1/f)$. The conditions of the experiments were: supporting electrolyte 0.05 mol L^{-1} citrate–phosphate buffer pH 2.2, c(zanamivir) $=3.6\times10^{-5}$ mol L^{-1}, amplitude $E_{sw}=50$ mV, $\Delta E=-9$ mV.

variety of experimental conditions. This type of dependence can be obtained by plotting $\log(I_p/f)$ vs. $\log(1/f)$ and its linearity can be regarded as a diagnostic criterion for the hydrogen catalytic evolution reaction in SWV [18].

Considering the above results, it can be assumed that the voltammetric response obtained at the mercury electrode in the presence of Zan. is a result of hydrogen catalytic evolution catalyzed by an adsorbed catalyst.

3.2. Analytical Application

3.2.1. Validation of the Proposed Method

In order to develop a voltammetric method for Zan. determination using the hydrogen evolution current at potential ca. −1.33 V, we selected the square wave voltammetry technique as one of the most selective and sensitive. The influence of amplitude and frequency on the

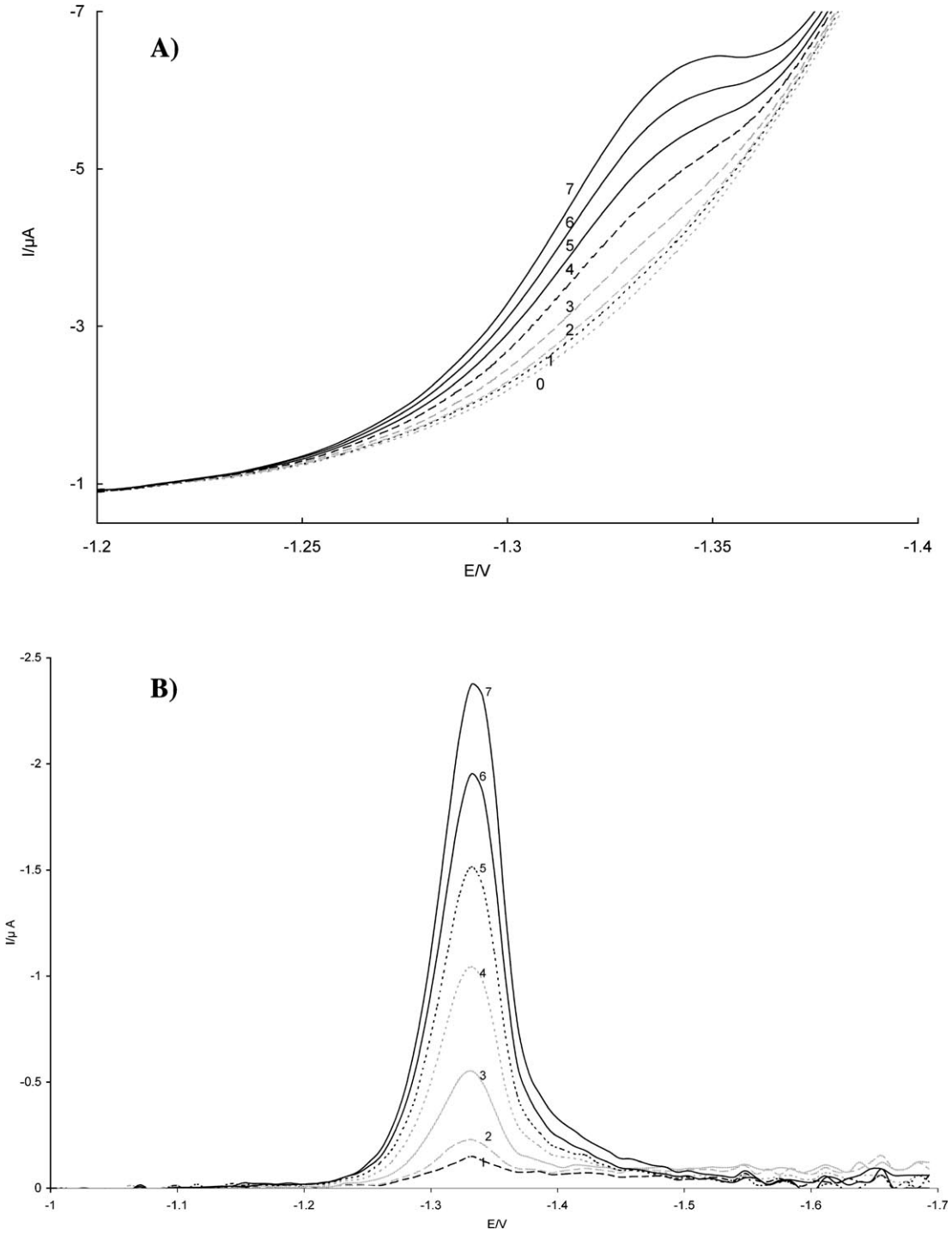

Fig. 5. SW AdS voltammograms of zanamivir recorded in 0.05 mol L^{-1} citrate–phosphate buffer at pH 2.2 (A). (B) Zanamivir voltammograms after subtraction of the supporting electrolyte current; c(Zan.) = 0) blank, 1) 4.8×10^{-7}, 2) 9.6×10^{-7}, 3) 2.4×10^{-6}, 4) 4.8×10^{-6}, 5) 7.2×10^{-6}, 6) 9.6×10^{-6}, 7) 1.2×10^{-5} mol L^{-1}. The other experimental conditions were f = 250 Hz E_{sw} = 40 mV, ΔE = −9 mV.

SWV peak current was studied. For further experiments, the amplitude 40 mV and frequency 250 Hz were chosen. The applicability of the SWV as the analytical methods for Zan. determination was tested. Good correlation between the peak current and Zan. concentration was obtained in the range 0.48–12 μmol L^{-1} (Figure 5). The plot leveled off at higher concentrations, as expected for a process that is limited by adsorption of the compound.

The quantitative and statistic parameters obtained for the validation of the method are collected in Table 1. The lowest detectable concentration and the lowest quantitative concentration of Zan. were estimated based on the following equations: $LOD = 3\,s/m$ and $LOQ = 10\,s/m$. Abbreviation *s* represents the standard deviation of the peak current (six runs) and *m* represents the slope of the related calibration curve [31].

The repeatability (1 day) of the SW voltammetric procedure was assessed on the basis of six measurements at a single Zan. concentration. In the concentration range 0.48–12 μmol L^{-1} the *RSD* of the net SW peak current changed from 9.78 % to 5.01 %.

Precision and accuracy of the method were investigated by determination of Zan. for different concentrations in the linear range. The results are presented in Table 2.

Table 1. Regresion data of calibration line for quantitative determination of zanamivir by SW AdSV in supporting electrolyte and diluted urine.

	SW AdSV supporting electrolyte	SW AdSV diluted urine
Measured potential (V)	−1.33	−1.33
Linear concentration range (μmol L^{-1})	0.48–12	0.48–12
Slope of calibration graph (A/μmol L^{-1})	0.187	0.1795
RSD of slope	0.053	0.028
Intercept (μA)	0.052	0.187
RSD of intercept	0.18	0.03
Correlation coefficient, *r*	0.998	0.995
Number of measurements	6	6
LOD (μmol L^{-1})	0.15	0.12
LOQ (μmol L^{-1})	0.48	0.4
Repeatability of peak current (*RSD*%) for $c = 0.48$ μmol L^{-1}	2.8	6.03
Repeatability of peak current (*RSD*%) for $c = 12$ μmol L^{-1}	2.5	5.71

3.2.2. Determination of Zan. in Diluted Urine

The optimized voltammetric procedure was successfully applied for Zan. determination in diluted urine. Besides the urine dilution, no extraction steps have been undertaken prior to the voltammetric measurements. Calibration curves were made for Zan. in this medium showing the linear dependence between peak current and concentration in the range of 0.48–12 μM. The characteristic of the calibration plots are summarized in Table 1. In order to check the accuracy, precision and selectivity of the developed method, a recovery study was carried out. As can be seen in Fig. 6, the noise peak found from the biological material appears about −70 mV from the Zan. peak and does not interfere with the studied signal. This confirms the results of accuracy and precision collected in Table 2. The method is sufficiently accurate and precise in order to be applied to determination of Zan. in spiked urine.

Table 2. Accuracy and precision obtained by SW AdSV.

Added (μmol L^{-1})	Found (μmol L^{-1}) [b]	Precision *RSD* (%)	Accuracy [a] (%)
Supporting electrolyte			
0.48	0.44 ± 0.03	6.03	91.18
0.96	0.88 ± 0.06	6.58	92.11
2.40	2.48 ± 0.25	9.78	103.2
7.20	7.44 ± 0.39	5.01	103.31
9.60	9.59 ± 0.54	5.35	99.90
12.00	11.62 ± 0.70	5.71	96.82
Diluted urine			
0.48	0.45 ± 0.03	6.58	94.68
0.96	1.03 ± 0.07	6.70	107.58
2.40	2.67 ± 0.24	8.87	111.30
7.20	7.32 ± 0.33	4.58	101.67
9.60	9.84 ± 0.36	3.64	102.55
12.00	11.96 ± 0.33	2.76	99.68
Spiked urine			
0.96	1.00 ± 0.08	8.34	104.2

[a] Accuracy = [(Found − Added)/Added] × 100 %. [b] $t(S/n^{1/2})$, $p = 95\,\%$

3.2.3. Determination of Zan. in Spiked Urine

The recovery results of Zan. in spiked urine are given in Table 2. The method of standard addition was used to determine Zan. in this medium. The concentration of the additions in the voltammetric sample amounted as follow: 2.40×10^{-7} mol L^{-1}, 1.44×10^{-6} mol L^{-1}, 3.82×10^{-6} mol L^{-1}. The variation of the SW peak current versus Zan. concentration in studied spiked urine is represented by the following linear equation: $I\ (\mu A) = 0.185\ C\ (\mu mol\,L^{-1}) + 0.186\ (\mu A)$, *RSD* of intercept 9.3 %, *RSD* of slope 0.5 %.

4. Conclusions

Zanamivir as the neraminidase inhibitor plays an important role in the treatment of A H1N1 and A H5N1influenza. The electrochemical behavior of Zan. was established and studied for the first time. Zan. is electrochemically inactive at HMDE, but is adsorbed at the electrode exhibiting effective catalytic activity toward hydrogen evolution reaction, which forms the basis for its quantitative voltammetric determination.

The behaviour at HMDE provides a useful tool for detection and quantification of the drug at a low level of concentration in biological fluids. This work shows that the Zan. concentration in human urine can be determined using the voltammetric technique. The procedure of de-

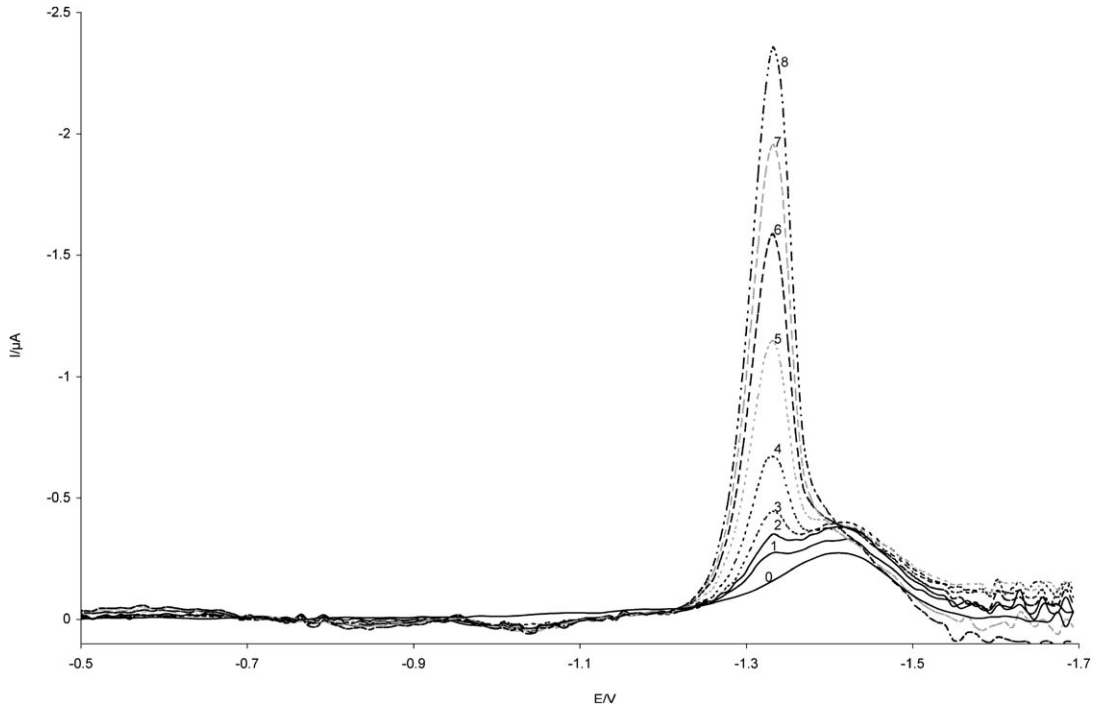

Fig. 6. Square wave voltammograms (after subtracting the supporting electrolyte current) of zanamivir in diluted urine samples. The supporting electrolyte was a 0.05 mol L^{-1} citrate – phosphate buffer at pH 2.2. c(Zan.) = 0) diluted urine 1) 4.8×10^{-7}, 2) 7.2×10^{-7}, 3) 1.2×10^{-6}, 4) 2.4×10^{-6}, 5) 4.8×10^{-6}, 6) 7.2×10^{-6}, 7) 9.6×10^{-6}, 8) 1.2×10^{-5} mol L^{-1}. Other experimental conditions were: f = 250 Hz, E_{sw} = 40 mV, ΔE = −9 mV.

termination of Zan. in urine requires only dilution of the sample prior to the voltammetric scan. Urine is a composite matrix, depending on the person and eating habits partially changing the composition. The concentration of the component is rather high as for the voltammetric techniques, so the dilution of urine is an important component of the procedure.

With this study it was not intended to show the pharmacodynamic properties of Zan. since only healthy volunteers were used for the sample collection and the results may be of no significance. It only shows that there is a great possibility of monitoring Zan. making the method useful for pharmacokinetic and pharmacodynamic purposes. It could also be adopted for quality control laboratory studies.

Acknowledgements

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References

[1] http://en.wikipedia.org/wiki/AH1N1.
[2] N. Lindegardh, *J. Chromatogr. B* **2008**, *1215*, 145.
[3] W. J. Brouillette, V. R. Atigadda, M. Luo, G. M. Air, Y. S. Babu, S. Bantia, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1901.
[4] M. Luo, G. M. Air, W. J. Brouillette, *J. Infectious Diseases* **1997**, *176*, S62.
[5] M. J. Jedrzejas, S. Singh, W. J. Brouillette, W. G. Laver, G. M. Air, M. Luo, *Biochemistry* **1995**, *34*, 3144.
[6] S. Singh, M. J. Jedrzejas, G. M. Air, M. Luo, W. G. Laver, W. J. Brouillette, *J. Med. Chem.* **1995**, *38*, 3217.
[7] D. M. Ryan, J. Ticehurst, M. H. Dempsey, *Antimicrob. Agents Chemother.* **1995**, *39*, 2583.
[8] V. R. Atigadda, W. J. Brouillette, F. Duarte, S. M. Ali, Y. S. Babu, S. Bantia, P. Chand, N. Chu, J. A. Montgomery, D. A. Walsh, E. A. Sudbeck, J. Finely, M. Luo, G. M. Air, G. W. Laver, *J. Med. Chem.* **1999**, *42*, 2332.
[9] V. R. Atigadda, W. J. Brouillette, F. Duarte, Y. S. Babu, S. Bantia, P. Chand, N. Chu, J. A. Montgomery, D. A. Walsh, E. Sudbeck, J. Finely, G. M. Air, M. Luo, G. W. Laver, *Bioorg. Med. Chem.* **1999**, *7*, 2487.
[10] E. A. Sudbeck, M. J. Jedrzejas, S. Singh, W. J. Broullette, G. M. Air, W. G. Laver, Y. S. Babu, S. Bantia, P. Chand, N. Chu, J. A. Montgomery, D. A. Walsh, M. Luo, *J. Mol. Biol.* **1997**, *267*, 584.
[11] M. Von Itzstein, Y. Wu, G. B. Kok, M. S. Peg, J. C. Dyason, B. Jin, T. Van Phan, M. L. Synthe, H. F. White, S. W. Oliver, P. G. Coleman, V. J. Varghese, J. M. Cameron, C. R. Pen, *Nature* **1993**, *363*, 418. in W. J. Brouillette, V. R. Atigadda, M. Luo, G. M. Air, Y. S. Babu, S. Bantia, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1901.
[12] D. M. Morris, B. K. Hussey, S. L. Geurin, K. A. Selinger, *J. Pharm. Biomed. Anal.* **1995**, *14*, 191.
[13] R. J. Stubbs, J. Harker, *J. Chromatogr. B, Biomed. Appl.* **1995**, *670*, 279.
[14] N. Erk, *J. Liq. Chromatogr. Relat. Tech.* **2004**, *27*, 1541.
[15] G. D. Allen, S. T. Brookes, A. Barrow, J. A. Dunn, C. M. Grosse, *J. Chromatogr. B, Biomed. Appl.* **1999**, *732*, 383.

[16] T. M. Baughman, W. L. Wright, K. A. Hutton, *J. Chromatogr. B, Anal. Technol.* in *Biomed. And Life Sc.* **2007**, *852*, 505.
[17] S. Skrzypek, W. Ciesielski, A. Sokołowski, S. Yilmaz, D. Kaźmierczak, *Talanta* **2005**, *66*, 1146.
[18] V. Mirceski, S. Skrzypek, W. Ciesielski, Adam Sokołowski, *J. Electroanal. Chem.* **2005**, *585*, 97.
[19] S. Skrzypek, V. Mirceski, W. Ciesielski, A. Sokołowski, R. Zakrzewski, *J. Pharm. Biomed. Anal.* **2007**, *45*, 275.
[20] S. Skrzypek, W. Ciesielski, S. Yilmaz, *Chem. Anal. (Warsaw)* **2007**, *52*, 1071.
[21] F. G. Banica, A. Ion, in *Encyclopedia of Analytical Chemistry* (Ed: R. A. Meyers), Wiley, Chichester **2000**, pp. 11115–11144.
[22] M. Brzezina, P. Zuman, in *Polarography in Medicine, Biochemistry and Pharmacy*, Interscience, New York **1958**, pp. 351–380.
[23] J. Wang, J. Zadeii, M. S. Lin, *J. Electroanal. Chem.* **1987**, *237*, 281.
[24] G. Somer, Z. Almas, *J. Electroanal. Chem.* **2006**, *593*, 170.
[25] M. Lovrić, in *Electroanalysis Methods, Guide to Experiments and Applications* (Ed: F. Scholz), Springer, Heidelberg **2002**, pp. 111–133.
[26] F. G. Banica, N. Spataru, T. Spataru, *Electroanalysis* **1997**, *9*, 1341.
[27] A. Calusaru, *Analyst* **1990**, *115*, 1223.
[28] B. Dogan, B. Uslu, S. Suzen , S. A. Ozkan, *Electroanalysis* **2005**, *17*, 1886.
[29] V. Mirčeski, R. Gulaboski, *Electroanalysis* **2001**, *13*, 1326.
[30] V. Mirčeski, R. Gulaboski, *J. Solid State Electrochem.* **2003**, *7*, 157.
[31] L. B.O dos Santos, G. Abate, J. S. Masini, *Talanta* **2004**, *62*, 667.