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Adsorptive stripping voltammetric determination of cefetamet in human urine

Mara Aleksić *, Ljiljana Milovanović, Vera Kapetanović

Faculty of Pharmacy, Institute of Physical and Analytical Chemistry, University of Belgrade, P.O. Box 146, Vojvode Stepe 450, 11000 Belgrade, Yugoslavia

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Abstract

On the basis of previously established mechanism of cefetamet (CEF) reduction, two methods were suggested for CEF determination—differential pulse polarographic and differential pulse adsorptive stripping voltammetric method. Two pH values were chosen, 2.0 and 8.4, where the electrochemical process was defined as one four-electron and two two-electron processes, respectively. The methods were performed in Britton–Robinson (BR) buffer and the corresponding calibration graphs were constructed and statistical parameters were evaluated. Applying the AdSV method at pH 2.0 linearity was achieved from 2×10^{-8} to 2×10^{-7} M with limit detection and limit determination of 4×10^{-9} and 1.4×10^{-8} M, respectively. At pH 8.4, the linearity was obtained between 6×10^{-8} and 6×10^{-7} M, with limit detection and limit determination of 1.5×10^{-8} and 5×10^{-8} M, respectively. Since the AdSV method enabled lower concentrations of CEF to be determined, this method was tested for CEF determination in spiked urine samples, and DPP method was used as a comparative one.
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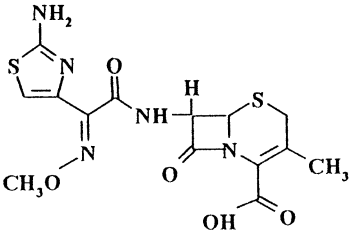
Keywords: Cefetamet; Adsorptive stripping voltammetry; Urine determination

1. Introduction

Cefetamet (CEF), is a cephalosporin, an antibiotic containing a six-membered dihydrothiazine ring, to which a β-lactam ring is fused. Like other cephalosporins bearing an alkoxyimino grouping on C-7 in the side chain, it yields a polarographic reduction wave [1].



Cefetamet

To achieve a proper and most efficient use of electroanalytical methods it is necessary, perhaps

* Corresponding author. Tel.: +381-11-39-70379; fax: +381-11-39-74349.
E-mail address: m.alexic@eunet.yu (M. Aleksić).

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even more importantly than for other analytical methods, to understand at least the principles of the nature of the processes involved. In our previous papers the mechanism of the CEF reduction was studied [2,3]. On the basis of that previously established mechanism, the new analytical method is proposed as its application to a real system.

In more acidic solutions, i.e. pH < 5, the reduction of the protonated form of CEF occurs, like that of the other oximes [4–7], in a simple four-electron step to produce an amine. The reduction of the protonated form of CEF at pH 5–10.5 occurs in two steps. At pH 5–6 these two steps are poorly separated, and their presence is manifested by a decrease of the slope of the polarographic i–E curves in this pH range. In the two step reduction pathway the N–OCH_3 group is being protonated (Eq. (1)), than N–O bond is cleaved (Eq. (2)), yielding an imine which is subsequently protonated (Eq. (3)) and reduced to an amine (Eq. (4)), as follows:

$$R_2C{=}NOCH_3 + H^+ \Leftrightarrow R_2C{=}NH^+OCH_3 \quad (1)$$

$$R_2C{=}NH^+OCH_3 + 2e + H^+ \Rightarrow R_2C{=}NH + CH_3OH \quad (2)$$

$$R_2C{=}NH + H^+ \Leftrightarrow R_2C{=}NH_2^+ \quad (3)$$

$$R_2C{=}NH_2^+ + 2e + 2H^+ \Rightarrow R_2CHNH_2 \quad (4)$$

Occurrence of a single four-electron wave is caused by the vicinity of the reduction potentials of oxime and intermediate imine. On contrary, splitting is occurring when the reduction of oxime happens at more positive potentials related to reduction potential of imine.

The study of mechanism reduction of CEF is mostly complicated by effects of adsorption at mercury surface, already observed for cephalosporins [8–14]. The adsorption of CEF at mercury, Pt and Au electrodes was also studied in our previous paper [15].

Knowing the mechanism of reduction and adsorption properties of CEF our idea was to use these findings to achieve an increase in sensitivity and possibility of CEF determination by applying the adsorptive stripping method. Adsorptive preconcentration followed by differential-pulse cathodic stripping showed one wave at ~ -0.5 V being the most sensitive for analytical determination of CEF at pH 2.0 and 8.4. Methods developed enable the CEF determination in human urine sample. As a comparative method a differential pulse polarographic method was used.

No previous application of adsorptive stripping voltammetry (AdSV) for the determination of CEF has been reported. Nevertheless, only two methods for CEF determination are mentioned in literature, HPLC [16] and DPP method [17]. The advantages of the proposed method related to the HPLC method are: no prior separation is needed in electrochemical methods (the selectivity of the method), the low cost of the method, and simplicity as well as the short time procedure consuming. As a consequence, the potential sources of the possible experimental errors in AdSV method are minimized.

2. Experimental

2.1. Apparatus and reagents

Polarographic analyzer PAR 174A connected with three-electrode cell (DME, SCE and Pt) was used. Dropping times of 2 s, scan rate 2 mV/s, and the mercury column height of 80 cm were used for direct current (dc) polarographic measurements.

Voltammetric and polarographic measurements were performed at AMEL 433-A computerized polarographic analyzer. Three electrode system was employed: static mercury (AdSV) and dropping mercury electrode (dc and DPP) as working ones, and Ag/AgCl and Pt-wire as reference and auxiliary electrodes.

A Radiometer pH meter, PHM 220, with appropriate standard buffer solutions was used.

CEF sodium was donated by Hoffmann LaRoche (Basel, Switzerland). Boric, orthophosphoric, acetic acids and sodium-hydroxide were p.a. (Merck). All other reagents and chemicals used in this study were of analytical grade.

The Britton–Robinson (BR) universal buffer was prepared from stock buffer solutions of 0.04 M boric, orthophosphoric and acetic acids with the appropriate volumes of 0.2 M NaOH.

Double distilled water was used throughout.

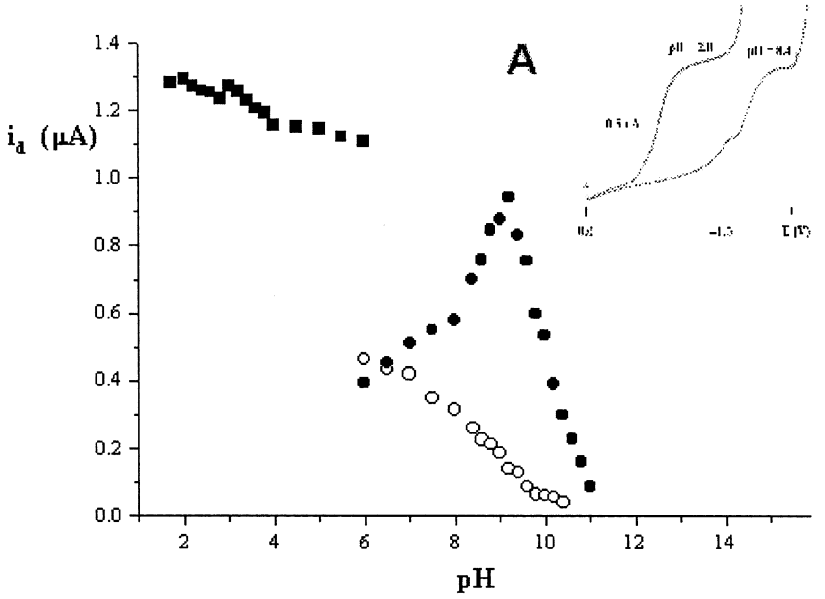

Fig. 1. Dependence of wave currents of 2×10^{-4} M CEF on pH (BR buffer): ■, one four-electron wave; ○, first two-electron wave; ●, second two-electron wave. A attached, dc curves of 2×10^{-4} M CEF at pH 2.0 and 8.4.

All experiments were done at room temperature.

2.2. Polarographic investigation

2.2.1. Standard solutions

A stock solution (S_1) of CEF of 1×10^{-3} M was prepared by dissolving CEF standard in bidistilled water.

A stock solution (S_2) was prepared by dissolving 25 mg of CEF standard in 100 ml volumetric flask.

More dilute solutions were prepared daily from stock solutions S_1 and S_2.

2.2.2. Procedure for dc polarographic investigation

In electrochemical cell 12 ml of BR buffer was transferred, deaerated for 10 min with nitrogen. Prior the entering the voltammetric cell nitrogen was passed, successively, through a solution of chromium (II) ions in dilute hydrochloric acid containing heavily amalgamated zinc granules, distilled water and molecular sieves.

Then, 3.0 ml of CEF stock solution was added to make its final concentration of 2×10^{-4} M. The solution was purged with nitrogen for another 3 min and the current–voltage curves were recorded.

2.3. Cyclic voltammetric investigation

2.3.1. Standard solutions

The same stock solution of CEF (S_1) was used as in polarography.

2.3.2. Procedure for voltammetric investigation

In voltammetric cell 9.5 ml of BR was transferred, purged with nitrogen for 10 min, than 0.5 ml of CEF stock solution (S_1) was added making the final concentration of CEF 5×10^{-5} M, then 2 more min was purged with nitrogen and voltammograms were recorded.

2.4. Adsorptive stripping investigation

2.4.1. Procedure for adsorptive stripping voltammetry

The procedure is the same as given for voltammetric investigation (Section 2.3.2).

2.4.2. Procedure of making calibration graphs

A 10 ml aliquot of the corresponding supporting electrolyte solution five and ten times diluted BR of pH 8.4 and 2.0, respectively, was placed in a

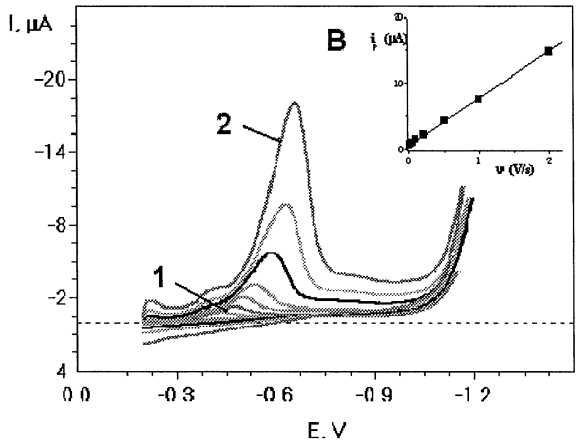

Fig. 2. The representative CV curves of 5×10^{-5} M CEF obtained at different scan rates, at pH 2.0; scan rates (v) ranged from 5 (1) to 2000 mV/s (2). B attached, the dependence of i_p vs. v.

voltammetric cell and deaerated for 10 min with high purity nitrogen. The required amount (20 μl portions) of diluted stock solution of CEF (5×10^{-5} and 5×10^{-6} M at pH 8.4 and 2.0, respectively), was added after recording the baseline. When a fresh mercury drop had formed, voltammograms were recorded after a certain time of adsorptive accumulation at a selected potential in a stirred solution.

Calibration graphs were constructed using data from these measurements and evaluated by the least-squares linear regression method. Measurements were performed at room temperature.

2.5. Procedure of investigations in urine sample

A stock solution S_2 and diluted urine solution (1:10) were used. The urine samples were taken from the healthy children (five volunteers were included).

2.5.1. Procedure of making calibration graphs—AdSV

A 9.0 ml aliquot of the corresponding supporting electrolyte solution and 1.0 ml of urine solution was placed in a voltammetric cell and deaerated for 10 min with high purity nitrogen. The required amount (20 μl portions) of stock solution of CEF (S_2), or diluted stock solution of CEF was added after recording the baseline. When a fresh mercury drop had formed, voltammograms were recorded after a certain time of adsorptive accumulation at a selected potential in a stirred solution.

Calibration graphs were constructed using data from these measurements and evaluated by the least-squares linear regression method. Measurements were performed at room temperature.

2.5.2. Procedure of making calibration graphs—DPP

A 9.0 ml aliquot of the corresponding supporting electrolyte solution and 1.0 ml of urine solution was placed in a voltammetric cell and deaerated for 10 min with high purity nitrogen. The required amount (60 μl portions) of stock solution of CEF (S_2), was added and the polarograms were recorded after recording the baseline.

Calibration graphs were constructed using data from these measurements and evaluated by the least-squares linear regression method. Measurements were performed at room temperature.

2.6. Determination of CEF in urine

2.6.1. Procedure of CEF determination in urine

A 9.0 ml aliquot of the corresponding supporting electrolyte solution and 1.0 ml of urine solution was placed in a voltammetric cell and deaerated for 10 min with high purity nitrogen. The baseline was scanned prior the solution was spiked with required amounts of stock solution of CEF (S_2). When a fresh mercury drop had formed, voltammograms were recorded after a certain time of adsorptive accumulation at a selected potential in a stirred solution. Determination of CEF in spiked urine was done for each concentration determination repeated five times.

3. Results and discussion

To develop a reliable electrochemical method for the determination of CEF, the variations of polarographic current–voltage curves, corresponding to the reduction of the methoxyimino

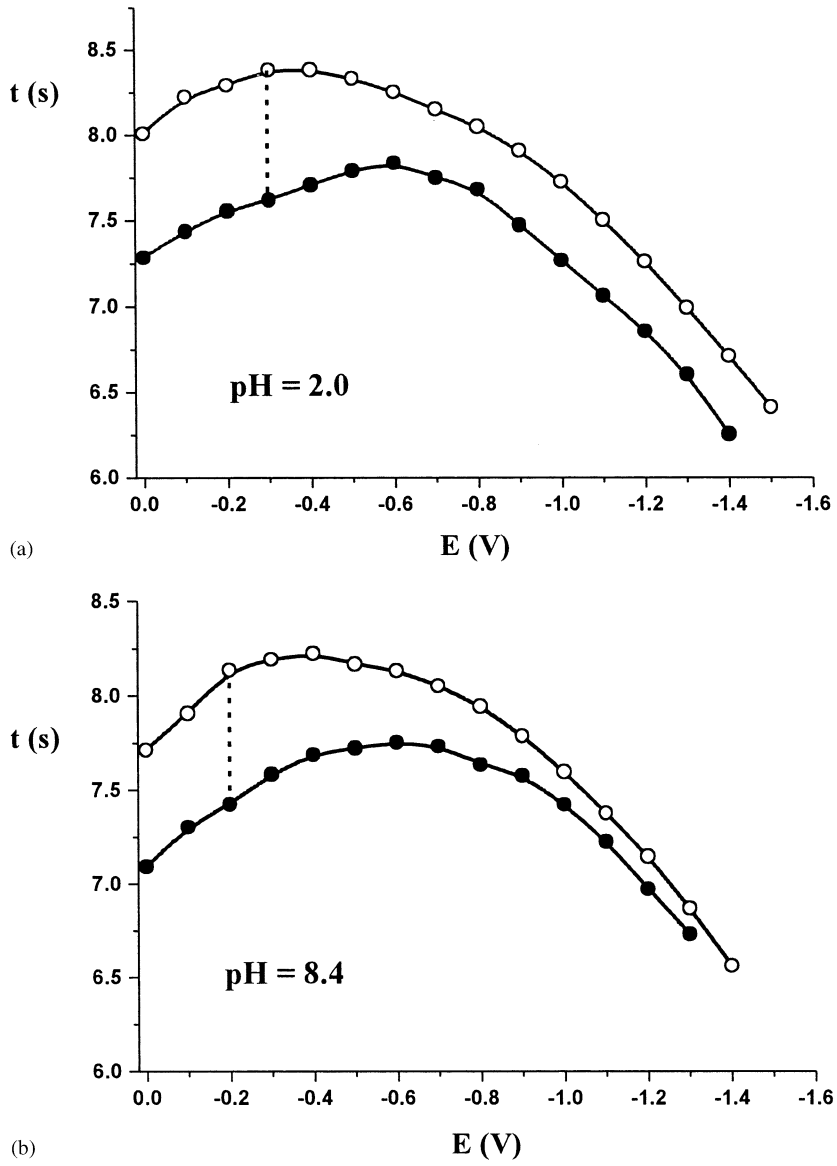

Fig. 3. Electrocapillary curves at: (a) pH 2.0: ○, BR buffer; ●, BR buffer in the presence of 1×10^{-4} M CEF. (b) pH 8.4: ○, BR buffer; ●, BR buffer in the presence of 1×10^{-4} M CEF.

group, were studied as a function of pH. Fig. 1, represents dependence of wave currents of 2×10^{-4} M CEF versus pH in BR buffer ranged from 1.7 to 11.0. As seen from Fig. 1, only one wave due to the one step reduction is present in acidic medium, while in neutral and alkaline medium it splits into two two-electron waves (Fig. 1A attached).

Two pH values were chosen for further investigation, the first one, 2.0 in acid medium where only one wave exists (overall reduction process), and 8.4 in an alkaline medium, where two step reduction process occurs. At pH chosen well formed and separated waves were obtained.

The stability of CEF solutions (5×10^{-4} M in BR buffer at pH 2 and 8.4) was checked using

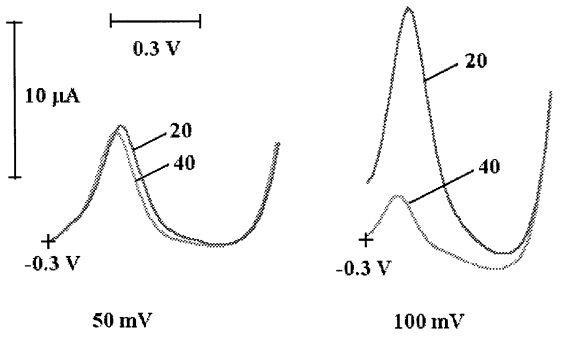

Fig. 4. The effect of the pulse amplitude and pulse width on the AdS voltammogram of CEF.

spectrophotometric method. No change in absorbance was found within 3 days.

At those pH values the nature of electrode process was studied by using cyclic voltammetry. At Fig. 2 the representative CV curves are given at different scan rates (ν varies from 5 to 2000 mV/s).

The effect of scan rate on the peak current is also presented (Fig. 2B attached). The linear dependence of $i_p = f(\nu)$ shows the adsorption controlled process.

The surface active behavior of the investigated substance, and consequently, the possibility of applying the adsorptive stripping voltammetric technique were investigated by measuring electrocapillary curves [18]. Fig. 3, shows electrocapillary curves of CEF and those for supporting electrolyte at pH values of analytical importance. The mercury dropping time at a determined potential was measured as the interval required for 50 mercury drops to spontaneously form at the outlet of the capillary, 0.04 mm diameter, and a mercury reservoir height of 80 cm. The CEF concentration was 1×10^{-4} M. Analysis of electrocapillary curves showed the maximum decrease of electrocapillary maximum at -0.3 and -0.2 V for pH 2.0 and 8.4, respectively. These findings suggested these potentials as the best for analytical purposes.

In order to establish the optimum conditions for the AdSV determination of CEF, the effect of the following parameters were investigated: the modulation pulse amplitude, pulse width, the mercury drop size, the scan rate, the effect of accumulation time, and stirring of the investigated solution.

3.1. The effect of the pulse amplitude and pulse width

Pulse amplitude was varied from 50 to 200 mV, and its increase causes the increase of the peak height. At each value of pulse amplitude, the effect of the pulse width of 20 and 40 ms, was investigated. The observed effects are presented in Fig. 4. For low values of pulse amplitude, no significant influence of the pulse width was noticed. On the contrary, for higher values of pulse amplitude a great influence of pulse width is obvious. According to these findings, the pulse amplitude of 100 mV, and pulse width of 20 ms were chosen for further investigation.

3.2. The effect of the mercury drop size

The optimum mercury drop size was find to be 60 a.u., since the peak obtained under this condition is well formed, and no significant increase of its height was observed with further increasing of the drop size.

3.3. The effect of the scan rate

The effect of the scan rate on the analytical signal was studied in a 5×10^{-5} M solution of CEF. Adsorption-stripping cycles (with 60 s accumulation time) carried out for increasing values of the scan rate, ν, gave rise to peaks with intensities that showed a linear increase with the scan rate between 5 and 300 mV/s. Scan rate of 200 mV/s was chosen.

3.4. The effect of accumulation time

The effect of accumulation time on the peak height was investigated at two pH values both with and without stirring (Fig. 5a and b). Generally, the peak height increases with accumulation time, but the observed non-linear dependences suggest the saturation of the electrode surface. In both cases peak current was higher when the accumulation was performed with stirring. For this reason all further investigations were done with stirring only. On this basis, a 60 s (pH 2.0), and 120 s (pH 8.4) deposition time were adopted.

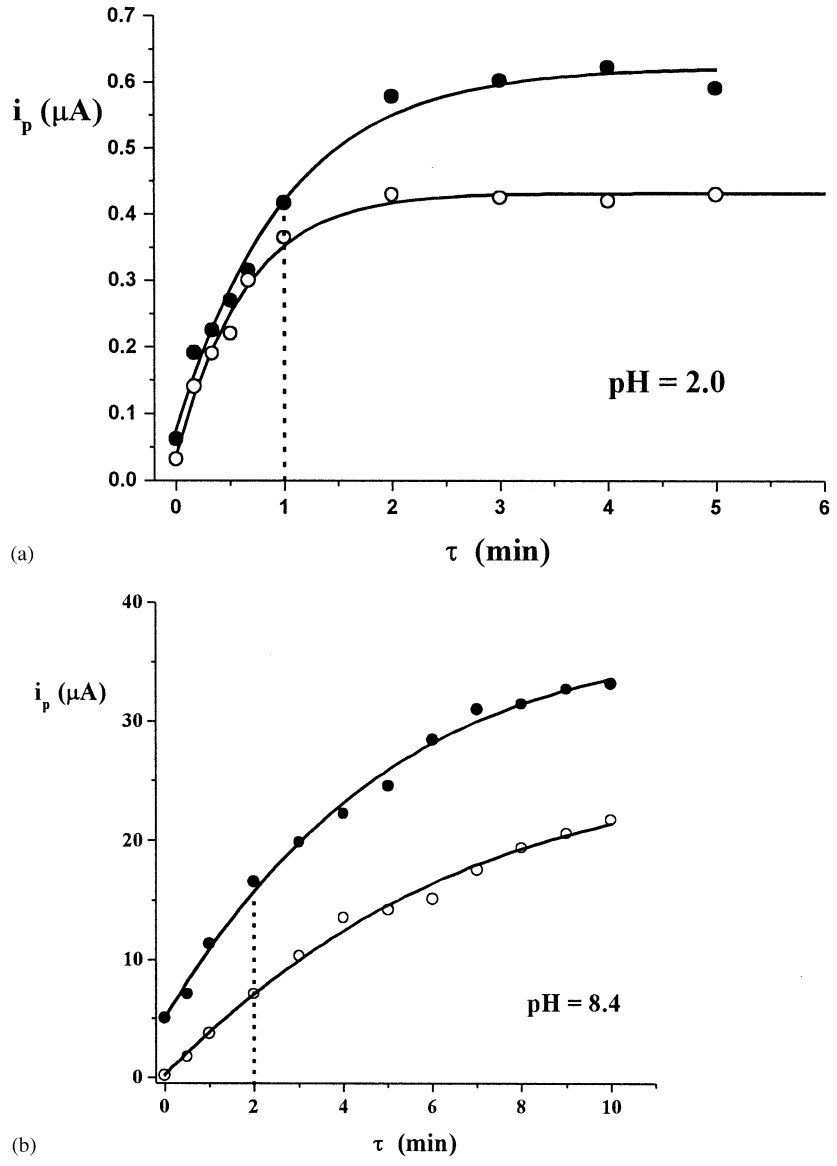

Fig. 5. The effect of the accumulation time on the peak current of the AdSV of CEF at: (a) pH 2.0: ●, 5×10^{-7} M CEF with stirring; ○, 5×10^{-7} M CEF without stirring. (b) pH 8.4: ●, 1×10^{-6} M CEF with stirring; ○, 1×10^{-6} M CEF without stirring.

Under the defined experimental conditions the linear dependence of i_p versus CEF concentration was achieved from 2×10^{-8} to 2×10^{-7} M at pH 2.0, and from 6×10^{-8} to 6×10^{-7} M at pH 8.4, by using ten time diluted supporting electrolyte. Concentration dependence was followed at $E_p \sim -0.5$ V at both pH values and obtained results are presented in Table 1.

The AdSV method established in BR buffer solution was applied for CEF determination in humane urine samples. For this purpose, urine samples were spiked with CEF, and the corresponding voltammograms were scanned. The baseline was scanned with BR buffer containing urine sample. The results of investigations in urine samples and the optimal conditions established for

Table 1
Statistical parameters for AdSV determination of CEF in BR buffer

pH	Concentration range (M)	Regression equation y (A): x (M)	S[a]	S[b]	S.D.	r	Detection limit (M)	Determination limit (M)
2.0	2×10^{-8}–2×10^{-7}	$y = 2.6 \times 10^{-9} + 0.44x$	7.74×10^{-10}	0.026	6.2×10^{-10}	0.9988	4×10^{-9}	1.4×10^{-8}
8.4	6×10^{-8}–6×10^{-7}	$y = -1.1 \times 10^{-8} + 0.42x$	1.91×10^{-9}	0.0083	2.1×10^{-9}	0.9982	1.5×10^{-8}	5×10^{-8}

[a] Standard deviation of the intercept.
[b] Standard deviation of the slope.

Table 2
Statistical parameters for AdSV and DPP determination of CEF in human urine

Method	pH	Concentration range (μg/ml)	Regression equation $y = i_p$ (μA), x = c (μg/ml)	S[a]	S[b]	r	Detection limit (μg/ml)	Determination limit (μg/ml)
AdSV	2.0	0.08–0.80	$y = 0.0143 + 4.5308x$	0.009	0.036	0.9996	0.023	0.076
		2.0–10.0	$y = -0.1251 + 1.6093x$	0.085	0.028	0.9982	0.46	1.62
	8.4	0.8–5.0	$y = -0.0385 + 3.8054x$	0.019	0.229	0.9930	0.26	0.67
DPP	2.0	5.0–50.0	$y = -4.3583 + 2.1655x$	0.486	0.018	0.9995	1.18	3.94
	8.4	4.5–45.0	$y = -1.0436 + 0.5642x$	0.156	0.007	0.9996	2.20	4.12

[a] Standard deviation of the intercept.
[b] Standard deviation of the slope.

Table 3
Results obtained for CEF determination in urine samples

Method	pH	Spiked concentration (μg/ml)	Found concentration (μg/ml)	R.S.D. (%) $n = 5$	Recovery (%)
ADSV	2.0	0.500	0.488	5.30	97.6
		4.000	4.046	2.97	101.2
	8.4	1.500	1.436	6.65	95.7
		4.000	3.902	5.16	97.5
DPP	2.0	10.00	10.07	1.09	100.7
		30.00	29.72	1.53	99.1
	8.4	10.00	9.88	2.19	98.8
		30.00	29.52	1.91	98.4

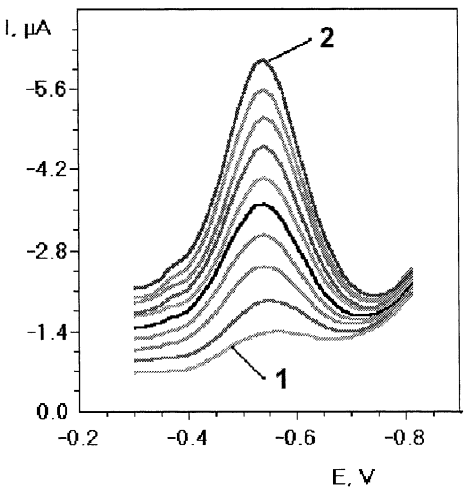

Fig. 6. AdSV curves of CEF in human urine at pH 2.0 obtained in the concentration range from 0.08 (1) to 0.80 μg/ml (2).

CEF determination (concentration range, linearity of the calibration curves including the relative standard deviation (R.S.D.) values for the slope and intercept, detection limit and determination limit) are summarized in Table 2.

During the actual analysis the analytical response was checked through the peak potential and its height. No change in peak potential was observed within an hour while its height changed about $\pm 5\%$.

Repetition of analysis of three urine samples spiked with different CEF concentration (5.0, 10.0 and 20.0 μg/ml) at pH 2.0 and 8.4 allowed an estimation of the relative errors and the R.S.D. which did not exceed 3.2 and 4.5% ($n = 5$), respectively.

On the basis of the experimental conditions defined and presented in Table 2, determination of CEF in urine sample was done by applying AdSV and DPP methods, simultaneously (Table 3). The representative AdSV curves obtained in urine sample concentration ranging from 0.08 to 0.8 μg/ml, are shown in Fig. 6.

The precision of the AdSV method was checked at pH 2 for CEF concentration of 0.35 and 5.5 μg/ml, and at pH 8.4 for CEF concentration of 3.5 μg/ml. Five determinations were performed in all cases, and the R.S.D. were 2.1, 1.6% at pH 2 and 3.2% at pH 8.4. The precision of the DPP method was investigated at pH 2 and 8.4 for CEF concentration of 30 μg/ml with R.S.D. of 0.95 and 1.05%, respectively.

Slightly higher noise of the buffer supporting electrolyte was obtained when urine was spiked in buffer solution. The suppression of the noise was obtained when "full" buffer solution was applied (no dilutions have been made). As a result, a lower sensitivity of the method was achieved under these conditions.

Specificity of the proposed analytical method was checked as its ability to measure specifically only the analyte, not the components that maybe expected in the urine matrix as well. In accordance with this, the blank urine sample was scanned before and after the addition of CEF. Since no analytical response was obtained in the pure urine sample at the working potential (regarded to CEF), it might be concluded that there was no interference of the components of the urine matrix. This means that the method proposed shows the selectivity at the same time.

Since the analytical response was almost the same in the pH range from 2 to 3 and temperature range from 20 to 25 °C, it can be concluded that the robustness of the proposed method, regarded to those factors is quite satisfactory.

4. Conclusion

Both methods applied gave an excellent analytical response. The AdSV method enables the lower concentrations of CEF to be determined. Since the determination was performed at pH 2.0 and 8.4, respectively, the results obtained indicated the acid medium as better one for CEF determination in urine sample. Comparison of the method suggested and the official HPLC method, shows the advantages of the prior one due to its simplicity, low cost, short time consuming, selectivity and its specificity.

Acknowledgements

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