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Short Communication

Analytical investigations of cephalosporins — II. Polarographic behaviour of ceftriaxone, cefuroxime, cefotaxime and ceftizoxime and assay of their formulations

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Introduction

Ceftriaxone [1], cefuroxime [2], cefotaxime [3] and ceftizoxime [4] are semi-synthetic cephalosporins with a broad antibacterial spectrum (Table 1). These antibiotics are administered by intravenous or intramuscular injection. In addition to microbiological methods, HPLC [5] and reversed-phase liquid column chromatography (RPLCC) [6] have been used for cefotaxime and DPP [7–8], RPLCC [6], HPLC [5] and fluorimetric [9] methods have been used for cefuroxime in pharmaceutical products. Furthermore, DPP has been used for the determination of degradation products of cephalosporin derivatives.

Electrochemical techniques are uniquely suited for the assay of compounds that contain reducible or oxidizible functions. These include those cephalosporins with an ether or a thioether linkage to a substituent at the 3-position. The presence of other substituents that contain an electroactive group make a polarographic determination possible. In the present study polarographic properties of cephalosporins were investigated and an electroanalytical method was developed. The results of this method were compared with the results of a modified colorimetric hydroxylamine method based on that of the USP XX [10] and the Code of Federal Regulations [11] for cephalosporins.

Experimental

Reagents and apparatus

All reagents and solvents in this study were of analytical reagent grade. The polarographic experiments were carried out using a differential pulse polarograph

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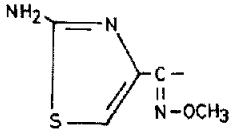
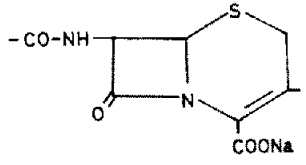
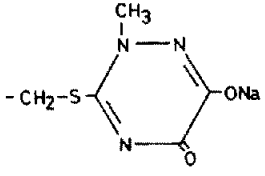
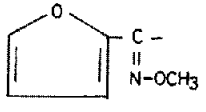
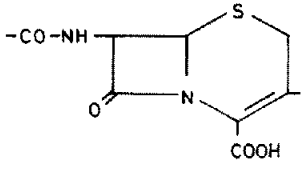
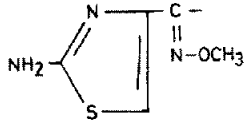
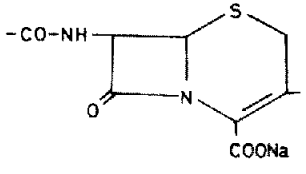
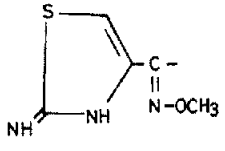
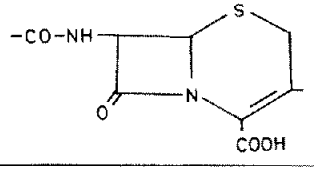
Table 1
Chemical formulae of cephalosporins

Compound	R_1-group	Main structure of cephalosporins	R_2-group
Ceftriaxone			
Cefuroxime			$-CH_2-O-CONH_2$
Cefotaxime			$-CH_2-O-COCH_3$
Ceftizoxime			$-H$

Metrohm, E 506 (Herisau, Switzerland). A pulse amplitude of 200 mV was applied to each mercury drop during the determination. The current was measured during 25 ms before the start of the pulse. The electronically regulated dropping time was 2 s. Visible spectra were recorded using a Beckmann Model B spectrophotometer (Beckmann Instruments Inc., Fullerton, Calif., USA).

Materials

Ceftriaxone working standard, Rocephin (Roche, Basle); cefuroxime working standard, Zinacef (Hoechst A.G., FRG); cefotaxime working standard, Claforan (Hoechst A.G., FRG); ceftizoxime working standard (Boehringer, Mannheim, FRG).

Polarographic method

Stock solutions used in the polarographic analyses were prepared by dissolving 0.001 M cephalosporin in dimethyl formamide (DMF) purified by liquid chromatography on basic alumina (activity 1) and silica gel 60 (0.063–0.2 mm) (Merck, Darmstadt, FRG), or in DMF and methanol (MeOH). Solutions of the desired concentrations were obtained from the stock solution by diluting with appropriate buffer solutions to volume. Stock solutions were stored below 10°C in the dark.

Britton Robinson buffer solution. The stock solution contained 0.04 M acetic acid, 0.04 M phosphoric acid and 0.04 M boric acid. The pH (1.81–11.98) of the solution was adjusted by adding appropriate volumes of 0.2 M NaOH dropwise to about 100 ml of the stock solution.

Acetate buffer solution (Walpole). The solution (pH 4.2) contained 2.9 ml of 0.2 M acetic acid and 17.1 ml of 0.2 M sodium acetate (3 H_2O).

Citrate buffer solution. The solution (pH 2.0) contained 9.8 ml of 0.1 M citric acid and 0.2 ml of 0.2 M disodium monohydrogenphosphate. The ionic strength was adjusted to 0.1 with potassium chloride.

Application of the polarographic method to the cephalosporins in vials. Pharmaceutical formulations that contained 0.5, 1 and 2 g of cephalosporin were diluted to volume with sterile water for injections; 0.1 ml aliquots were transferred by pipette into volumetric flasks and diluted to 10, 25 and 50 ml respectively with DMF or DMF-MeOH (60:40, v/v). A 1 ml aliquot of each solution was transferred into a polarographic cell at 20°C ±0.10°C and 19 ml of the appropriate buffer solution was added. The height of the mercury reservoir was 60 cm and determinations were performed by differential pulse polarography.

Hydroxylamine method

Ni-*Hydroxylamine reagent.* A 6.95 g portion of hydroxylamine HCl and a 2.90 g portion of $Ni(No_3)_2H_2O$ were dissolved in about 30 ml of distilled water. The pH was adjusted to 6.2 ± 0.05 with 10 M NaOH and the solution was diluted to 50 ml with distilled water. This solution contained 2 M hydroxylamine and 0.2 M Ni.

Iron (III) *reagent.* A 30 g sample of $FeNH_4(SO_4)_2$,12 H_2O was dissolved in 2.7 ml of concentrated H_2SO_4 and the solution was diluted to 100 ml with distilled water. This solution contained 0.622 M iron (III).

Procedure. A 20 mg sample of each cephalosporin (ceftriaxone, cefuroxime, cefotaxime or ceftizoxime) was dissolved in 1% $NaHCO_3$ and diluted to 20 ml with the same solvent. Successive samples (0.5, 1, 1.5, 2, 2.5 and 3 ml) of this solution were transferred by pipette into stoppered test tubes. All volumes were adjusted to 3 ml with 1% $NaHCO_3$. A 2 ml aliquot of Ni-hydroxylamine reagent was added to each test solution and the mixture was allowed to stand at room temperature for 40 min. During this period, the test tubes were stoppered and the contents of each tube were mixed for about 5 min with a shaker. After 40 min, 5 ml of iron (III) reagent was added to each tube and the mixture was allowed to stand for 15 min; again the stoppered test tubes were shaken for 5 min. After 15 min, the solutions were centrifuged; this was necessary because of turbidity produced by nickel (II) salts.

Blank solutions were prepared under the same conditions without the drug. The absorbance of each test solution was measured at 460 nm against the blank solution and linear calibration plots were drawn.

Application of the Ni-*hydroxylamine method to the cephalosporin vials.* Samples (0.5, 1 and 2 g) of each cephalosporin were dissolved in 1% $NaHCO_3$ and 0.1 ml of each of these stock solutions was transferred into five separate 20 ml volumetric flasks by a Hamilton syringe and diluted to volume. A 3 ml portion of each solution was transferred by pipette into stoppered test tubes, the procedure used for the preparation of the standard curve was repeated and absorbances were measured at 460 nm against the blank solution. Quantitative determinations were performed with reference to the linear calibration plots.

Analysis of pharmaceutical formulations. Pharmaceutical formulations of the three cephalosporins were diluted to the appropriate volumes; five samples were examined for each cephalosporin. DPP and colorimetric determinations were carried out.

Results and Discussion

Differential pulse polarography of the cephalosporins ceftriaxone, cefuroxime and cefotaxime give two polarographic waves whereas ceftizoxime gives a single wave (Fig. 1). Data for those cephalosporins studied which give polarographic peaks are summarized in Table 2. Calibration graphs were linear (Fig. 2).

For ceftriaxone, the regression equation for peak 1 was: $y = 4.666x + 7.54$; standard error (S.E.) in gradient = 0.099; $n = 5$; correlation coefficient $r = 0.9993$. The relative standard deviation (R.S.D.) at 45.6 μg/ml was 0.22% ($n = 5$). The regression equation for peak 2 was: $y = 2.059x + 4.87$; the S.E. in gradient = 0.112; $n = 5$; $r = 0.9956$. The R.S.D. at 45.6 μg/ml was 0.98% ($n = 5$).

For cefuroxime, the regression equation for peak 1 was: $y = 4.224x - 3.52$; the S.E. in gradient = 0.097; $n = 5$; $r = 0.9992$. The R.S.D. at 26.8 μg/ml was 0.45% ($n = 5$).

For cefotaxime, the regression equation for peak 1 was: $y = 5.365x + 1.8$; the S.E. in gradient = 0.061; $n = 5$; $r = 0.9998$. The R.S.D. at 31.5 μg/ml was 0.29% ($n = 5$). The regression equation for peak 2 was: $y = 5.460x + 9.6$; the S.E. in gradient = 0.127; $n = 5$; $r = 0.9992$. The R.S.D. at 31.5 μg/ml was 0.54% ($n = 5$).

For ceftizoxime, the regression equation for the peak was: $y = 1.911 + 3.11$; the S.E. in gradient = 0.027; $n = 5$; $r = 0.9997$. The R.S.D. at 75.4 μg/ml was 0.34% ($n = 5$).

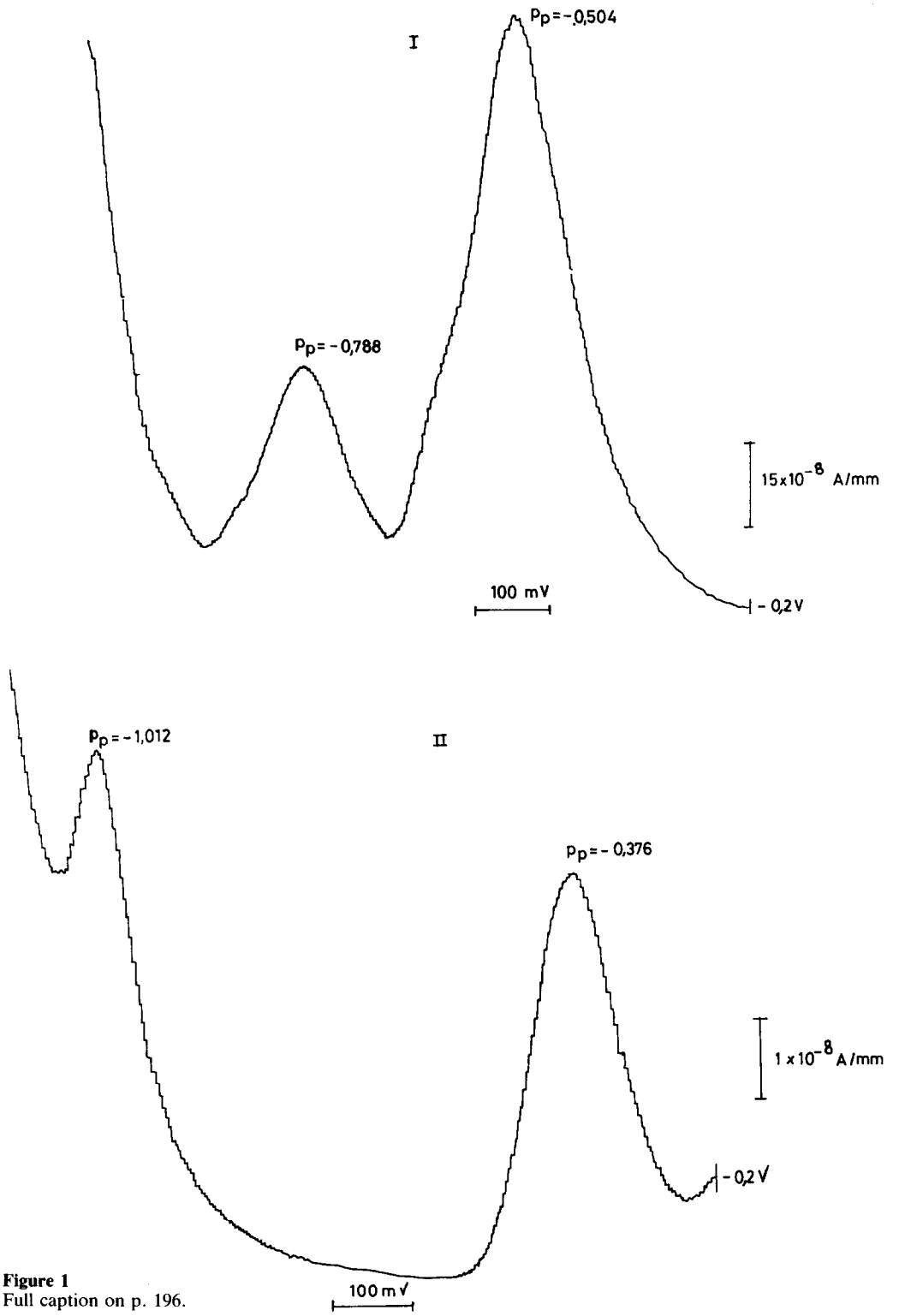


Figure 1
Full caption on p. 196.

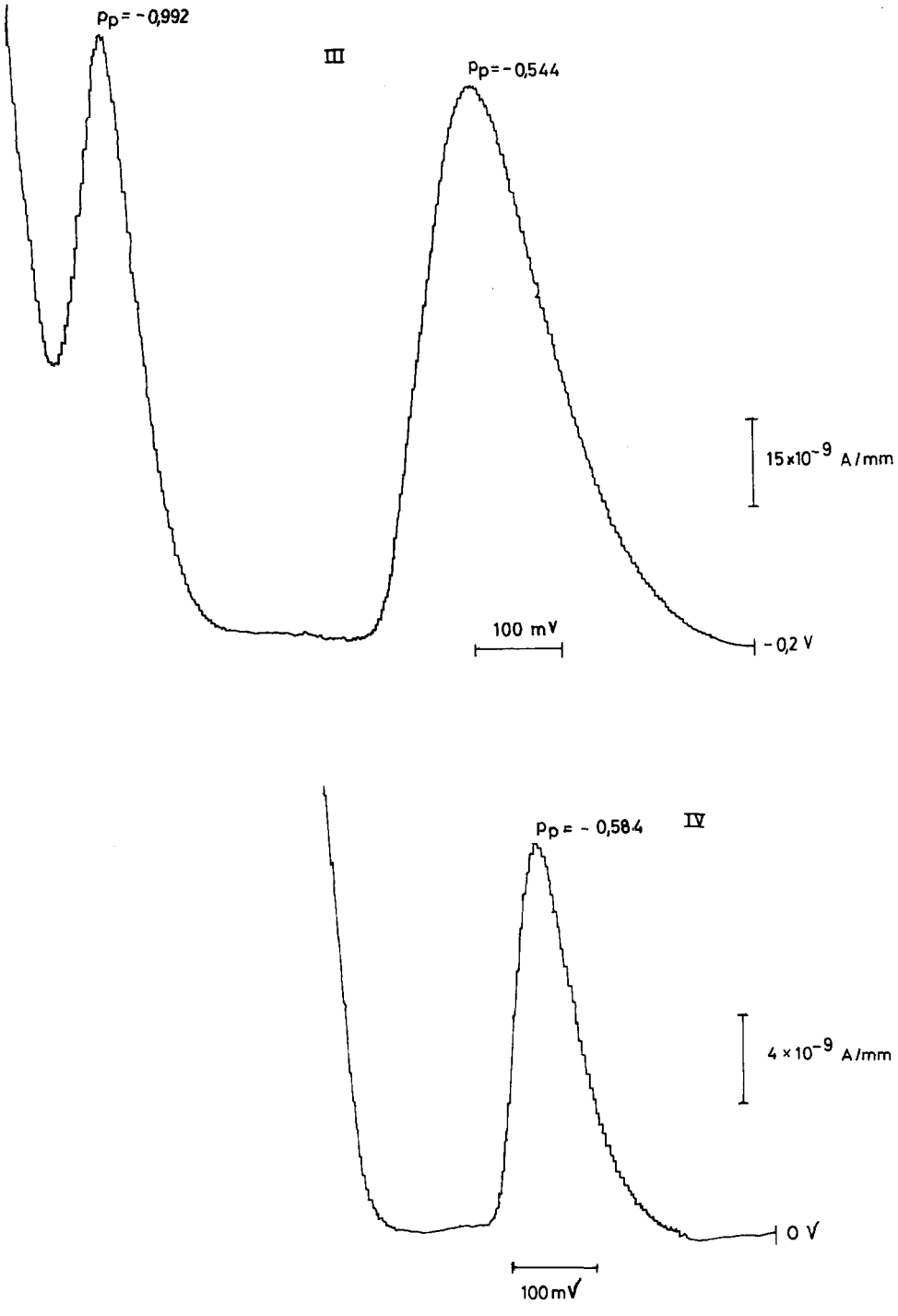


Figure 1
Differential pulse polarograms of cephalosporins in citrate buffer pH 2.0. (I) Ceftriaxone in 5% DMF–MeOH (60:40, v/v); c = 45.6 μg/ml. (II) Cefuroxime in 5% DMF; c = 26.8 μg/ml. (III) Cefotaxime in 5% DMF; c = 31.5 μg/ml. (IV) Ceftizoxime in 5% DMF–MeOH (60:40 v/v); c = 75.4 μg/ml.

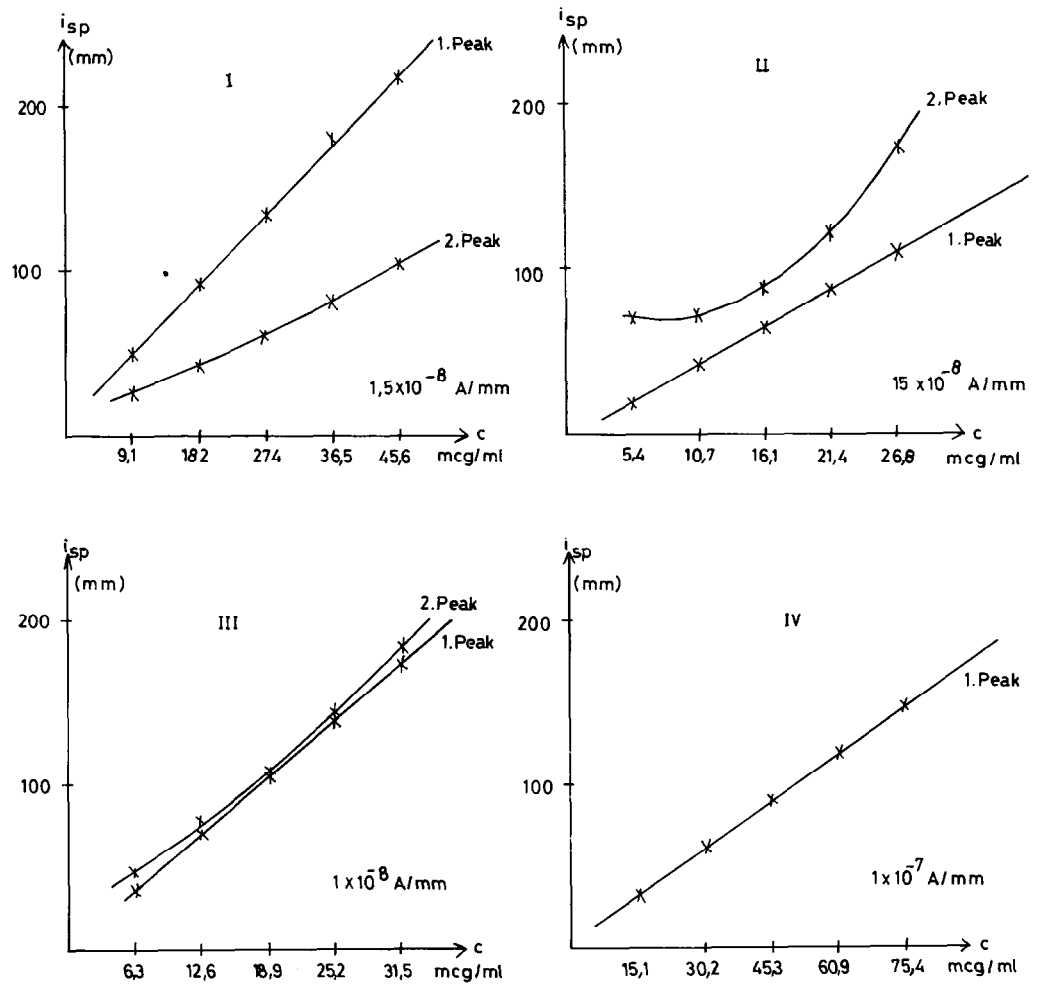


Figure 2
Relationships between peak heights and concentrations of cephalosporins in citrate buffer pH 2.0. (I) Ceftriaxone in 5% DMF–MeOH (60:40, v/v). (II) Cefuroxime in 5% DMF. (III) Cefotaxime in 5% DMF. (IV) Ceftizoxime in 5% DMF–MeOH (60:40, v/v).

Since peak currents were highly dependent on pH, standards and samples had to be measured in the same buffer. In general, peak currents decreased with an increase in pH. The three cephalosporins which have a substituted 3-methyl group gave a differential pulse peak at about 1.0 V at pH 2.0–4.0.

Ochiai *et al.* [12, 13] used preparative electrochemical reduction to show that cephalosporins substituted at the 3-methyl position are reduced according to Scheme 1.

In the present study, ceftizoxime, which does not possess a substituent at the C_3 position, does not give a reduction peak at the related potential and this confirms the validity of the scheme.

Polarographic peaks observed approximately at −0.4 to −0.5V for four of the cephalosporins are probably due to the methoxyimino group substituted at the 7-

Scheme 1

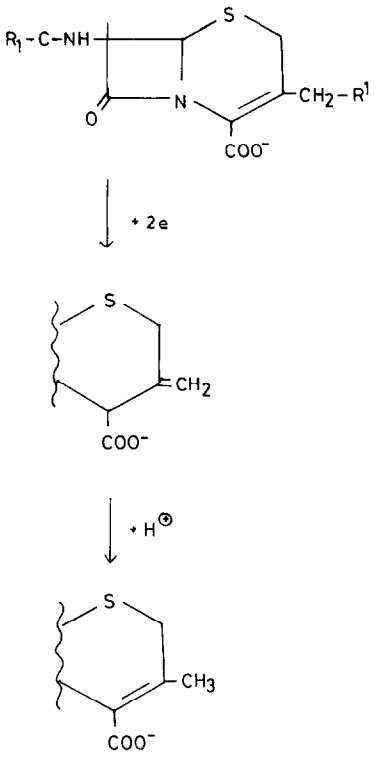


Table 2
Polarographic data for cephalosporins

Compound	Supporting electrolyte	No. of peaks	p_p	Polarographic current	No. of electrons	Reducible bonds
Ceftriaxone	5% DMF – MeOH (60:40, v/v)	2	1. peak −0.504 V 2. peak −0.788 V	1. peak Diffusion current k.c 2. peak	1. peak 2e 2. peak 2e	−C=N− Azomethine group 2. peak −CH_2−R Leaving group
Cefuroxime	10% DMF	2	1. peak −0.376 V 2. peak −1.012 V	1. peak Diffusion current 2. peak Diffusion current k.c	1. peak 2e 2. peak 2e	1. peak −C=N− Azomethine group 2. peak −CH_2−R Leaving group
Cefotaxime	10% DMF	2	1. peak −0.544 V 2. peak −0.992 V	1. peak Diffusion current k.c 2. peak Diffusion current k.c	1. peak 2e 2. peak 2e	1. peak −C=N− Azomethine group 2. peak −CH_2−R Leaving group
Ceftizoxime	5% DMF – MeOH (60:40, v/v)	1	1. peak −0.584	1. peak Diffusion current k.c	1. peak 2e	1. peak −C=N− Azomethine group

Table 3
Results for the determination of cephalosporins in vials

Cephalosporins	Pharmaceutical formulation	Amount of cephalosporin (mg)			
		Polarographic		Spectrophotometric	
		C	$S_{rel}(\%)$	C	$S_{rel}(\%)$
Ceftriaxone	Rocephin 0.25	250	0.79	249	0.79
(as Di-Na-Salt)	Rocephin 0.5	500	0.41	503	0.36
	Rocephin 1.0	1009	0.36	1019	0.95
	Rocephin 2.0	2011	0.21	2007	0.7
Cefuroxime	Zinacef 250	249	0.85	246	1.0
(as Na-Salt)	Zinacef 750	761	0.46	764	0.83
	Zinacef 1500	1509	0.29	1516	0.91
Cefotaxime	Clarofan 0.5	507	0.61	512	0.74
(as Na-Salt)	Clarofan 1.0	1020	0.39	1026	0.49
	Clarofan 2.0	2008	0.29	2011	0.47

position. Furthermore, peak currents in this polarographic reduction depending on the $2e + 2H^+$ transfer are greater than the second peak currents and are more suitable for analytical purposes. Results for the determination of cephalosporins in vials are presented in Table 3.

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