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Short communication

Voltammetric behavior of Cefixime and Cefpodoxime Proxetil and determination in pharmaceutical formulations and urine

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Abstract

Electrochemical reduction behavior of cephalosporins, Cefixime (CF) and Cefpodoxime Proxetil (CP) have been studied by using different voltammetric techniques in Britton-Robinson buffer system. Two well defined cathodic waves are observed for both the compounds in the entire pH range. Number of electrons transferred in the reduction process was calculated and the reduction mechanism is proposed. The results indicate that the process of both the compounds is irreversible and diffusion-controlled. The peak currents for CF and CP are found to be linear over the range of concentration 6.0×10^{-8} to 1.2×10^{-5} mol l^{-1} and 8.8×10^{-8} to 1.1×10^{-5} mol l^{-1}, respectively. The lower detection limits are found to be 4.6×10^{-8} and 8.52×10^{-8} mol l^{-1} for the two compounds. A differential pulse voltammetric method has been developed for the determination of these drugs in pharmaceutical formulations and urine samples.
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Keywords: Cefixime; Cefpodoxime Proxetil; Differential pulse voltammetry; Pharmaceutical formulations; Urine samples

1. Introduction

Cephalosporins are antibiotics of β-lactum ring with a broad spectrum of antimicrobial and antibacterial properties. These are effective, resistance and have pharmacokinetic properties [1–3]. Cefixime (CF) and Cefpodoxime Proxetil (CP) (Fig. 1) belong to the third generation cephalosporins. Nowadays these are extensively used in clinical practice and therapy. Therefore there is a need for simple, sensitive and accurate method for their evaluation in pure solutions and body fluids. Various techniques have been utilized for the determination of cephalosporins in body fluids and dosage forms, these includes derivative spectrophotometric [4–6] and chromatographic [7–11] techniques. However, although the selectivity and the detection limit have been improved in these methods, these are rather time consuming methods and require large number of complicated steps to follow on for analysis. Therefore a simple and easy technique has been urgently desired for the detection of cephalosporins. For the present purpose, the desirable technique for the analysis of cephalosporins should be rapid, simple and of high sensitivity in analysis.

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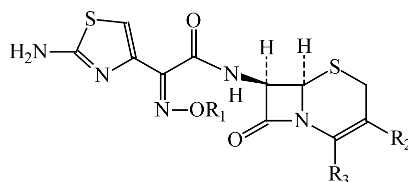


Compound	R1	R2	R3
Cefixime	$—CH_2COOH$	$—CH{=}CH_2$	$—COOH$
Cefpodoxime Proxetil	$—OCH_3$	$—CH_2OCH_3$	$—COO—CH(CH_3)—O—C(=O)—O—CH(CH_3)_2$

Fig. 1. Structure of CF and CP.

Therefore the use of voltammetric method is required for the present study, because it has such advantages that handy-size equipment is now commercially available. The technique is rather time saving and quantitative and qualitative with satisfactory sensitivity, which can be achieved by selecting a mode of the technique, depending on the required detection limit.

Polarographic techniques such as differential pulse polarography [12–14] and adsorptive stripping voltammetry [15] were employed for the determination of cephalosporins such as Ceftazidime, Ceftizoxime, Ceftriaxone, Cefazolin, Cefuroxime, Ceftriaxone and Cefotaxime.

The third generation cephalosporins are the main families of β-lactum antibiotics. Cephalosporins have an advantage of being applicable to penicillin-allergic patients and being active against penicillin resistant micro-organisms in some instances and are also used for therapy of variety of infections which develop in intensive care units. Thus due to biological and scientific values of the title compounds, the study of these compounds is of more importance. There is no published information concerning voltammetric behavior and determination of CF and CP. The analytical problems described here for this determination are of considerable value to analytical chemistry. The analyte used in the present analysis is very stable. The purpose of the present work was to study the voltammetric reduction behavior of CF and CP by employing different voltammetric techniques and to establish the methodology for their trace determination by using differential pulse voltammetry (DPV) in pharmaceutical formulations and urine samples.

2. Experimental

2.1. Apparatus

DPV and cyclic voltammetry (CV) measurements were performed with Metrohm 757 VA Computerace (Herisau, Switzerland) controlled by running electrochemical analysis software, output was a Hewlett Packard plotter. Three electrode assembly cell consisted of dropping mercury electrode, hanging mercury drop electrode (HMDE) as working electrodes, an Ag/AgCl (Metrohm 6.0728.000) as a reference electrode and glassy carbon (Metrohm 6.1247.000) as an auxiliary electrode. pH measurements were carried out with Metrohm 632 pH meter. Controlled potential electrolysis was done with techno potentiostat (Model PS-603). IR spectra was determined with a Perkin-Elmer 1600. All measurements were made at room temperature.

2.2. Reagents and solutions

The compounds, CF and CP were obtained from Micro Labs India Ltd (Hosur). Standard stock solutions (1×10^{-3} mol l^{-1}) were prepared by dissolving an appropriate amount of electroactive species in water and methanol. The standard

stock solutions were protected from light through out the experimental procedures. Britton-Robinson (BR) buffer solutions were prepared with 0.04 M acetic acid; 0.04 M boric acid and 0.04 M *ortho*-phosphoric acid were mixed with addition of 0.2 M NaOH to obtain desired pH. All chemicals and solvents used were of anal R grade. Triple distilled water was used for measurements. Oroken granule (Pharmuka) containing 40 mg, Excef-DT powder (Ind-Swift) containing 50 mg, Ceftas powder (Intas) containing 100, 200, 400 mg of CF and Monocef powder (Aristo) containing 200 mg, Cefpodem powder (Stancare) containing 100, 200 mg of CP were taken as formulations. Desired concentrations of solutions were prepared daily from the stock solutions.

2.3. Voltammetric procedure

Ten millilitre of the BR buffer solution was deoxygenated in the cell with nitrogen gas. An aliquot of standard solution of the electroactive species was added to the buffer containing in the cell. After recording the voltammograms small increments (0.2 ml) of standard solution were added and voltammograms were recorded after each addition under the same conditions. The optimum conditions for CF and CP in pH 2.0 were found to be of drop time 2 s, pulse amplitude 50 mV and applied potential of −0.586, −1.01 and −0.675, −1.04 V, respectively. The above described analytical procedure has been employed for the determination of these drugs in pharmaceutical formulations and urine samples.

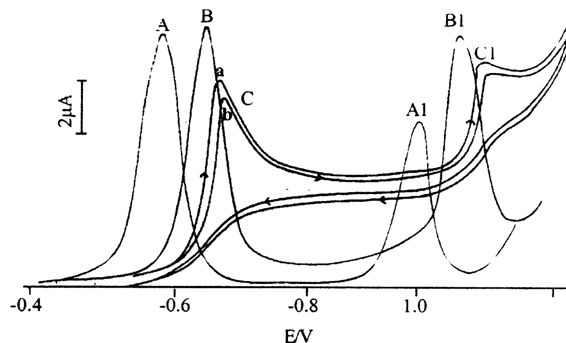
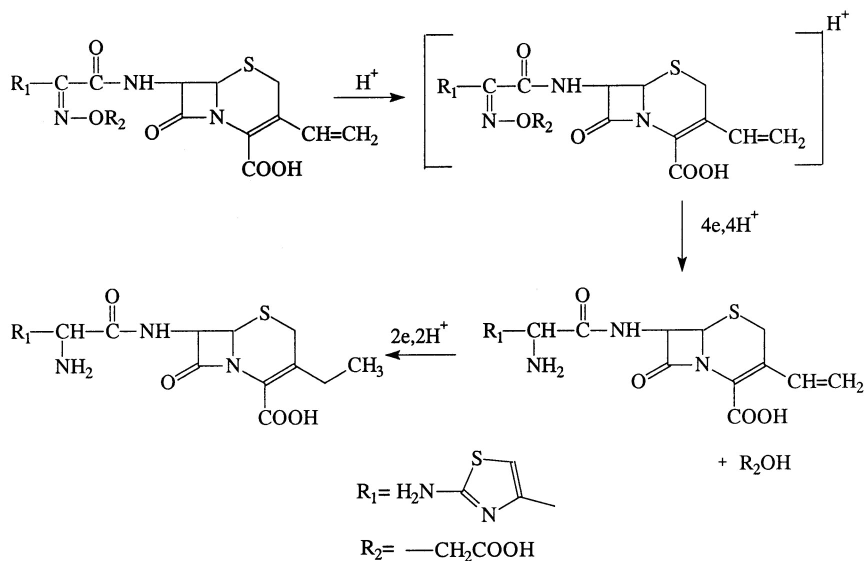


Fig. 2. Differential pulse voltammograms of CF (A and A1 are the first and second peaks) and CP (B and B1 are the first and second peaks) in pH 2.0 concentration 10^{-5} mol l^{-1}, drop time 2 s, pulse amplitude 50 mV; cyclic voltammogram (C and C1 are the first and second peaks) of CF in pH 4.0 a) first cycle b) second cycle of concentration 10^{-5} mol l^{-1} and scan rate of 50 mV s^{-1}.

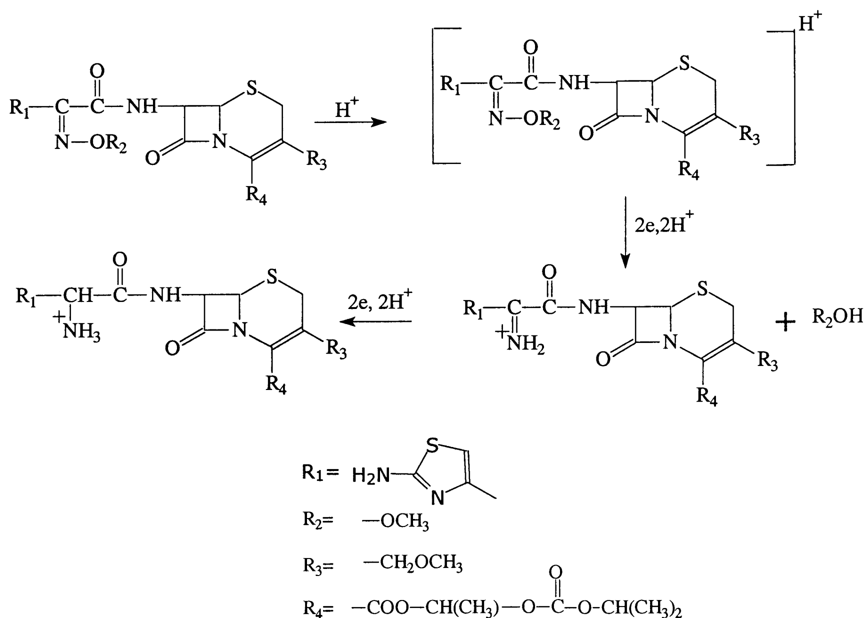
3. Results and discussions

3.1. Differential pulse voltammetry and cyclic voltammetry

The voltammetric behavior of CF and CP have been examined in pH range 2.0–12.0 by using DPV and CV. CF was found to give two well defined peaks in the entire buffer system and these are attributed to the reduction of azomethine and unsaturated C=C bond groups in four and two electron process. CP has been found to give two peaks in the entire buffer system and these peaks are attributed to the reduction of azomethine group by two electron process in two steps. The DPV and CV of CF and CP are shown in Fig. 2. The reversibility of the reduction process was studied at HMDE using CV, where no anodic peak was observed in the pH range for both compounds. The nature of the reduction process was found to be diffusion-controlled and adsorption free in the buffer system studied, as evidenced from the linear plots of i_m vs $t^{2/3}$ [16,17] (where i_m is the maximum current in DPV, t is the drop time) and i_p vs $v^{1/2}$ (i_p is the peak current in CV, and v is the scan rate) relationships. The peak potential (E_m) values of CF and CP are dependent on pH and shifts towards a more negative potential along with an increase in the pH of the buffer system, indicating proton involvement in the electrode process and shifting of E_m values towards more negative potentials on increasing the concentration of the electroactive species, indicating the irreversibility of the electrode process. In the basic buffers of pH > 8 the curves are very poor due to the non-availability of protons and are not useful for analytical purpose.



Scheme 1.



Scheme 2.

3.2. Controlled potential electrolysis

Controlled potential electrolysis was carried out for the identification of reduction products. Two millilitre of 1×10^{-3} mol l^{-1} solution of the electroactive species were placed in the cell and the electrolysis were carried out at a potential -0.586, -1.01 and -1.04 V (vs Ag/$AgCl^{-}$) for respective compounds at pH 2.0. During the electrolysis, solutions were continuously stirred

Table 1
Differential pulse voltammetric data of CF and CP in pharmaceutical formulations

Sample	Labeled amount (mg)	Average amount found (mg)	Average recovery[a]	
			%	±S.D.
CF				
Oroken	40	39.94	99.85	±0.024[b]
Excef-DT	50	49.92	99.84	±0.036
Cefspan	100	99.95	99.95	±0.031
Ceftas	100	100.04	100.04	±0.039
	200	200.01	100.01	±0.050
	400	400.03	100.01	±0.073
CP				
Monocef	200	199.93	99.97	±0.027
Cefpodem	100	99.93	99.93	±0.035
	200	199.93	99.97	±0.036

Drop time 2 s, pulse amplitude 50 mV.
[a] Each value is an average of three determinations.
[b] Compared with HPTLC [11] method.

and purged with nitrogen. After the electrolysis the solutions was extracted three times with 20 ml of ethyl acetate. The combined extracts were dried over anhydrous sodium sulphate and the solvent removed by evaporation. The results indicate that the product is saturated product and the products were confirmed as amino group through IR spectra in which presence of peak in the range of 3250 cm^{-1} responsible for reduction of C=N and in the range of 3625–3375 cm^{-1} responsible for reduction of unsaturated C=C group.

Millicoulometry is employed to find out the number of electrons involved in the electrode process using the method of De Vries and Kroon [18] and were found to be six and four for respective compounds in the entire buffer system. Based on the above results the following mechanism may be assigned for the electrochemical reduction of CF (Scheme 1) and CP (Scheme 2).

3.3. Analysis

For the purpose of analysis the optimum pH for both the compounds CF and CP was pH 2.0 where the first peak was sharp and reproducible and was preferred for the analysis. Standard addition and calibration methods are used for the estimation of the drugs in pharmaceutical formulations and in urine samples. The peak current for CF and CP is found to be linear over the range of concentration 6.0×10^{-8} to 1.2×10^{-5} mol l^{-1} and 8.8×10^{-8} to 1.1×10^{-5} mol l^{-1}. The lower detection limit is found to be 4.6×10^{-8} and 8.52×10^{-8} mol l^{-1}. The detection limit was calculated using the expression $dl = 3\ S.D./m$ where S.D. is the standard deviation and 'm' is the slope of the calibration plot.

3.3.1. Analysis of pharmaceutical formulations

The five tablets of different formulations, namely Oroken 40 mg, Excef-DT 50 mg, Cefspan 100 mg, Ceftas 100, 200, 400 mg of CF and Monocef 200 mg, Cefpodem 100, 200 mg of CP containing different amounts were thoroughly ground to a fine powder. A quantity equivalent to one tablet was weighed, dissolved in water or methanol. The excipients was separated by filtration and the precipitates washed three times with water or methanol. The solution and washing water were transferred quantitatively in to the 100-ml volummetric flask and diluted with water or methanol up to the mark. Known volumes (0.5 ml) of the clear supernatant liquid was diluted to 10 ml with supporting electrolyte and subjected to

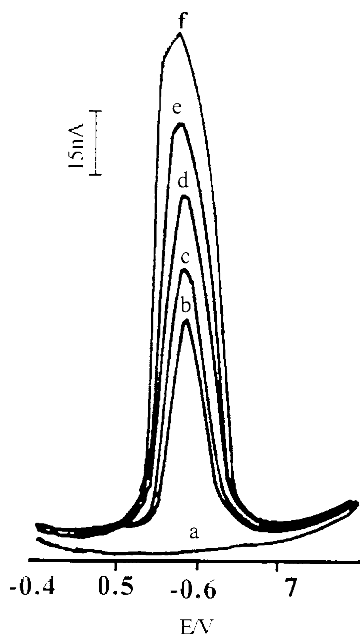


Fig. 3. Differential pulse voltammograms for blank urine sample (a), the successive addition of CF to urine sample (b–f) of drop time 2 s, pulse amplitude 50 mV in pH 2.0.

polarography. The amount of the compound in the tablets was calculated by standard addition method. The accurate and precise results obtained for the formulation of CF namely Oroken in the present method was compared with those obtained with HPTLC [17] and the results were found to be in good agreement with that of the previously reported method, the remaining formulations of CF and CP were found to be in good agreement with that of the declared values of each drug taken from different formulations. The results obtained for the determination of drug in pharmaceutical formulations are given in Table 1.

3.3.2. Determination of urine samples

Collected urine samples were spiked with different concentrations of drugs. To avoid urine interference solid–liquid extraction was carried out using Waters Associates sep-pak C_{18} cartridge, previously activated with 5 ml of methanol and rinsed twice with 3 ml of water; then the catridge was buffered at pH 2.0. One millilitre of spiked urine sample was passed through the catridge where the drug was separated and was retained. The catridge was washed with 4 ml of water or methanol (1:3 v/v) and then rinsed with 2 ml of water. The elution was carried out with two 2 ml of 40% methanol and the combined eluent was evaporated in rotatory vacuum evaporator. To this 10 ml of buffer solution was added and voltammograms were recorded. The standard

Table 2
Determination in urine samples of pulse amplitude of 50 mV and drop time of 2 s

Sample	Amount added (μM)	Average amount found (μM)	Average recovery[a]	
			%	±S.D.
CF				
	5	4.91	98.20	±0.025
	10	9.92	99.20	±0.016
	15	14.93	99.53	±0.016
	20	19.91	99.55	±0.026
	25	24.94	99.76	±0.020
	30	29.92	99.73	±0.025
CP				
	5	4.94	98.80	±0.025
	10	9.89	98.90	±0.036
	15	14.91	99.40	±0.036
	20	19.94	99.70	±0.040
	25	24.92	99.68	±0.020
	30	29.94	99.80	±0.031

[a] Each value is an average of three determinations.

addition method was applied for the determination of these drugs in urine samples. Differential pulse voltammetric determination of CF in urine sample is shown in Fig. 3 at pH 2.0. Analytical results obtained for CF and CP in urine samples were presented in Table 2.

Excretion studies in urine samples were carried out for the drugs CF and CP. CF (100 mg) and CP (200 mg) were given orally to six patients. A few hours after oral administration CF and CP has been distributed in body tissues, blood, serum and urine. Urine samples were collected from the patients for up to 24 h.

By employing the proposed technique, the DPV signal of the drug was seen after 2–3 h and the excreted drug through urine was found to be nearly 45–50% and 30–35%, respectively. The results are compared with the amount excreted given in the Indian drug review book [19] and it was found to be in good agreement. From the above studies it was found that the major amount of the drug is excreted through urine.

3.3.3. Interference studies

The potential interference of some urine ingredients was tested by their electroactivity. In separate experiments, the following substances were added to the buffer solution, urea, NaCl, Na_2SO_4, NH_4Cl, $NaHCO_3$, trisodium citrate, oxalic acid and tetrasodium phosphate. Their concentration in the cell was made approximately 200 times smaller then their usual physiological concentration in urine. None of the above compounds under the above conditions did affect the peaks of the drugs.

3.4. Calibration graph and detection limit

A linear relationship was found over the concentration range 6×10^{-8} to 1.2×10^{-5} mol l^{-1} and 8.8×10^{-8} to 1.1×10^{-5} mol l^{-1} for CF and CP, respectively, the limit of detection is given by the expression LOD = 3 S.D./m [20], where 'S.D.' is the standard deviation of replicate determination values, 'm' is the slope of the calibration curve. Now S.D. = 1.35×10^{-3} and 1.42×10^{-3} μA, $m = 8.8 \times 10^4$ and 5×10^4 μA mol l^{-1}, hence

Table 3
Regression equation and statistical parameters of voltammetric determination of CF and CP by DPV

Compound	Calibration curve equation (mol l^{-1})	r^2	Linear range concentration (mol l^{-1})	R.S.D (%)		LOD (nmol l^{-1})	LOQ (nmol l^{-1})
				Repeatability[a] (n)	Reproducibility[b] (n)		
CF	$8.8 \times 10^4 x - 0.16$	0.998	6×10^{-8} to 1.2×10^{-5}	4.51 (15)	4.79 (15)	0.46	0.015
CP	$5.0 \times 10^4 x + 0.06$	0.999	8.8×10^{-8} to 1.1×10^{-5}	3.0 (15)	3.21 (15)	0.85	0.028

[a] Same day, same standard solution, same analyst, n, number of assays.
[b] Different days, different standard solution, different analysts, n, number of assays.

$LOD = 3 \times S.D./m = 3 \times 1.35 \times 10^{-3}/8.8 \times 10^{4} = 4.6 \times 10^{-8}$ mol l^{-1} and $3 \times 1.42 \times 10^{-3}/5 \times 10^{4} = 8.52 \times 10^{-8}$ mol l^{-1}. The LOQ is defined as 10 S.D./m [20], and was found to be 1.53×10^{-7} and 2.84×10^{-7} mol l^{-1}, respectively. The calibration curve equations and statistical parameters of voltammetric determination of CF and CP are given in Table 3.

4. Conclusions

DPV provides a very sensitive and selective method for the determination of CF and CP. The analytical results obtained by DPV are adequately accurate and precise and are in good agreement with those obtained by other technique HPTLC. The main advantage of new method is its higher sensitivity and because of the possibility of higher sample dilution, less influence of matrix effects. Consequently, the present method becomes a good analytical alternative for determining CF and CP in pharmaceutical formulations and spiked urine samples.

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