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Direct determination of metformin in urine by adsorptive catalytic square-wave voltammetry

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Abstract

A new adsorptive catalytic voltammetric method for voltammetric determination of metformin based on the catalytic hydrogen evolution reaction at a hanging mercury drop electrode was developed. The electrode reaction was analyzed under conditions of linear sweep voltammetry (LSV), differential pulse voltammetry (DPV) and Osteryoung-type square-wave voltammetry (SWV). The peak current depends on pH of the medium, concentration and chemical composition of the buffer solution, and instrumental parameters. The optimal conditions for quantitative determination were obtained in an acetate buffer at pH 4.7. The voltammetric procedure was characterized with respect to the repeatability, precision and the recovery. The detection and quantification limits were found to be 1.8×10^{-8} and 5.9×10^{-8} mol l^{-1} for SWV, 3.2×10^{-8} and 1.0×10^{-7} mol l^{-1} for DPV, and 7.7×10^{-8} and 2.5×10^{-7} mol l^{-1} for LSV, respectively. The SW voltammetric method, as the most sensitive one, was applied for determination of metformin in human urine. The voltammetric method has been validated by using HPLC with UV detection.
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Keywords: Metformin; Hydrogen evolution; Catalytic mechanism; Urine analysis; Square-wave voltammetry; High performance liquid chromatography

1. Introduction

Metformin, hydrochloride (*N*,*N*-dimethylimidodicarbonimidicdiamide hydrochloride), is an effective hypoglycemic drug that lowers blood glucose concentration by decreasing hepatic glucose production and increasing glucose disposal in skeletal muscles [1]. The compound contains biguanide group and its chemical structure is shown in Fig. 1.

Analytical methods for quantitative determination of metformin are based on HPLC [2–11] fast liquid chromatography–tandem mass spectrometry [12], planar chromatography [13], capillary electrophoresis [14], NMR spectrometry [15], UV-spectrophotometry [16], NIR spectroscopy [17], colorimetry [18], conductometry [19], and volumetric analysis with visual endpoint detection [20]. Each method enables determination of metformin at different concentration levels. Among the most sensitive ones are the HPLC methods with precolumn fluorescence derivatization [3] (LOD = 1.3 pmol per 20 μl injection volume) and with the tandem mass spectrometric detection [12] (LOD = 10 ng ml^{-1}). Methods based on colorimetry [18] or conductometry [19] enable determination of metformin down to 10^{-4} mol l^{-1}. On the other hand, electroanalytical methods are highly sensitive, simple, and require low-cost instrumentation. However, there are only a few studies on voltammetric behaviour of metformin. Liu and Li [21] determined metformin hydrochloride in the concentration range from 1×10^{-9} to 1×10^{-6} mol l^{-1} on a glassy carbon electrode using the oxidation of metformin at about 0.37 V versus Ag/AgCl. Tian and Song developed a method for voltammetric determination of metformin by using a paste electrode [22] (LOD = 6.5×10^{-7} mol l^{-1}). In spite of the well-known utility of voltammetric methods for determination of drug traces at a hanging mercury drop electrode (HMDE), the voltammetric properties of metformin at HMDE are yet unknown.

In the present study, the voltammetric properties of metformin at the HMDE were investigated using linear sweep

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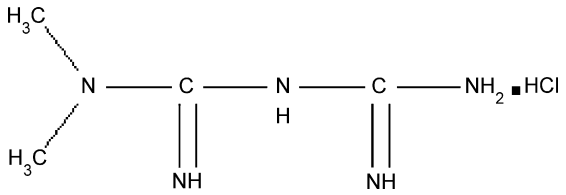

Fig. 1. Chemical structure of metformin.

voltammetry (LSV), differential pulse voltammetry (DPV) and Osteryoung-type square-wave voltammetry (SWV). The aim of the work is to characterize the electrode reaction of metformin at the HMDE and to develop voltammetric method for its quantitative determination. Though metformin is electrochemically inactive on the HMDE, it is adsorbed at the HMDE exhibiting effective catalytic activity toward hydrogen evolution reaction, which forms the basis for its quantitative voltammetric determination.

Among various catalytic electrode mechanisms, those based on the hydrogen evolution reaction are the most sensitive ones [23]. 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 [24], microcrystals of platinum metals [25], platinum complexes with organic ligands [26] or transition metal complexes [27]. Catalytic electrode reaction based on the hydrogen evolution has been proven to be of particular analytical utility for determination of compounds of pharmacological relevance [28] and anticancer alkaloids [29].

In a recent study, we have provided a theoretical background for hydrogen catalytic electrode mechanism under conditions of SWV [30]. SWV is both fast and sensitive electroanalytical technique that enables inspection of the electrode mechanism [31]. In the present study, it has been demonstrated that SWV exhibits the highest sensitivity towards metformin concentration compared to other voltammetric techniques used. Hence, the SW voltammetric method has been applied for metformin determination in human urine, following dilution of the sample only. The proposed adsorptive catalytic SW voltammetric method is fast and simple, being a promising alternative for quantitative metformin determination. The voltammetric method was validated by using HPLC.

2. Experimental

2.1. Instrumentation

The voltammetric experiments were performed on a microAutolab/GPES (General Purpose Electrochemical System, Version 4.8, Eco Chemie) computer-controlled electrochemical system. A controlled growth mercury drop electrode (Entech s.c, Cracow, Poland) was used. All potentials were referred versus Ag/AgCl (3 mol l^{-1} KCl) reference electrode. The counter electrode was a platinum wire. Operating conditions were: scan rate 100 mV s^{-1} and step potential −3 mV for LSV; pulse amplitude −40 mV, step potential −4 mV for DPV; and pulse amplitude E_{sw} = 80 mV, frequency f = 40 Hz, and step potential ΔE = −4 mV for SWV.

The chromatographic experiments were conducted using an analytical column Econosil C18 (250 mm × 4.6 mm i.d., 10 μm, Alltech). The mobile phase for HPLC analysis consisted of acetonitrile, phosphate buffer (pH 6.0) and water (60:12:28; v/v/v). The flow-rate was 1.2 ml/min at ambient temperature. The eluate was monitored at a wavelength of λ = 234 nm using a variable wavelength LC spectrophotometer (Waters 2487 Dual λ). The mobile phase delivery system was a Multisolvent Delivery System Model 600E (Waters). Test samples were applied to the HPLC column with a Rheodyne 7725i injector (20 μl loop). The chromatographic system and recorder were connected with bus-SaT/In Module (Waters). The chromatograms were integrated with Millennium software (Waters).

In addition, the following equipment was also used: pH-meter type N-517 (Mera-Elwro, Poland), and electronics scales type MC 1 (Sartorius, Germany).

2.2. Reagents and solutions

Metformin hydrochloride was a gift of "Polfa" Kutno, Poland. A fresh stock solution of 1×10^{-3} mol l^{-1} metformin was prepared daily by dissolving of 8.3 mg of the compound in 50 ml water. 0.2 mol l^{-1} acetate buffers (pH 4.3–5.5), 0.2 mol l^{-1} MOPS buffers (pH 6.5–7.9), 0.04 mol l^{-1} Britton–Robinson buffers (pH 3.8–8.0) and 0.04 mol l^{-1} phosphate buffer (pH 6.0) were used as supporting electrolytes.

HPLC-grade acetonitrile were obtained from Sigma–Aldrich (Steinheim, Germany) or LAB-SCAN Analytical Sciences (Dublin, Ireland). All other chemicals were analytical grade (POCh SA Gliwice, Poland, Merck or Sigma–Aldrich). The metformin tablets ("Metformax", 500.0 mg, "Polfa" Kutno, Poland) were purchased from the local pharmacy. All solutions were prepared with triply distilled water.

2.3. Analysis procedures

The general procedure used to obtain cathodic 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, required volume of metformin aqueous solution was added by means of a micropipette. Then, the solution was de-oxygenated for 20 s, and after formation of a new drop of Hg, an accumulation period of 15 s was applied without steering at an accumulation potential of 0 V, and a negative-going potential scan from 0 to −1.75 V was applied. To obtain a well-shaped voltammetric peak for quantitative determination, after subtraction of the blank current, the baseline was fitted with a polynomial function and extrapolated under the metformin peak.

2.4. Analysis in spiked urine samples

To investigate possible interferences from the urine, known amounts of the metformin were added into 1 ml of metformin-free urine sample, which was further diluted up to 25 ml with

water. Sixty microliters 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 metformin. The spiked urine was analyzed using the standard addition method and the recoveries obtained after six replicate experiments were calculated.

2.5. Analysis of patient's urine samples

Patient's urine (morning, mid-stream urine) was obtained daily from a volunteer who took three times a day, every 8 h, "Metformax" tablets containing 500 mg of metformin hydrochloride. Before voltammetric measurements, the urine sample was diluted 1:25 with water. Sixty microliters of this solution was placed in the voltammetric cell containing 10 ml of 0.01 mol l^{-1} acetate buffer as a supporting electrolyte and voltammograms were recorded.

2.6. Validation by HPLC method

One milliliter of spiked metformin urine was diluted with water to 10 ml. The calibration curve was prepared by adding a known amount of metformin (within the range from 2×10^{-8} to 4×10^{-6} mol) to 1 ml of metformin-free urine sample and diluted with water to 10 ml. The sample was further analyzed according to the procedure described in Section 2.1. The area of the chromatographic peak corresponding to metformin was plotted against the concentration of metformin in the urine sample.

A 1 ml of the patient's urine sample was diluted with water to 10 ml and the solution was processed further as described in Section 2.1. To determine the concentration of metformin in the sample, the standard addition method was used by adding 1, 1.5 and 2 μmol of metformin to 1 ml of the urine sample.

3. Results and discussion

3.1. Mechanism of the electrode process

Cyclic voltammogram of 1×10^{-5} mol l^{-1} metformin recorded at 100 mV s^{-1} in a MOPS buffer at pH 6.7 is shown in Fig. 2. The voltammogram consists of a single cathodic peak at rather negative potential of −1.55 V versus Ag/AgCl reference electrode. Since no anodic peak can be observed at a scan rate $v \leq 1$ V/s, the electrode process appears totally irreversible. Such type of an electrode process occurring at the negative end of the potential window at the HMDE is frequently related to the hydrogen evolution reaction [32–34], catalyzed by species adsorbed on the electrode surface. Hence, the electrode mechanism is expected to be particularly sensitive to the concentration and chemical nature of the proton donor compounds, pH of the medium, as well as the surface concentration of the catalyst, i.e., metformin.

We have recently studied in detail such electrode mechanism under conditions of SWV [30]. Hydrogen evolution reaction in the presence of an adsorbed catalyst can be represented by the following reaction scheme:

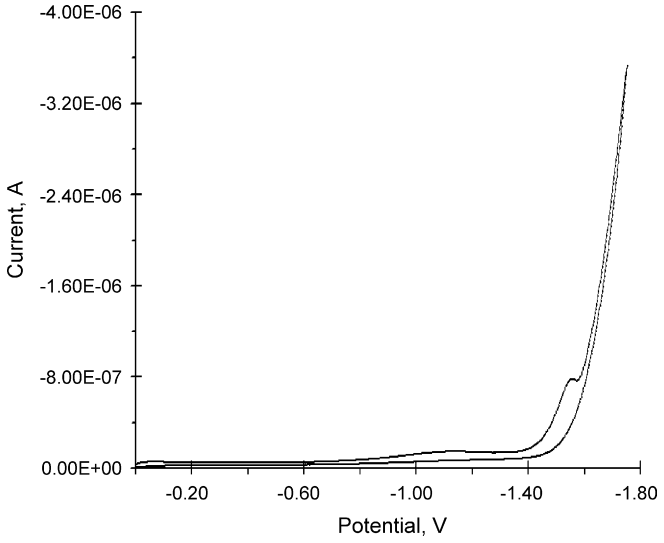

Fig. 2. Cyclic voltammogram of 5×10^{-5} mol l^{-1} metformin recorded in 0.01 mol l^{-1} MOPS buffer at pH 6.7 and scan rate 100 mV/s.

$$\mathrm{Cat_{(ads)} + H^{+}_{(aq)} \underset{k_d}{\overset{k'_p}{\longleftrightarrow}} CatH^{+}_{(ads)}} \quad \text{(I)}$$

$$\mathrm{CatH^{+}_{(ads)} + e^{-} \longrightarrow Cat_{(ads)} + H_{(aq)}} \quad \text{(II)}$$

Here k'_p and k_d are rate constants of the protonation and dissociation reactions, respectively. 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 $k_p = k'_p c(H^+)$. Note that in the present experimental system, the adsorbed metformin plays the role of a catalyst. The mechanism of hydrogen evolution reaction consists of the preceding chemical reaction (I) and the electrode reaction (II) that regenerates the initial electroinactive reactant $Cat_{(ads)}$.

By theoretical analysis of this mechanism, it has been found that the voltammetric response depends mainly on the equilibrium of reaction (I) established prior to the voltammetric experiment, the rate of protonation reaction (I), and the time window of the voltammetric experiment, represented by the frequency of the SW potential modulation. Consequently, pH of the medium is particularly important parameter since it affects the equilibrium of reaction (I) and the kinetics of the protonation reaction. Fig. 3 shows the effect of pH on the net SW peak current of metformin measured in Britton–Robinson buffers.

In the interval $2.6 \leq pH \leq 8.0$ the overall dependence of the net SW peak current, I_p, versus pH is non-linear, with a maximum located around pH 4.1. At pH lower than 3.5, the net peak current cannot be precisely measured due to significant overlapping with the uncatalyzed hydrogen evolution current. However, at pH > 4.1, 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 reactant $CatH^{+}_{(ads)}$ on the electrode surface. At the same time, an increase of pH from 3.4 to 7.7 causes a negative shift of the

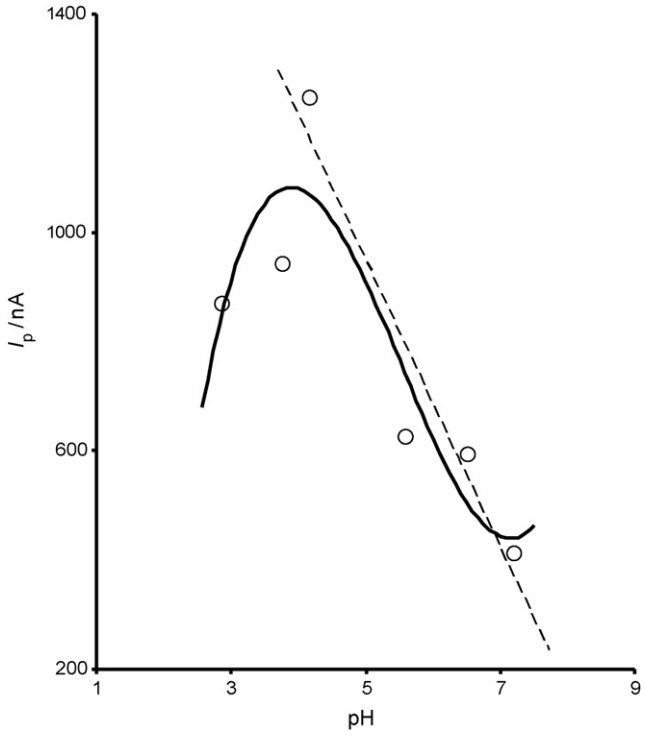

Fig. 3. Dependence of the net SW peak current of 1×10^{-5} mol l^{-1} metformin on pH of Britton–Robinson buffers (v_{buffer}:v_{water} = 1:9). The parameters of the potential modulation were: frequency f = 40 Hz, amplitude E_{sw} = 80 mV, and scan increment $\Delta E = -4$ mV.

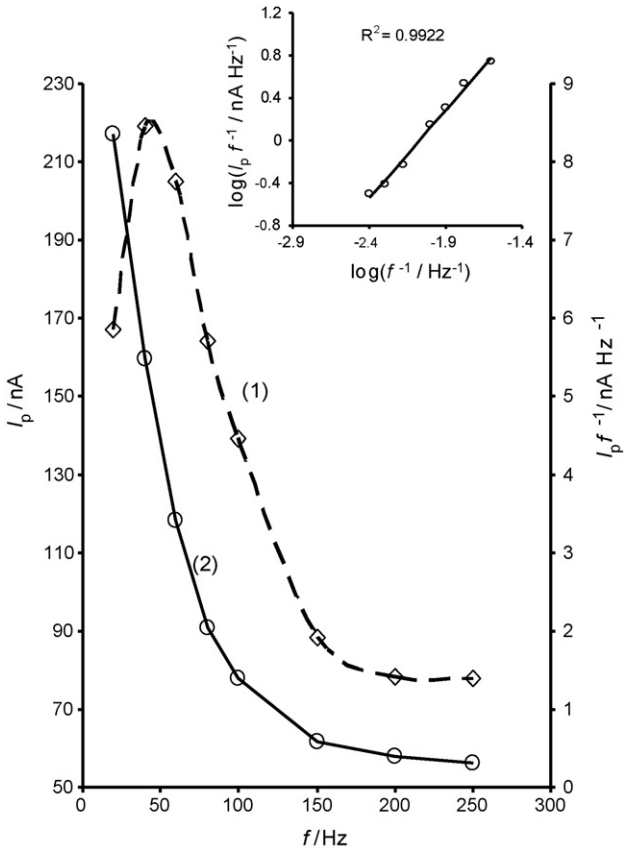

Fig. 4. The influence of the frequency (f) on the net peak current (I_p) (curve 1 left axis) and the ratio I_p/f (curve 2, right axis). The inset shows the dependence of log(I_p/f) on the log(1/f). The conditions of the experiments were: supporting electrolyte 0.01 mol l^{-1} MOPS buffer, c(metformin) = 5×10^{-5} mol l^{-1}, initial potential of the square-wave modulation $E_i = -0.8$ V, amplitude E_{sw} = 25 mV, $\Delta E = -4$ mV.

peak potential with a slope of about 20 mV per pH unit. Though a positive slope was predicted by the theoretical simulations, the experimental value of 20 mV differs from the theoretical value of 30 mV [30]. The analysis of the response by varying the frequency of the potential modulation provides further information on the nature of the electrode mechanism. In the previous theoretical analysis [30], it was found that the overall catalytic effect depends on the dimensionless catalytic parameter defined as $\kappa_{cat} = k_p/f$. The magnitude of the peak increases non-linearly with κ_{cat}. Therefore, an increase of the frequency is expected to cause a decrease in the net peak current. On the other hand, an increase of the frequency causes enhancement of the net peak current, which is a general property of most of the reversible and quasireversible electrode mechanisms in SWV [31]. Therefore, the overall dependence of the net peak current on the frequency is a parabolic function, as a compromise between the two opposite effects of the frequency. This dependence is illustrated by curve 1 in Fig. 4.

Plotting the ratio, I_p/f versus the f, a descending curve can be observed, which represents the influence of the catalytic parameter only (see curve 2 in Fig. 4). This type of curve is typical for all catalytic mechanisms [35,36]. By numerical simulations of the voltammetric response [30], it has been established that the most typical feature of the current catalytic mechanism is the linear dependence between log(I_p/f) versus log(κ_{cat}), which holds under a large variety of experimental conditions. It can be regarded as a diagnostic criterion for hydrogen catalytic evolution reaction in SWV. In the experimental analysis, studying a single electrode reaction attributed with particular protonation rate constant k_p, the latter type of dependence can be the obtained by plotting log(I_p/f) versus log(1/f). The inset of Fig. 4 depicts this dependence measured in the presence of metformin.

Besides the peak current, the position of the peak is highly sensitive to the frequency. In agreement with the theoretical prediction, the position of the peak shifts toward more negative potentials by increasing of the frequency. The dependence E_p versus log(f) is a line with a slope of -101 ± 3 mV. The theoretical value of this slope is $-2.303\ RT/2\alpha F$ [30]. Hence, the electron transfer coefficient of the electrode reaction studied is $\alpha = 0.57 \pm 0.01$.

All these results confirm that the voltammetric response obtained at the mercury electrode in the presence of metformin is a result of hydrogen catalytic evolution catalyzed by adsorbed metformin.

3.2. Analytical application and validation of the proposed method

For determination of metformin the influence of pH from 2.6 to 8.0 was examined. Among the studied electrolytes were MOPS, Britton–Robinson, acetate, and phosphate buffers. Considering the peak shape and sensitivity to the metformin con-

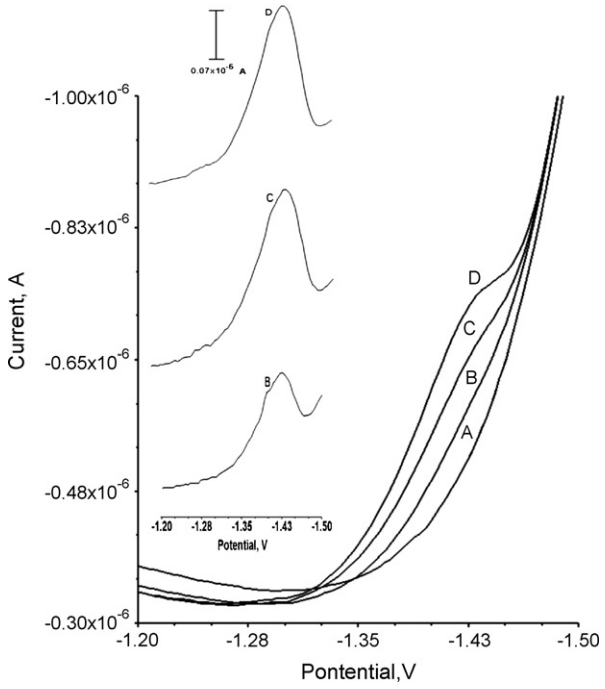

Fig. 5. Square-wave voltammograms of metformin recorded in 0.01 mol l^{-1} acetate buffer at pH 4.7. Concentration of metformin was: (A) blank, (B) 4×10^{-7} mol l^{-1}, (C) 8×10^{-7} mol l^{-1}, and (D) 10×10^{-7} mol l^{-1}. The inset shows metformin voltammograms after subtraction of the blank current. The other experimental conditions conditions were: $E_i = 0$ V, $f = 40$ Hz, $E_{sw} = 80$ mV, $\Delta E = -4$ mV.

centration, the optimal response for quantitative determination of metformin was obtained in acetate buffer at pH 4.7.

The applicability of the SWV as an analytical method for the determination of metformin was tested over metformin concentration range from 5×10^{-8} to 4×10^{-6} mol l^{-1} and accumulation time of 15 s (Fig. 5). At concentration higher than 4×10^{-6} mol l^{-1} the electrode is saturated by adsorbed metformin, giving rise to deviations in the linearity of the calibration line. Accordingly, the maximal concentration limit can be extended toward higher concentration by decreasing the accumulation time. Besides SWV, the determination of metformin was performed with linear sweep and differential pulse voltammetry, too. The analytical characteristics of the method carried out with three different voltammetric techniques are summarized in Table 1.

Table 2
Recovery and precision obtained by SWV in supporting electrolyte and spiked urine*

Added [$\times 10^6$ mol l^{-1}]	Found $\bar{x} \pm t_{0.95}\bar{s}$ [$\times 10^{-6}$ mol l^{-1}]	Precision R.S.D.	Recovery%[a]
0.1	0.110 ± 0.004	0.039	110
0.8	0.836 ± 0.001	0.002	105
2	1.950 ± 0.007	0.003	97.5
0.4*	0.378 ± 0.001	0.036	94.5

* Concentration of metformin spiked to urine sample.
[a] Recovery = 100% + [(found − added)/added] × 100%.

Validation of the voltammetric procedure was performed by determining the limit of detection (LOD), limit of quantification (LOQ), repeatability, recovery and precision of the method. The limits of detection and quantification were calculated according to Long and Winefordner method [37].

According to parameters listed in Table 1, 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 to differential pulse or linear sweep voltammetry. The repeatability (1 day) of the SW voltammetric procedure was assessed on the basis of six measurements at a single metformin concentration. In the concentration range of 0.1×10^{-6} to 1×10^{-6} mol l^{-1} the R.S.D. of the net SW peak current changed from 5.3 to 0.5%, respectively.

Precision and recovery of the method were investigated by determination of metformin at three different concentrations in the linear range. Results are presented in Table 2.

3.3. Analysis of urine

The recovery results of metformin in spiked urine are given in Table 2. The optimized voltammetric procedure was successfully applied for determination of metformin in patient's urine. Besides the urine dilution, no extraction steps have been undertaken prior to the voltammetric measurements. Fig. 6 illustrates the response of urine sample together with successive standard additions of metformin ($6 \times 10^{-7}, 8 \times 10^{-7}, 10 \times 10^{-7}$ mol l^{-1}) into diluted patient's urine. Sometimes voltammetric techniques could pose difficulties in the analysis of biological fluids containing reducible substances. As can be seen from Fig. 6, no reduction of other compounds occurs within the potential region of metformin response.

Table 1
Quantitative determination of metformin in 0.01 mol l^{-1} acetate buffer at pH 4.7 by linear sweep voltammetry (LSV), differential pulse voltammetry (DPV) and square-wave voltammetry (SWV)

	SWV	DPV	LSV
Linear concentration range (mol l^{-1})	1×10^{-7} to 2×10^{-6}	1×10^{-7} to 2×10^{-6}	2×10^{-7} to 2×10^{-6}
Slope of calibration curve (nA M^{-1})	2.07×10^8	1.06×10^8	5.58×10^7
R.S.D.% of slope	0.53	0.35	2.04
Intercept (nA)	4.09	7.36	23.00
R.S.D.% of intercept	4.98	6.03	4.34
Correlation coefficient	0.997	0.999	0.998
Number of measurements	6	6	6
LOD (mol l^{-1})	1.8×10^{-8}	3.2×10^{-8}	7.7×10^{-8}
LOQ (mol l^{-1})	5.9×10^{-8}	1.0×10^{-7}	2.5×10^{-7}

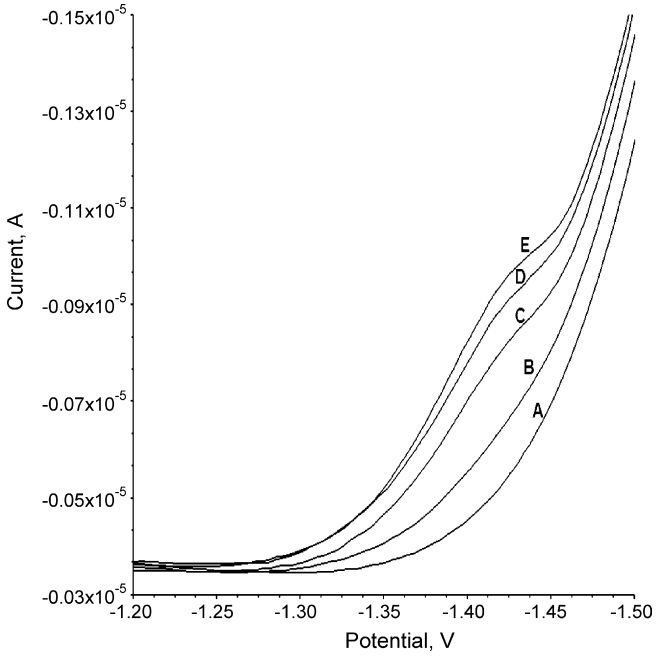

Fig. 6. Square-wave voltammograms obtained in the presence of metformin recorded in a diluted patient's urine samples: (A) blank, (B) sample of patient's urine, (C) as (B) + 6×10^{-7} mol l^{-1}, (D) as (B) + 8×10^{-7} mol l^{-}, and (E) as (B) + 1×10^{-6} mol l^{-1} of metformin. The supporting electrolyte was a 0.01 mol l^{-1} acetate buffer at pH 4.75. The other experimental conditions were: $E_i = 0$ V, $f = 40$ Hz, $E_{sw} = 80$ mV, $\Delta E = -4$ mV.

The variation of the net SW peak current versus metformin concentration (nM) in studied patient's urine is represented by the following linear equation: $I(\text{nA}) = 1 \times 10^8\ c(\text{mol l}^{-1}) + 44$ (R.S.D. of the intercept is 0.08, $n = 5$).

The HPLC method [11] was chosen as the analytical reference method in order to control the results obtained with SWV. Applying the chromatographic method, the retention of metformin was studied on various columns, with a mobile phase containing phosphate buffer at pH 6.0 and acetonitrile. The best results were found by using an Econosil C18-column with a mobile phase containing phosphate buffer, acetonitrile and water (12:60:28; v/v/v) at a flow-rate of 1.2 ml/min. Under these conditions, the peaks were well-shaped, the analysis time was short, and the resolution of metformin and urine peaks was satisfactory.

A urine sample (20 μl) containing known amount of metformin was treated as described in Section 2.6. No interferences were observed with the peak of metformin.

The relationship between detector response and metformin concentration was reproducible. The calibration lines over the concentration range from 4×10^{-5} to 4×10^{-3} mol of metformin in 1 l of urine (for 20 μl loop) are attributed with R.S.D. varying from 2.7% for 4×10^{-5} to 0.5% for to 4×10^{-3} mol of metformin in 1 l of urine. The equation for the linear regression line and coefficient of correlation for the peak area ratios was $y(\text{mAU s}) = 1.498 \times 10^6 c(\text{mol of metformin in 1 l of urine}) - 12.94$, $R^2 = 0.9997$ for urine sample.

The detection limit, defined as a minimal concentration that generates a signal-to-noise ratio of 3, was estimated by analyzing solutions of decreasing concentration of metformin. Before the analysis of each sample, water was processed through the chromatographic system in a corresponding procedure as applied for the samples. It was confirmed that there were no contaminants. The value of LOD was 2×10^{-5} mol l^{-1} of metformin in 1 l of urine. The limit of quantification was defined as the amount of the analyte required to generate a six times higher signal than the standard deviation of the background. Thus, the limit of quantification was 4×10^{-5} mol l^{-1}. After validation procedure, the urine samples were studied. At the same time, the same samples of patient's urine were analyzed with SW voltammetric method and the following results were obtained: 1.87×10^{-3} mol l^{-1} (R.S.D. 8.4%) for SWV and 1.82×10^{-3} mol l^{-1} (R.S.D. 3.8%) for HPLC. The results obtained with HPLC and SWV are in good agreement from which we can deduce that the biological matrix does not give rise to significant interferences to the voltammetric response of metformin. Moreover, the lower value of the slope of the regression line found by standard addition implies that urine does not contain significant amount of interfering species which could catalyse hydrogen reduction current.

4. Conclusion

A new square-wave voltammetric method for metformin determination based on hydrogen evolution reaction catalyzed by adsorbed metformin at a hanging mercury drop electrode was developed. The method was applied for the drug determination in urine samples. The most important advantage of the present method arises from the fact that common reducible substances present in the urine sample exhibit electrochemical activity at much more positive potentials than the response due to hydrogen evolution reaction. Hence, no significant interferences have been observed when the voltammetric method was applied to diluted urine sample. Nevertheless, it has to be emphasized that most of the voltammetric methods based on adsorptive accumulation applied to biological samples suffer from the competitive adsorption of various species present in the sample. For instance, significant interferences have been observed in the previous study concerned with determination of histamine H_2-receptor antagonist famotidine [34]. The interferences were primarily related to the competitive adsorption of proteins, as well as inactivation of the catalytic effect of famotidine due to chemical interactions with proteins and traces of heavy metal ions. Contrary to the complex molecule of famotidine, metformin is a much simpler molecule; its chemically active groups, i.e., the two imine groups (see Fig. 1), are engaged in strong interactions with the mercury surface, thus preventing additional significant interactions with other species present in the sample. Moreover, the small molecular dimension of metformin enables formation of a compact film of adsorbed molecules on the electrode surface, which persists toward the competitive adsorption of matrix molecules. The good agreement between the results obtained with the electrochemical and chromatographic method supports these conclusions. Of course, the significant dilution of the sample before the measurements can also play a significant role in diminishing the potential interferences. The proposed voltammetric procedure is fast, simple, and more cost effective than chromatographic methods. The measuring time is less

than 5 min, requiring only dilution of the sample prior to the voltammetric scan.

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