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Short communication

Adsorptive properties of cefpodoxime proxetil as a tool for a new method of its determination in urine

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Abstract

It was found that the reduction of the cefpodoxime proxetil (CP) molecule is strongly influenced by the adsorption. The adsorptive properties of CP were investigated in order to achieve an increase sensitivity of its determination. Validated adsorptive stripping differential pulse voltammetry is applied for the determination of low concentration of CP at pH 3.5 and 9.0 where the best pronounced adsorption effects were observed. The linearity of the calibration curves were achieved from 1×10^{-8} to 1×10^{-7} M with limit of detection (LOD) of 6.3×10^{-9} and 7.1×10^{-9} M, and limit of quantification (LOQ) of 2.1×10^{-8} and 2.3×10^{-8} M, at pH 3.5 and 9.0, respectively. The proposed method was tested for CP determination in spiked urine samples, enabling determination of low concentrations of CP.

Keywords: Cefpodoxime proxetil; Adsorptive stripping voltammetry; Urine determination

1. Introduction

Cefpodoxime proxetil, cephalosporin with methoxyimino group, belongs to the semi-synthetic third generation cephalosporins, being the drug of choice in Gram-negative bacillary infections. The electrochemical behaviour of large number of cephalosporins with methoxyimino group was reported by other authors [1–4]. The voltammetric behavior of CP was studied by Reddy et al. [5] in the pH range 2.0–12.0 using DPV and CV. They found that CP gave two peaks which are attributed to the reduction of azomethine group by two electron process in two steps. They reported that the reduction process is irreversible, diffusion controlled and adsorption free. The same authors [5] developed differential pulse voltammetric method for determination of this drug in pharmaceutical formulations and urine samples in acid medium (pH 2.0). In the basic buffers of pH >8, they claimed that the voltammetric curves are very poor due to the non-availability of protons and are not useful for analytical purpose.

In spite the results published by Reddy et al. [5], our investigations of the reduction of cephalosporins with methoxyimino group show some differences [6–10]. Reduction of the methoxyimino group is not adsorption free as stated by the authors [5], but can be preferably described as strongly influenced by the adsorption of the CP on the mercury surface. Since the adsorption effect of CP was proved both in acid and alkaline medium, analytical application of proposed AdSDPV method in pH >8 became possible. The other thing is that the methoxyimino group is not the only one electroactive site in CP molecule. The unsaturated C=C bond is also reduced in two-electron process [6,11].

Since no literature data have been found on adsorption of CP on the surface of the mercury electrode, the goal of this paper is to use the adsorption properties of CP as a tool for developing the AdSDPV method for determination of low concentrations of CP. The proposed method has been successfully applied for determination of this drug in urine sample.

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2. Experimental

2.1. Apparatus

Voltammetric measurements were performed at AMEL 433-A computerized polarographic analyzer with three electrode system: static mercury as working one, and Ag/AgCl and Pt-wire as reference and auxiliary electrodes. A magnetic stirrer and a stirring bar provided convective transport during the preconcentration step.

AdSDPV was performed under the following conditions: scan speed 200 mV s^{-1}, pulse amplitude 100 mV, pulse width 20 ms, mercury drop size 60 a.u. and scan range from −0.2 to −1.2 V in acidic medium and from −0.4 to −1.7 V in basic solutions. After recording the base-line, when a fresh mercury drop had formed, voltammograms were recorded after a certain time of adsorptive accumulation (80 and 50 s), and at a selected accumulation potential (−100 and −300 mV) at pH 3.5 and 9.0, respectively, in a stirred solution (300 rpm). The accumulation period in a stirred solution was followed by a 10 s settling period to allow for quiescence of the solution and uniform distribution of the deposited substance on the surface of the mercury drop.

Polarographic analyzer PAR 174A connected with three-electrode cell (DME, SCE and Pt) was used for measuring electrocapillary curves.

A Radiometer pH meter, PHM 220, with combined pH electrode Radiometer GK2401B and appropriate standard buffer solutions was used.

2.2. Reagents and solutions

Cefpodoxime proxetil was donated by Hoechst Marion Roussel Inc. (Kansas City, Kansas, USA). 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 [12].

Double distilled water was used throughout. Measurements were performed at room temperature.

A stock solution (S_1) of CP of 1×10^{-4} M was prepared by dissolving an accurate mass of the CP standard in bidistilled water. More dilute solutions were prepared daily from stock solution S_1.

The urine samples were taken from the healthy children (five volunteers were included). An urine stock solution (U_1) was full urine sample 10 times diluted with bidistilled water.

2.3. Procedure

A 15 ml aliquot of the corresponding supporting electrolyte solution (BR buffer only, or 13.5 ml of BR buffer and 1.5 ml of urine solution U_1) was placed in the voltammetric cell, and deaerated for 10 min with high purity nitrogen, and then the base-line was recorded. The required amount of stock solution S_1 or diluted stock solution S_1 (5×10^{-5} or 5×10^{-6} M) was added to voltammetric cell. The solution was purged with nitrogen for another 100 s and the current–voltage curves were recorded.

Calibration graphs were constructed using data from two series of measurements and evaluated by the least-squares linear regression method.

Urine solutions were directly analyzed, according to the above procedure, without any pretreatment or extraction step. Determination of CP in spiked urine was done for each concentration repeated five times.

3. Results and discussion

The C=N–OCH_3 group is reducible in the whole pH range investigated (1.8–13.0). In acidic and neutral medium, pH <7, one well developed and sharp voltammetric peak (I) is present. In the pH range from 7 to 10.5 this peak splits, and two peaks, II and III, are present. According to the peak current–pH dependence (Fig. 1), it is evident that all peaks show maxima at certain pH value. The maximum of the i_p versus pH curve indicates the pronounced adsorption at given pH. The dependence of the DPV peak I current versus pH shows maximum at pH = 3.5, and both peak currents II and III show the maximum at pH 9.0. The nature of the reduction process was studied by following the effect of the scan rate on the peak I, II and III current. Both dependencies i_p versus $\nu^{1/2}$ and i_p versus ν gave non-linear plots, indicating that the diffusion-controlled process is strongly influenced by the adsorption [13].

Besides those three peaks, two more peaks (IV and V), are present. The former one is due to the reduction of methoxymino group at pH >10, and the second one is attributed to the two electron reduction of the unsaturated C=C bond. Due to negative reduction potential which is only slightly more positive than the reduction of H^+ ions from the supporting electrolyte used, peaks IV and V are not suitable for analytical purpose.

3.1. Adsorptive character of the drug

The surface active behavior was investigated by measuring electrocapillary curves [14] of CP and those for supporting electrolyte at pH values of analytical importance (Fig. 2a and b). 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 CP concentration was 1×10^{-5} M. Analysis of electrocapillary curves showed the maximum decrease of electrocapillary maximum at −0.1 and −0.3 V for pH 3.5 and 9.0, respectively. These findings suggested above mentioned potentials as the best ones for analytical purposes.

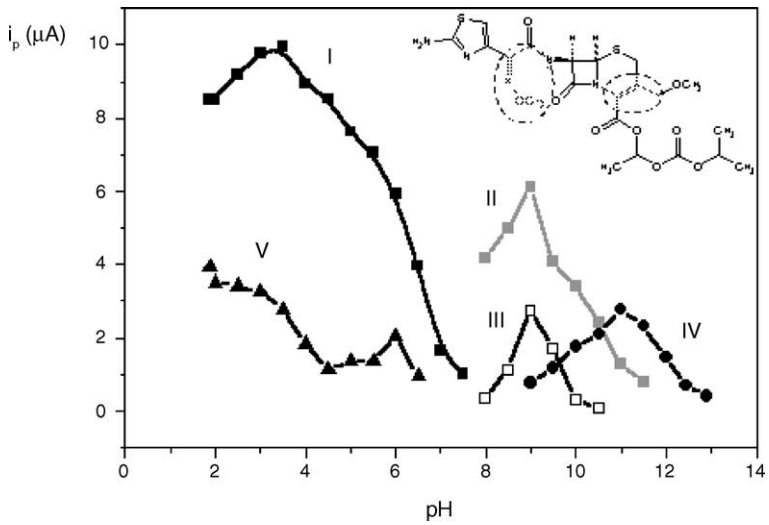

Fig. 1. Infuence of the pH on the DPV peak currents of 1×10^{-5} M CP in BR buffer.

The interfacial adsorptive character of the drug onto the mercury electrode surface was proved when recording the AdSDP voltammograms of CP without and after preconcentration period. The voltammetric signal obtained without accumulation was negligible compared to those obtained after a preconcentration step, indicating that CP adsorbs readily at the electrode surface, and a considerably increase in sensitivity can be gained by adsorptive accumulation prior to the voltammetric determination. The effect of accumulation time on the peak height was investigated at two pH values, 3.5 and 9.0 (Fig. 2c). On this basis, a 80 s (pH 3.5), and 50 s (pH 9.0) deposition time was adopted.

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.

3.2. The optimization of the analytical parameters

In order to establish the optimum conditions for AdSDPV determination of CP, the effect of the modulation of pulse

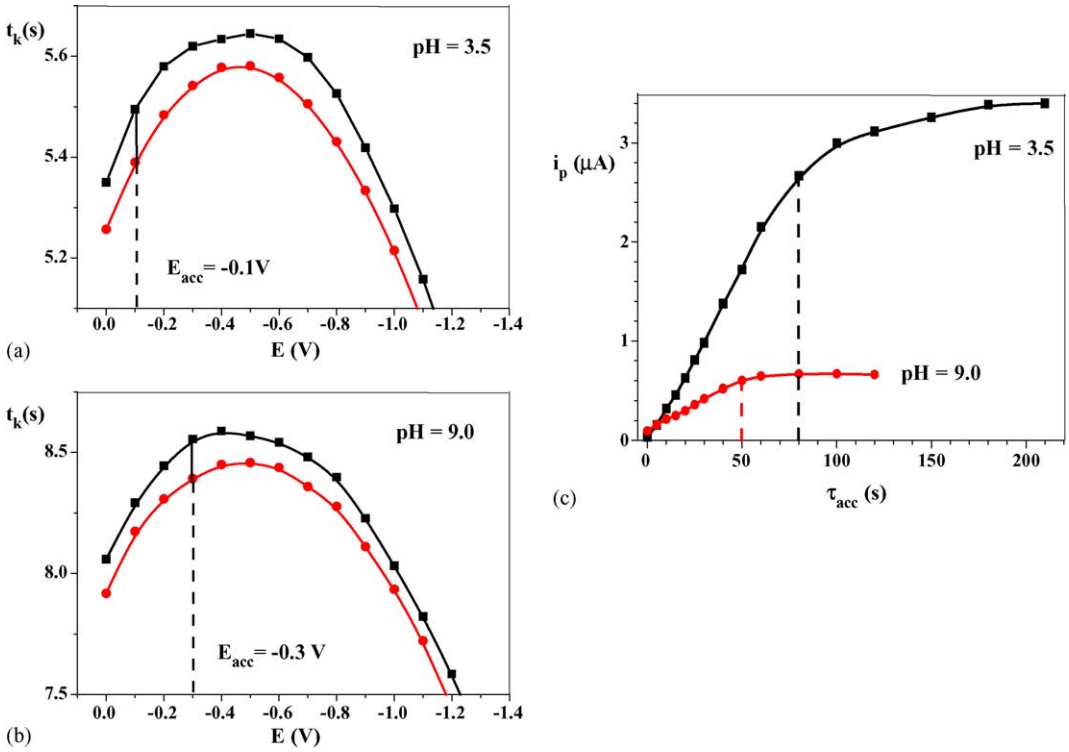

Fig. 2. Electrocapillary curves of: (■) BR buffer, (●) BR buffer in the presence of 1×10^{-5} M CP, at (a) pH = 3.5; (b) pH = 9.0. (c) The effect of the accumulation time on the peak current of the AdSDPV of 1×10^{-7} M CP, at pH = 3.5, and pH = 9.0.

amplitude, pulse width, scan speed, mercury drop size and stirring speed was investigated with 1×10^{-7} M CP solution, and the selected values are given in Section 2.1.

3.3. Analytical application

For analytical purposes the optimum pHs were found to be 3.5 and 9.0.

At pH 3.5 the only one peak (I), $E_p \approx -0.5$ V was followed. At pH 9.0 two peaks were followed, the peak II at $E_p \approx -1.0$ V, and peak III at $E_p \approx -1.2$ V. The linear dependence of peak current versus concentration was obtained only for peak at −1.2 V (Table 1). The same peaks were obtained and followed in spiked urine and the results are also presented in Table 1.

3.4. Validation of the analytical procedure

The proposed voltammetric procedure was applied for the determination of the different concentrations of standard solutions of the drug and linear calibration ranges were obtained from 1×10^{-8} to 1×10^{-7} M. The LOD = 3 S.D.$_a$/b and LOQ = 10S.D.$_a$/b, were calculated from the calibration graphs [15,16], where S.D.$_a$ is the standard deviation of intercept and b is the slope of the calibration graph. The obtained values are presented in Table 1. Comparing the results obtained by AdSDPV at two pH values for peak at −0.5 V (pH 3.5) and peak at −1.2 V (pH 9.0) it is seen that the concentration range and LOD and LOQ values are approximately the same. Good linearity was obtained for both pH values, confirming that pH 9.0 can be exploited for CP determination with equally good sensitivity as at pH 3.5.

Reproducibility of this method was examined by performing five replicate measurement for 5×10^{-8} M at both pH values. The obtained relative standard deviations were 2.8% (pH 3.5) and 3.2% (pH 9.0).

The AdSDPV method established in BR buffer solution was applied for CP determination in urine sample. Since slightly higher noise of the buffer supporting electrolyte was obtained when full urine was spiked in buffer solution, dilution of the urine was made. The noise was minimized as shown in Fig. 3, and no response of blank diluted urine sample was obtained at working potential related to CP (curve 1), enabling the determination of low concentration of CP in complex urine matrix. The results of investigations in urine samples and the optimal conditions established for CP determination are summarized in Table 1. The precision of the method applied was checked at both working pH values for CP concentration of 0.06 and 0.3 μg/ml. Five determination were performed for each concentration of CP, and the corresponding R.S.D. values are 4.72 and 5.23% with recovery values of 96.2 and 102.3% for pH 3.5 and 9.0, respectively.

Reproducibility of this method in urine sample was examined in the same way as with buffer solutions, and the

Table 1
Statistical parameters for AdSDPV determination of CP in BR buffer and urine sample

AdSDPV	pH	Peak potential (V)	Linear concentration range (M)	Regression equation y (μA), x (M)	S_a	S_b	R	S.D. (μA)	N	LOD (M)	LOQ (M)
BR buffer	3.5	−0.5	1×10^{-8} to 1×10^{-7}	$y = -0.0382 + 1.859 \times 10^{7}x$	0.0391	6.30×10^{6}	0.9954	0.0572	10	6.3×10^{-9}	2.1×10^{-8}
	9.0	−1.2	1×10^{-8} to 8×10^{-8}	$y = -0.2123 + 3.365 \times 10^{7}x$	0.0792	3.67×10^{6}	0.9941	0.05185	8	7.1×10^{-9}	2.3×10^{-8}
Urine samples	3.5	−0.5	5×10^{-8} to 1.8×10^{-7}	$y = -0.100 + 3.114 \times 10^{6}x$	0.0077	8.27×10^{4}	0.9982	0.00666	7	7.4×10^{-9}	2.4×10^{-8}
	9.0	−1.2	1×10^{-7} to 2×10^{-6}	$y = -0.004 + 3.416 \times 10^{5}x$	0.0062	5.43×10^{5}	0.9987	0.01173	12	5.4×10^{-8}	1.8×10^{-7}

S_a Standard deviation of the intercept; S_b standard deviation of the slope.

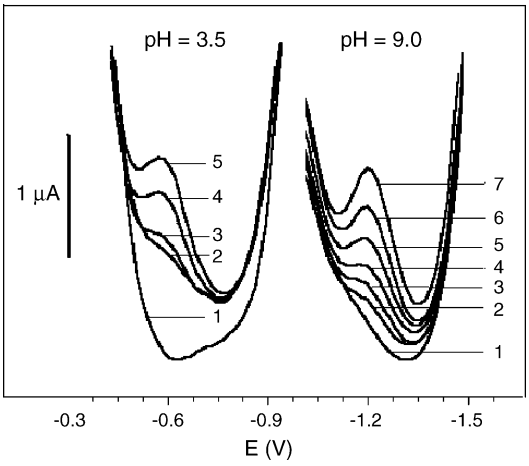

Fig. 3. AdSDPV curves of CP in urine samples. At pH = 3.5 (1) supporting electrolyte—10 times diluted urine solution U_1 in BR buffer; (2) 5×10^{-8} M; (3) 7×10^{-8} M; (4) 1×10^{-7} M; (5) 1.5×10^{-7} M. At pH = 9.0 (1) supporting electrolyte—10 times diluted urine solution U_1 in BR buffer; (2) 1×10^{-7} M; (3) 2×10^{-7} M; (4) 4×10^{-7} M; (5) 6×10^{-7} M; (6) 8×10^{-7} M; (7) 1×10^{-6} M.

obtained R.S.D. values were 3.2% at pH 3.5 and 3.7% at pH 9.0.

The robustness [16] of the method proposed was examined by evaluating the influence of small variations of some most important operational parameters such as pH (3.3–3.7 and 8.8–9.2); accumulation potential (from −0.05 to −0.15 V and from −0.250 to −0.350 V); accumulation time (50–60 s and 80–100 s) and stirring speed (200–400 rpm) on the recovery of the CP concentration of 5×10^{-8} M. Difference between the recovery obtained under the chosen experimental conditions and the recovery obtained within the studied range of variation of the operational parameters was between 1.4 and 2.1%. The most pronounced influence was observed with the change of accumulation potential (2.1%) and pH (1.9%). The obtained results show that none of these variables significantly affects the determination of CP, so the proposed procedure can be considered robust.

The results obtained by day-to-day were found reproducible since there is no significant difference in S.D. and R.S.D. values.

Specifity of the proposed method was checked as its ability to measure specifically only the analyte, not the components that maybe expected in urine matrix as well. In accordance with this, the blank urine sample was scanned before the addition of CP. Since no analytical response was obtained in urine sample (Fig. 3), at the working potential (regarded to CP), it might be concluded that there was no interference of the components of the urine matrix. This also shows the selectivity of the method.

4. Conclusions

The novelty of these investigations is based on the adsorption effects of CP at mercury surface which enables the applying of the AdSDPV method not previously described in literature for its determination.

AdSDPV provides very sensitive and selective method for the determination of CP. The main advantage of AdSDPV is its higher sensitivity and possibility of higher sample dilution when the influence of matrix effects is minimized. The optimized procedures showed clear advantages such as short period of real time of sample analysis and no pretreatment or time consuming extraction steps were required prior the analysis.

The AdSDPV method applied can be good analytical alternative for determining CP in spiked urine samples.

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