

Determination of Some Cephalosporins by Adsorptive Stripping Voltammetry

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Cephapirin, cefamandole and cefmetazole were determined using cathodic stripping voltammetric technique by adsorptive collection on a hanging mercury drop electrode (HMDE) phosphate buffer pH 5.5 and 7 was used for cephapirin and cefamandole, while Britton-Robinson buffer pH 7 was used for cefmetazole. The adsorptive cathodic peaks were observed at - 1.46, -0.98 and -1.00 V vs. Ag/AgCl reference electrode for cephapirin, cefamandole and cefmetazole, respectively. The effect of various parameters such as pH, preconcentration time, deposition potential, supporting electrolytes, possible interferences and other variables were investigated for the examined drugs. Statistical analysis was carried out for the obtained results. Good linearity's were obtained. The proposed method was successfully applied to the analysis of the studied drugs in their available pharmaceutical formulations and in biological samples (serum and urine). The interference of some amino acids urea, ascorbic acid and some metal ions was investigated.

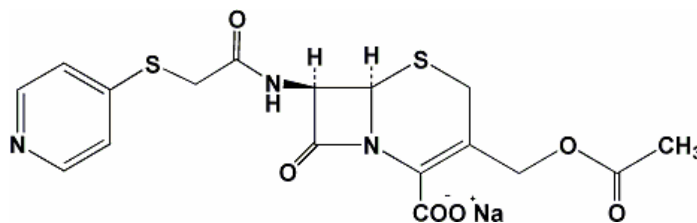
Keywords: Cephapirin, Cefamandole, Cefmetazole, Adsorptive stripping voltammetry

1. INTRODUCTION

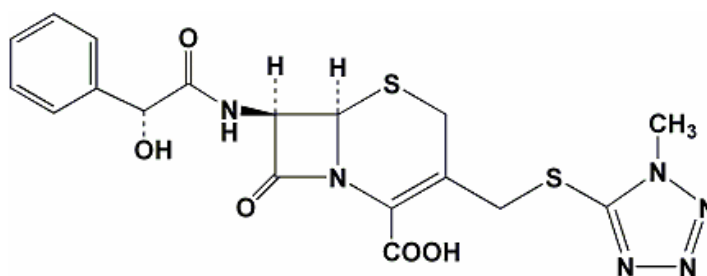
Cephapirin, cefamandole and cefmetazole are cephalosporins derivatives of the first and second generation. They are closely related in structure and in their antibactericidal action mechanism to penicillins.

Various methods have been proposed for the investigation of cephalosporins, including spectrophotometric [1-4], fluorimetric [5] and electroanalytical [6-14]. The pharmacopoeial methods for analyzing the investigated cephalosporins are chromatographic methods[15], which are expensive.

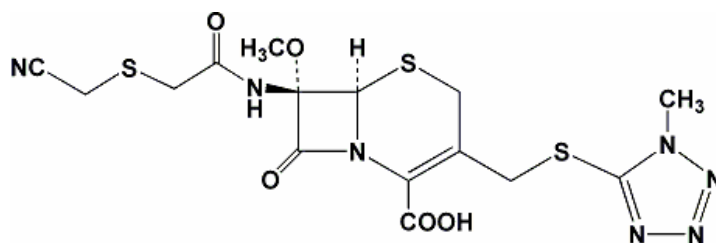
The aim of this work is to develop a simple electroanalytical method for determination of these drugs in pharmaceutical dosage forms and in biological fluids. The structural formulas for the studied drugs are.



Cephapirin (I)



Cefamandole (II)



Cefmetazole (III)

2. EXPERIMENTAL PART

2.1. Apparatus

Cathodic stripping and cyclic voltammetric measurements were carried out using EG&G PAR (model 263 polarographic analyzer). A cell model 303A static mercury drop electrode (SMDE) was connected to the potentiostat, a hanging mercury dropping electrode (HMDE) was used as a working electrode, silver/silver chloride (saturated KCl) as a reference electrode and a platinum wire was used as a counter electrode.

The pH of the solutions was tested using a Thermo Orion Model 420 A plus digital pH-meter.

2.2. Chemicals

Cephapirin sodium salt, cefamandole and cefmetazole were obtained from Sigma-Aldrich (Steinheim Germany) and used as received without further purification. Stock solutions were prepared daily by dissolving an appropriate amounts of the drugs in double distilled water. All other solutions were prepared using double-distilled water and analytical grade reagents. Urine and serum samples from healthy volunteers were used in the analysis.

2.3. Dosage forms

Cefatrexyl vials (cephapirin sodium) were obtained from Bristol-Mayers Squibb Co., (Cairo, Egypt). Mandol vials (cefamandole nafate) from Eli Lilly Italy S.P.A.

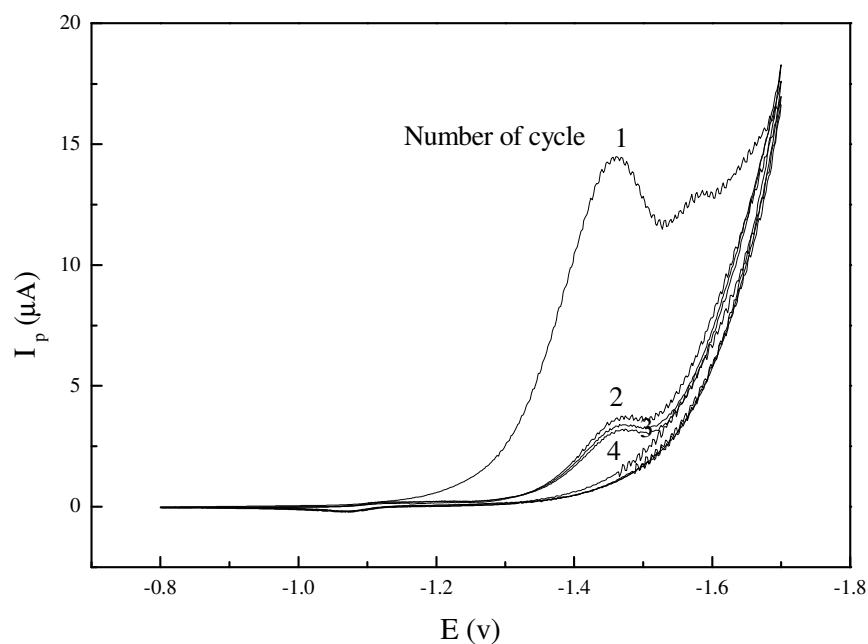


Figure 1. Repetitive cyclic voltammograms for $1 \times 10^{-5} \text{ mol dm}^{-3}$ cephapirin in phosphate buffer (pH 5.5), accumulation potential 0.0 V, scan rate 100 mV/s and 30 s deposition time.

3. RESULTS AND DISCUSSION

3.1. Cyclic Voltammetry

In the present work, the cyclic voltammetric measurements, using 30 s deposition time and 0.0, - 0.5 and - 0.65 V accumulation potential for cephapirin, cefamandole and cefmetazole respectively,

showed only one defined cathodic reduction peak at - 1.46, -0.98 and -1.00 V for cephalosporin, cefamandole and cefmetazole, respectively. The repetitive cyclic voltammograms of 1×10^{-5} mol dm⁻³ cephalosporin in phosphate buffer (pH 5.5) were shown in Fig. 1, which indicates the rapid desorption of the desorbed form in which the reduction peak current decreased sharply in the second and third cycles. A linear sweep voltammetric technique with high scan rate is preferable in such cases.

3.2. Effect of supporting electrolyte

The peak responses for the studied drugs were affected by the type of supporting electrolytes which were used. Different supporting electrolytes were examined, including: potassium chloride, sodium nitrate, sodium phosphate, borax, sodium citrate, Britton-Robinson, and phosphate buffers. The highest peak current and the best peak shape were obtained in the presence of phosphate buffer for cephalosporin and cefamandole, while for cefmetazole, Britton-Robinson buffer gave the best result. The effect of pH on the peak current was examined over the range from 2 to 12. The results showed that the best peak response was observed at pH 5.5, 7 and 7 for cephalosporin (Fig. 2), cefamandole and cefmetazole, respectively. Phosphate buffer pH 5.5 and 7 were used for further work for cephalosporin and cefamandole, while Britton-Robinson pH 7 was selected for the determination of cefmetazole. The peak potential shifted to more negative values by increasing the pH (Fig. 3).

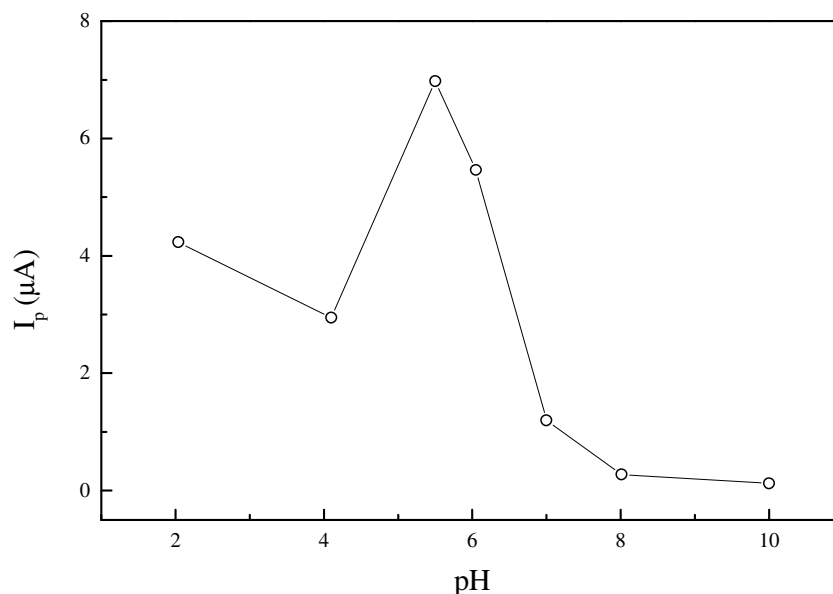


Figure 2. Influence of pH on the peak current of 1×10^{-4} mol dm⁻³ cephalosporin, at 0.0 V deposition potential, 30 s deposition time and scan rate 100 mV/s, in phosphate buffer.

3.3. Effect of deposition potential

The effect of deposition potential on the peak current was studied over the range from 0.0 to -1.4 V, the maximum values for the resulted peak current were 0.0, -0.5 and -0.65 V for cephalosporin and

cefamandole and cefmetazole, respectively, therefore these potentials were used for further investigations.

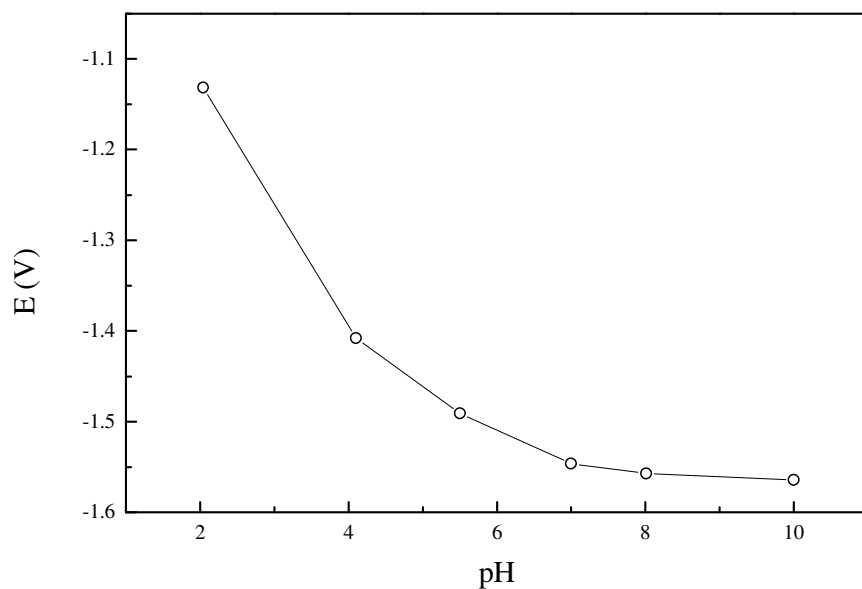


Figure 3. Influence of pH on the peak potential of 1×10^{-4} mol dm^{-3} cephalixin, at 0.0 V deposition potential, 30 s deposition time and scan rate 100 mV/s, in phosphate buffer.

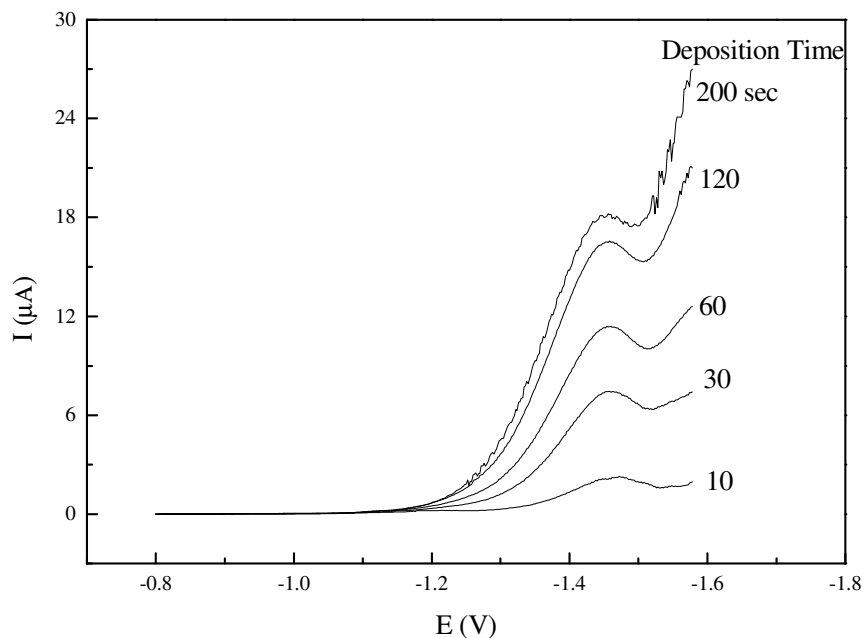


Figure 4. Typical cathodic stripping voltammograms of 5×10^{-6} mol dm^{-3} cephalixin at different deposition times, scan rate 100 mV/s and preconcentration potential 0.0 V in phosphate buffer (pH 5.5).

3.4. Effect of accumulation time

The influence of accumulation time on the peak height for the studied drugs was examined at different accumulation times over the range from 0.0 to 200 s. The resulted peak current increased with the increase of the accumulation time. Fig. 4 showed the resulted voltammograms for 1×10^{-6} mol dm⁻³ for cephapirin. The results were treated statistically using different regression modes (linear, power, exponential and logarithmic) to select the most suitable one for the collected results (Table 1). From the obtained data the linear regression mode is the most suitable for the determination of 5×10^{-6} - 1×10^{-4} mol dm⁻³ cephapirin using accumulation time range from 1 to 30 s. On plotting the peak current (I_p) versus the square root of time (\sqrt{t}), a straight line was obtained with a correlation coefficient of 0.9917, this behavior is expected for response controlled by adsorption [16].

Table 1. Characteristics of current-time curves established using different cephapirin concentrations in phosphate buffer (5.5), scan rate of 100 mV/s and 0.0 V preconcentration potential.

Regression mode	[Cephapirin] mol dm ⁻³	Deposition time range (s)	Correlation coefficient (μ A)	Slope (μ A/s)	Intercept (μ A)
Linear Y = A + Bx i.e. I = A + Bt	5×10^{-6}	1-30	0.99454	1.049	-0.872
	1×10^{-4}	1-30	0.99553	1.961	7.631
Power Y = Ax ^B i.e. I = At ^B or ln I = lnA + Bt	5×10^{-6}	1-30	0.98886	0.441	-0.316
	1×10^{-4}	1-30	0.91731	0.150	1.987
Exponential Y = Ae ^{Bx} i.e. I = Ae ^{Bt} or ln I = lnA + Bt	5×10^{-6}	1-30	0.99101	0.031	0.327
	1×10^{-4}	1-30	0.99396	0.025	1.200
Logarithmic Y = A + B ln x i.e. ln I = B ln t	5×10^{-6}	1-30	0.98886	0.441	-0.316
	1×10^{-4}	1-30	0.91731	0.150	1.987

3.5. Effect of scan rate

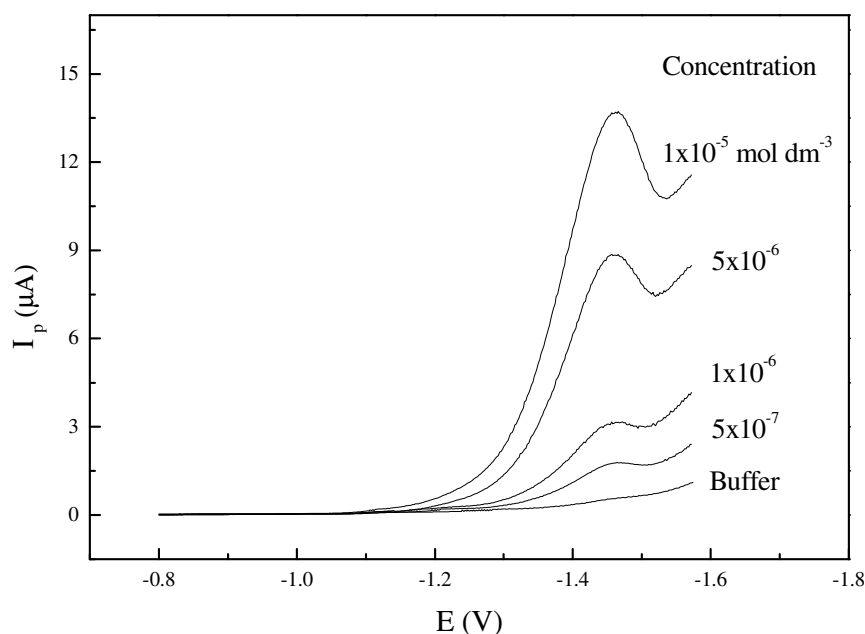
By increasing the scan rate, the peak current for 1×10^{-5} mol dm⁻³ cephapirin and cefamandole and cefmetazole increased from 25 to 150 mVs⁻¹. At higher values the peak shape distorted. Scan rate of 100 mVs⁻¹ was selected for further work.

On plotting Log I_p against Log V (Log scan rate) for 1×10^{-5} mol dm⁻³ cephapirin, a linear relation was observed over the range 25 mVs⁻¹ to 150 mVs⁻¹ with a slope of 1.1, with 0.992 correlation coefficient. A slope of 1.00 is expected for an ideal reaction of surface species [17].

3.6. Effect of concentration

The effect of different concentrations from each drug ranged from 2×10^{-8} mol dm⁻³ to 1×10^{-4} mol dm⁻³ on the resulted peak response was studied. Fig. 5 illustrates typical voltammograms for different cephalpirin concentrations, using 30s accumulation time 0.0 V deposition potential and 100 mVs⁻¹ scan rate. An increase in the peak current with increasing the concentration, with a linear relation from 1×10^{-7} mol dm⁻³ to 1×10^{-5} mol dm⁻³, correlation coefficient of 0.998 and a slope of 0.555 were obtained.

Figure 5. Typical cathodic stripping voltammograms for different concentrations of cephalpirin in



phosphate buffer (pH 5.5), accumulation potential 0.0 V and scan rate 100 mV/s, 30 s deposition time.

3.7. Validation of the method

Under the proposed experimental parameters mentioned above, the standard curves of the studied cephalosporins were established. The equation for the calibration graph $C_{\mu} = a + bc$, where C_{μ} is the current (in μA) and c is the concentration of the studied drugs. The data obtained from the least-squares analysis are given in Table 2. The detection limits calculated as $3\sigma/b$, where b is the slope and $\sigma = \text{SD}$ of the intercept and the quantitative limits also calculated [18-26] as $10\sigma/b$. It can be seen that cephalpirin can be detected at 1×10^{-7} mol dm⁻³ (correlation coefficient, $R = 0.998$ and $n = 5$), LoD for cefamandole = 1×10^{-8} mol dm⁻³ ($R = 0.998$ and $n = 5$), and LoD for cefmetazole = 1×10^{-8} mol dm⁻³ ($R = 0.999$ and $n = 5$). The linearity is ranged from (1×10^{-7} to 1×10^{-5}), (1×10^{-6} to 8×10^{-5}) and (1×10^{-6} to 1×10^{-4}) mol dm⁻³ for cephalpirin, cefamandole and cefmetazole, respectively.

Table 2. Analytical parameters of cathodic stripping voltammeter (CSV) for the determination of cephalirin, vefamandole and cefmetazole

Drug	Linearity range (mol dm ⁻³)	Correlation coefficient (R)	Slope (nA/mol)	LoD (mol dm ⁻³)
Cephapirin	5×10 ⁻⁶ -5×10 ⁻⁴	0.9982	0.21345	1×10 ⁻⁷
Cefamandole	3×10 ⁻⁵ -5×10 ⁻⁴	0.9983	5.539	1×10 ⁻⁸
Cefmetazole	5×10 ⁻⁶ -5×10 ⁻⁴	0.9983	4.45	1×10 ⁻⁸

LoD = Lower detection limit

3.8. Reproducibility

Successive measurements of 5×10⁻⁷, 1×10⁻⁵ and 5×10⁻⁷ mol dm⁻³ form for cephalirin, cefamandole and cefmetazole, respectively, using the optimized conditions for each drug were examined. The resulted voltammograms showed good reproducible resets with a relative standard deviation (RSD) of 0.24, 0.36 and 0.89 % for cephalirin, cefamandole and cefmetazole, respectively.

3.9. Application

3.9.1. I- In pharmaceutical dosage forms

The developed method is applied to the determination of for cephalirin, cefamandole and cefmetazole in the commercial dosage forms in the local market. Good recoveries were obtained in all determined preparations (Table 3).

Table 3. Analytical recovery of cephalirin, cefamandole and cefmetazole from their dosage forms (n = 5).

Dosage form	Claimed/ mg	% recovery
Cephatrexy l vial	500	97.3
Mandol vial	500	101.2

3.9.2. II- Analysis of biological fluids (serum and urine)

For the determination of the examined drugs in serum samples, one ml of human serum was diluted to 10 ml by the supporting electrolyte. Different concentrations from the drug were added and

the resulted voltammograms were recorded. On plotting the resulted current versus the concentration, a straight lines were observed with concentrations ranged from 5×10^{-5} - 5×10^{-4} , 9×10^{-5} - 5×10^{-4} and 5×10^{-5} - 5×10^{-4} mol dm⁻³ for cephalosporin, cefamandole and cefmetazole, respectively.

Cephalosporin, cefamandole and cefmetazole are extracted in urine through glomerular filtration, which approximately 20 to 30% of cephalosporin is rapidly metabolized to deacetylated form in the liver and about 60 to 70% of a dose is extracted in the urine [27]. Measurement of the drug in a spiked urine sample was demonstrated and the resulted voltammograms were recorded. On plotting the resulted current versus the concentration, a straight lines were observed with concentrations ranged from 5×10^{-6} - 5×10^{-4} , 3×10^{-5} - 5×10^{-4} and 5×10^{-6} - 5×10^{-4} mol dm⁻³ for cephalosporin, cefamandole and cefmetazole, respectively.

3.10. Interferences study

The influence of some metal ions, amino acids, urea and ascorbic acid, which are potent interfering compounds presented in biological samples on the response of the studied drugs were investigated. The resulted data were collected in Table 4.

Table 4. The effect of some metal ions, amino acids, urea and ascorbic acid (1×10^{-5} of each) on the signals for cephalosporin, cefamandole and cefmetazole.

Interferences Drug	Metal ions			Amino acids			Urea %	L- ascorbic acid %
	Copper (II) %	Lead (II) %	Cadmium (II) %	L- alanine %	L- valine %	Glycine %		
Cephalosporin [5×10^{-5} mol dm ⁻³]	-3.3	-13.9	- 3.9	- 6.1	- 15.2	- 0.8	17.3	5.6
Cefamandole [1×10^{-5} mol dm ⁻³]	-12	- 8.6	- 3.58	--	14.3	5.9	12.3	-22
Cefmetazole [1×10^{-6} mol dm ⁻³]	- 4.9	- 10.8	3.9	- 32.3	- 25.9	- 32	- 32	- 14.7

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