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Anodic voltammetry of cefotaxime

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Abstract

In this study electrooxidation of cefotaxime was investigated using specially activated glassy carbon (GC), platinum and carbon paste (CP) electrodes in different supporting electrolyte solutions and at different pHs. The data revealed that the shapes of the voltammograms and the numbers of the oxidation steps changed depending on the nature of the electrode. The nature of the supporting electrolyte was also important for the response of the electrode. From an analytical point of view, the activated GC electrode was the most favourable one. In 0.2 M H_3PO_4 with an activated GC electrode the calibration graph gave two lines with different slopes in the concentration ranges of 2×10^{-5}–1×10^{-4} and 2×10^{-4}–6×10^{-4}. The results of the recovery test and statistical analysis showed that the voltammetric method could be used for the determination of cefotaxime.

Keywords: Cefotaxime; Determination; Voltammetry; Glassy carbon electrode

1. Introduction

Cephalosporins are semi-synthetic antibiotics of the β-lactam family. β-Lactam antibiotics are used most frequently in human medicine on account of their broad antibacterial activity spectrum and low toxicity. Their activity is exerted through the inhibition of some enzyme reactions vital to bacteria which hinders the formation of the bacterial wall.

Penicillins, cephalosporins and cefamycins are the main families of β-lactam antibiotics. The microbiological activity, chemical structure, pharmacokinetic action and clinical use are very similar for the three families, but cephalosporins and cefamycins have the added advantage of being applicable to penicillin-allergic patients. They are also active against a large number of gram-positive bacteria including β-lactamase-producing staphylococci. Cefotaxime and cefsulodine are two such antibiotics of choice for the treatment of meningitis caused by gram-negative bacteria.

Cefotaxime, the structure of which is given in Fig. 1, is a third-generation cephalosporin.

Although the electrochemical reductions of cephalosporins have been widely studied [1–9], very few papers deal with their electrochemical oxidation [10].

In the present study the anodic voltammetry of cefotaxime was investigated using glassy carbon (GC), carbon paste (CP) and Pt electrodes in various electrolyte solutions having different pHs.

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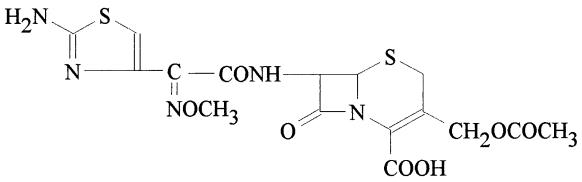

Fig. 1. Structure of cefotaxime.

The apparent rates of electron transfer are slower at GC electrodes than at metallic ones. Previous tests performed in our laboratory showed that the application of some pretreatment steps such as high-frequency square-wave and high-frequency triangular potential signals be-

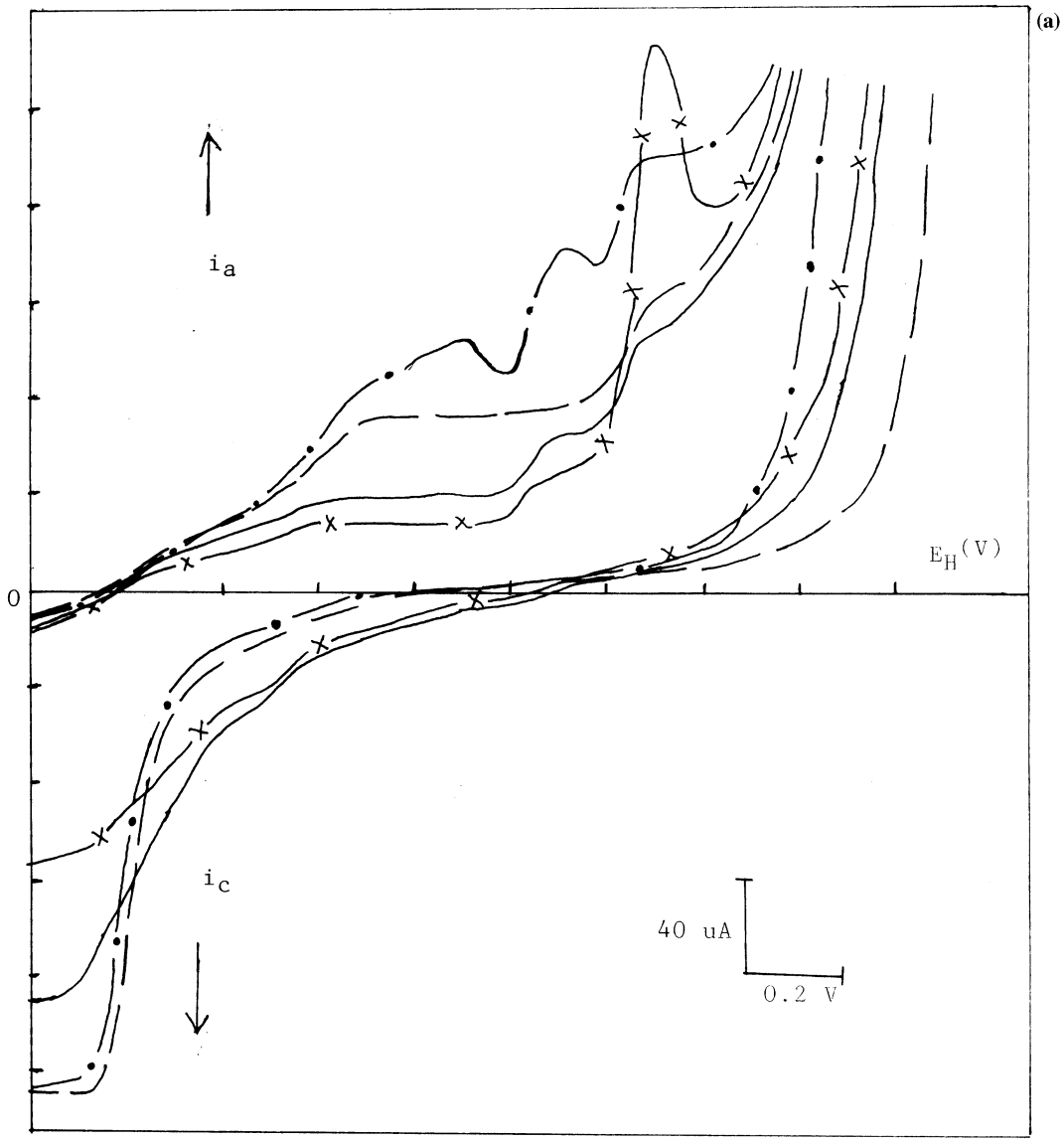

Fig. 2. (a) Voltammograms of cefotaxime obtained using activated GC electrode in 0.5 M H_2SO_4 and 0.2 M H_3PO_4. Scan rate 100 mV s^{-1}. (— — —) 0.5 M H_2SO_4; (– · –) 0.5 M $H_2SO_4 + 2 \times 10^{-4}$ M cefotaxime; (——) 0.2 M H_3PO_4; (– × –) 0.2 M $H_3PO_4 + 2 \times 10^{-4}$ M cefotaxime. (b) I_p–$V^{1/2}$ relation obtained for GC electrode in 0.2 M H_3PO_4 containing 2×10^{-4} M cefotaxime solution ($r = 0.9993$, $m = 5.95$, $n = -10.70$).

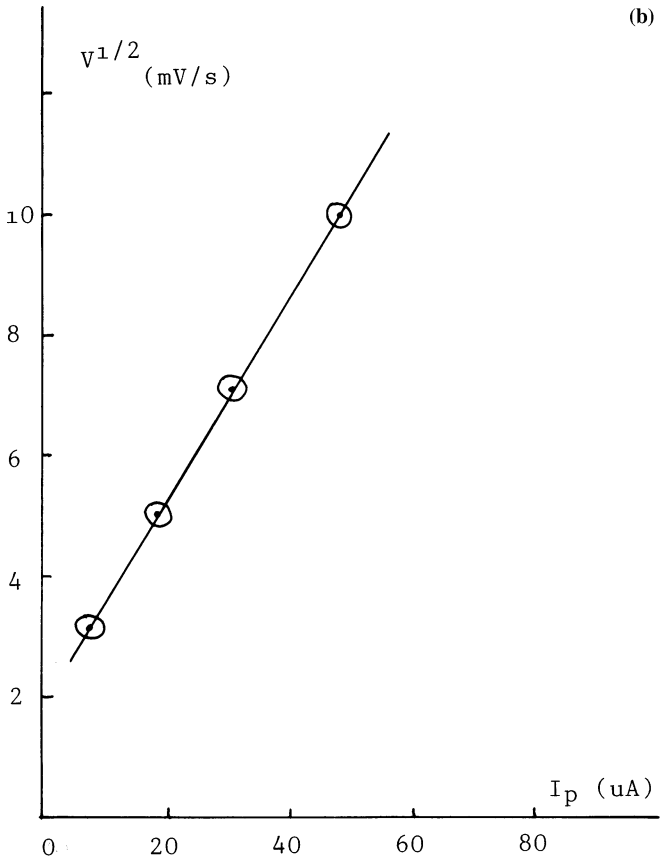

Fig. 2. (*Continued*)

tween ±6 V made the electrode active for many electrode reactions. The activated electrode was applied to the electroanalysis of some substances [11,12].

The present study revealed that the activated GC electrode was convenient for the electroanalysis of cefotaxime.

2. Experimental

2.1. Apparatus

The measurements were taken and the voltammograms were recorded with a PRG-3 polarograph and an EPL-2 recorder (Tacussel Electronique). A Wenking model HP-70 potentiostat and an Exact-type 250 function generator were employed for applying square-wave and high-frequency multiscan triangular signals. A GC electrode (Tacussel XM 540; area, 1.013 cm^2) and a platinum wire (Tacussel; diameter, 1 mm; length, 15.7 mm) and a CP electrode, prepared by mixing graphite powder (Aldrich) and mineral oil (Sigma), were used as working electrodes. A platinum wire and a saturated calomel electrode (Tacussel XR-100) were used as counter and reference electrodes, respectively. All the potentials in the text are given vs. normal hydrogen electrode.

2.2. Reagents

Cefotaxime sodium (generously provided by Bilim Drugs Industries, Istanbul, Turkey) was *used*

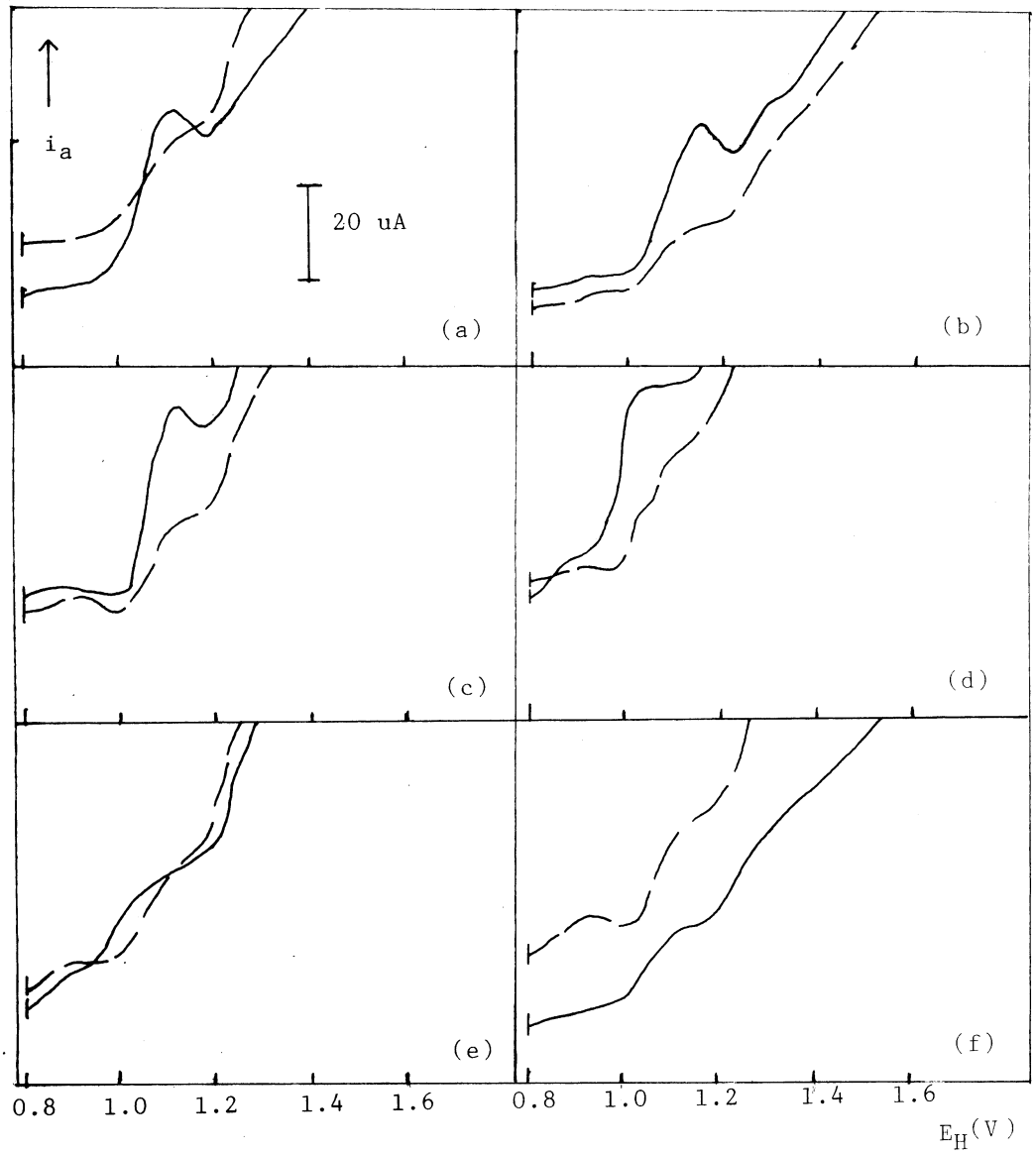

Fig. 3. (a–f) Voltammograms of cefotaxime obtained with activated GC electrode in acetate and phosphate buffers. (— — —) supporting electrolyte, (——) supporting electrolyte and 2×10^{-4} M cefotaxime. Scan rate, 100 mV s^{-1}. (a) pH 4.5 acetate buffer, (b) pH 5.4 phosphate buffer, (c) pH 6.4 phosphate buffer, (d) pH 7.4 phosphate buffer, (e) pH 8.5 phosphate buffer, (f) pH 10.0 phosphate buffer. Effects of pH on (g) the peak potential and (h) the peak current. Cefotaxime concentration, 2×10^{-4} M; scan rate, 100 mV s^{-1}; electrode, glassy carbon; solution, phosphate buffer.

without further purification. All other reagents were of analytical grade.

Stock solutions were prepared daily by dissolving cefotaxime in selected supporting electrolytes, namely sulphuric acid (0.5 M), phosphoric acid (0.2 M), acetate buffers (pH 3.5 and 4.7; 1.0 M), phosphate buffers (pH 5.0–10.00; 0.2 M). Double-distilled water was used to prepare the solutions.

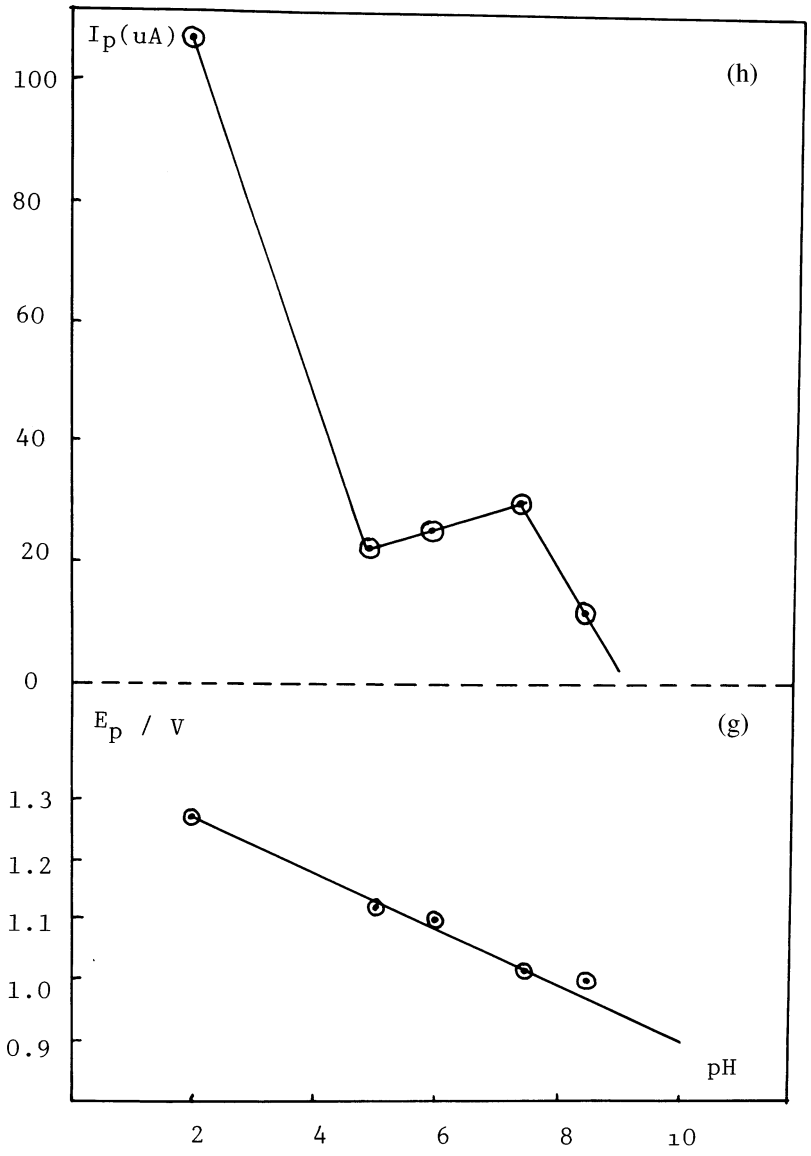

Fig. 3. (*Continued*)

2.3. Pretreatment of the electrodes

(a) The platinum electrode was oxidized in 0.5 M H_2SO_4 solution at the oxygen evolution potential for 5 min and then it was washed with double-distilled water after the circuit was disconnected, a potential of 400 mV was then applied until the current became zero.

(2) The GC electrode is rather inert in comparison with the metallic electrodes. In order to activate this electrode it is thus necessary to apply some pretreatment. Previous tests performed in our laboratory showed that the application of high-frequency (340 Hz) square-wave and high-frequency (3500 Hz) triangular potential signals between ± 6 V and subsequent application of

Table 1
Results of linear regression analysis of concentration–limiting current relationship at 1300 mV in the concentration ranges of (A)[a] 2×10^{-5}–1×10^{-4} and (B)[b] 2×10^{-4}–6×10^{-4}

Concentration (M)	Limiting current (μA)
Range A	
2×10^{-5}	25
4×10^{-5}	39
6×10^{-5}	53
8×10^{-5}	66
1×10^{-4}	80
Range B	
2×10^{-4}	107
4×10^{-4}	132
6×10^{-4}	153

Obtained with activated GC electrode in 0.2 M H_3PO_4 solution.
[a] Correlation coefficient = 0.9999; slope = 6.9×10^{5}; y-intercept = 11.5; SE of slope = 5.00×10^{3}; SE of intercept = 0.33.
[b] Correlation coefficient = 0.9885; slope = 1.15×10^{5}; y-intercept = 86.00; SE of slope = 1.73×10^{4}; SE of intercept = 7.48.

potentials of +1750 mV for 5 min and −750 mV for 2 or 3 s in 0.1 M $NaNO_3$ made the electrode active for many electrode reactions. This procedure was repeated until the voltammograms became reproducible. Once this active state was reached it was stable for nearly 1.5 months. Before each experiment this activated electrode was pretreated by the application of +1750 mV for 5 min and −750 mV for 2 or 3 s.

3. Results and discussion

Previous studies carried out on the series of cephalosporins and their decomposition products indicate that aminothiazole substituent on the side chain in position 7 of the Δ_3-cephem ring is the electroactive group that undergoes anodic oxidation in the case of cefotaxime [10].

Cathodic reduction of cephalosporins has been widely investigated and published but there are only few references in the literature dealing with the anodic oxidation of these compounds [10]. It was reported that cephalosporins were electroactive at the GC electrode [10]. Our experiments on cefotaxime revealed that the reproducibility of the voltammograms with the activated GC electrode in the oxidative direction was better than in the reductive direction. The tests were performed with scan rates of 10, 25, 50 and 100 mV s^{-1}. Regarding the reproducibility of the curves and the ratio of peak current to background current, the best results were obtained with 100 mV s^{-1}.

In 0.5 M H_2SO_4 solutions three steps were obtained, as shown in Fig. 2a. These steps are at the potentials where the reactions of GC functional groups take place. The first two steps appear as broad peaks at about 900 and 1100 mV. The third one beginning from 1150 mV reaches a limiting current region at about 1250 mV. At the reverse scan no clear reduction step related to the substance can be seen on the curves.

In 0.2 M H_3PO_4 the voltammograms have also three steps. The most pronounced one is a peak at about 1300 mV as can be seen in Fig. 2a. When the peak current of this peak was plotted against the square root of the scan rate, a linear graph was obtained (Fig. 2b) indicating the diffusion-controlled reaction. The peak current of this peak increases with the increase in cefotaxime concentration, and this relationship is as two linear sections with different slopes. For analytical purpose the first concentration region 2×10^{-5}–1×10^{-4} M is convenient (Table 1).

On the curves obtained in phosphate buffers (Fig. 3) the first two steps are ill defined, and the third is a well-defined peak-shaped step, the peak potential of which is 1150 mV at pH 5.4 and shifts towards less positive values with increase in pH (Fig. 3g). At pH 10 background current becomes higher than faradaic current; this may be because of the strong adsorption of the cefotaxime on the electrode. At the reverse scan no reduction peak was observed in phosphate buffer. The current of the third peak is most favourable in 0.2 M H_3PO_4 (Fig. 3h). There is not an important difference in shape between the curves obtained in acetate buffer of pH 4.5 and in phosphate buffers, but in acetate buffer the ratio of peak current to background current is less than in phosphate buffer.

With the CP electrode the curves obtained in 0.5 M H_2SO_4 and 0.2 M H_3PO_4 supporting electrolytes are given in Fig. 4. The third peak at

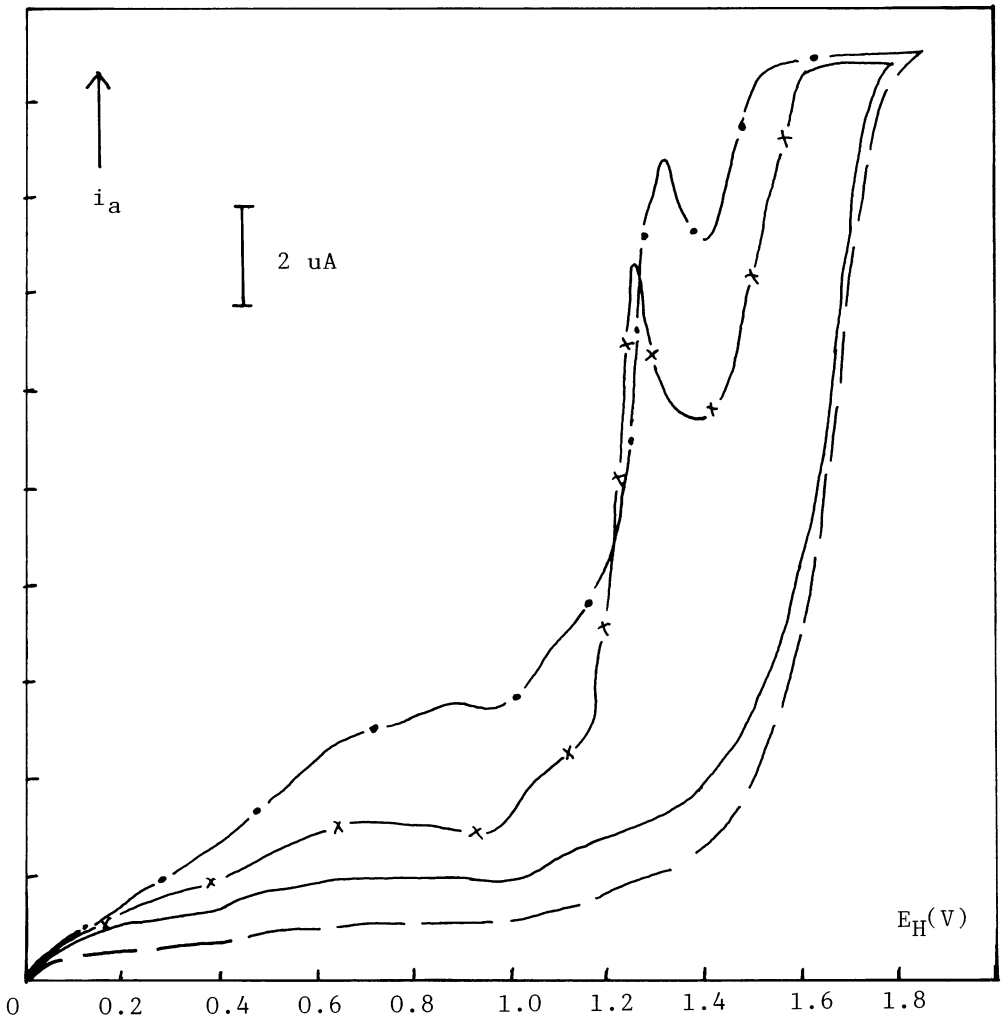

Fig. 4. Voltammograms of cefotaxime obtained with CP electrode in 0.5 M H_2SO_4 and 0.2 M H_3PO_4. Scan rate, 100 mV s^{-1}. (— — —) 0.5 M H_2SO_4, (– · –) 0.5 M $H_2SO_4 + 2 \times 10^{-4}$ M cefotaxime, (——) 0.2 M H_3PO_4, (– × –) 0.2 M $H_3PO_4 + 2 \times 10^{-4}$ M cefotaxime.

Table 2
Results of linear regression analysis of concentration–limiting current relationship at 1300 mV

Concentration (M)	Limiting current (μA)
2×10^{-5}	3.8
4×10^{-5}	5.0
6×10^{-5}	6.2
8×10^{-5}	7.5

Obtained with CP electrode in 0.2 M H_3PO_4 solution. Correlation coefficient = 0.9998; slope = 6.14×10^4; y-intercept = 2.55; SE of slope = 8.7×10^2; SE of intercept = 4.74×10^{-2}.

nearly 1250–1300 mV is sharp but the first and second peaks observed in Fig. 2 with the GC electrode, are not well defined in the case of the CP electrode. The shapes of the curves obtained in phosphate buffers having different pH values with CP electrode are similar to those obtained with the GC electrode. In 0.2 M H_3PO_4 the peak current of the third peak is linearly dependent on cefotaxime concentration (Table 2).

With the Pt electrode in 0.5 M H_2SO_4 solution two steps are seen on the curves (Fig. 5). The first one begins at 1000 mV and reaches a limiting

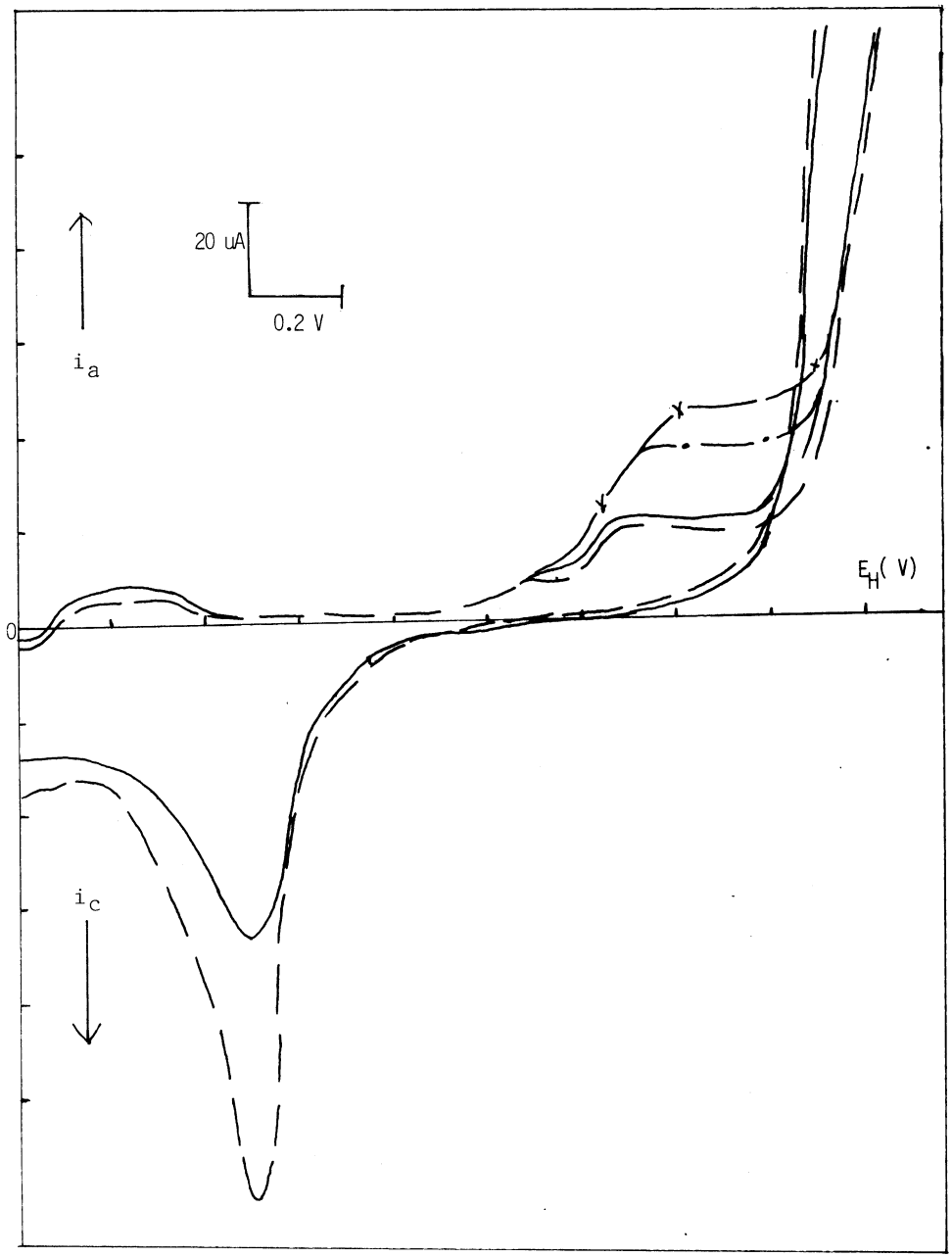

Fig. 5. Voltammograms of cefotaxime obtained with platinum electrode in 0.5 M H_2SO_4. Scan rate, 100 mV s^{-1}. (— — —) 0.5 M H_2SO_4, (——) 0.5 M $H_2SO_4 + 1 \times 10^{-5}$ M cefotaxime, (– · –) 0.5 M $H_2SO_4 + 2 \times 10^{-4}$ M cefotaxime, (– × –) 0.5 M $H_2SO_4 + 1 \times 10^{-3}$ M cefotaxime.

current region lying between 1050 and 1150 mV. The second step beginning from 1200 mV shows a limiting current region in the potential range of 1250–1650 mV. At higher cefotaxime concentrations two steps overlap and only one limiting current region appears, the beginning potential of

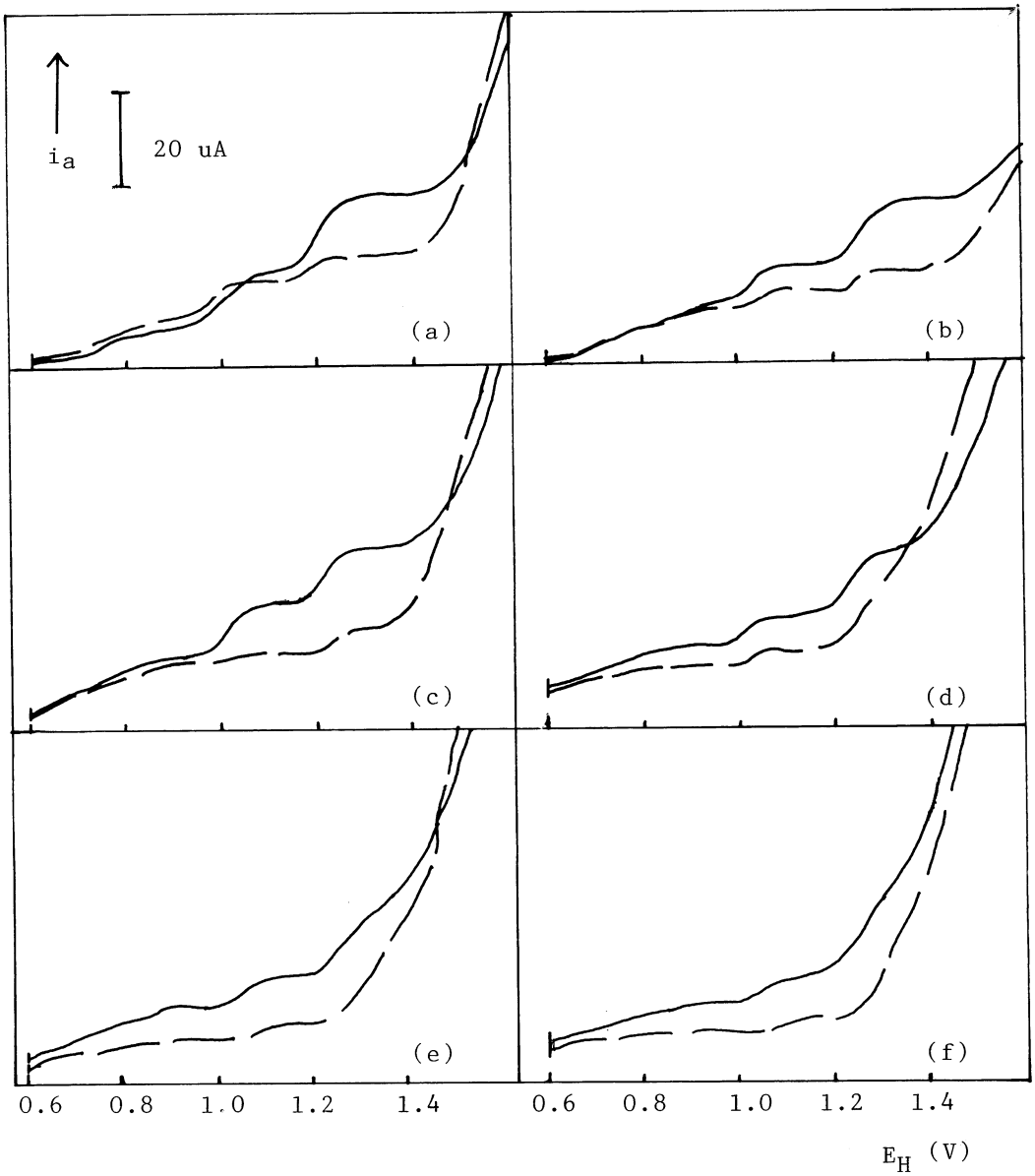

Fig. 6. Voltammograms of cefotaxime obtained with platinum electrode in acetate and phosphate buffers. (— — —) supporting electrolyte, (——) supporting electrolyte and 2×10^{-4} M cefotaxime. Scan rate, 100 mV s^{-1}. (a) pH 4.5 acetate buffer, (b) pH 5.4 phosphate buffer, (c) pH 6.4 phosphate buffer, (d) pH 7.4 phosphate buffer, (e) pH 8.5 phosphate buffer, (f) pH 10.0 phosphate buffer.

which shifts to more positive values with increase in cefotaxime concentration. On the reduction branch only one concentration is given as an example. Oxidation of cefotaxime takes place at the potential at which surface oxides form, and a reduction peak is seen at the potential where the reduction of the surface oxides takes place [13]. But the peak current of this reduction peak in the supporting electrolyte is higher than in cefotaxime solution. This can be because of the adsorbed

Table 3
Recovery results of cefotaxime vials by voltammetry in 0.2 M H_3PO_4 with GC electrode

Sample no.	Added (mg)	Found (mg)	Recovery (%)
1	25	23.8	96.0
2	25	23.8	96.0
3	25	24.7	99.0
4	25	25.7	102.8
5	25	25.7	102.8
6	25	24.0	96.0
7	25	24.2	96.8
8	25	24.8	99.2
9	25	23.8	96.0
10	25	24.8	99.2
Mean value		24.53	98.38
Standard deviation		0.738	2.707
Standard error		0.233	0.856
Relative standard deviation		3.01	2.75

materials which occupied the active sites on the electrode. In 0.2 M H_3PO_4 with this electrode for cefotaxime the ratio of faradaic to background current was found to be smaller than that in 0.5 M H_2SO_4 solution. This may be because of the difference in the structure of the surface film on Pt in phosphoric acid solution.

The curves obtained with the Pt electrode in acetate and phosphate buffers are given in Fig. 6. Generally two steps are seen on the curves. The first step begins at about 950 mV, and the beginning potential of the second step is about 1150 mV. There is not a large shift in the potential of the steps with the increase in pH, but when pH exceeds 8.5 the second step disappears.

A satisfactory linear current-concentration relationship could not be obtained with the Pt electrode in any of the electrolytes.

4. Analytical evaluation

The reliability of the voltammetric method was checked by recovery test as this drug is only available in vials containing the pure substance. The results of recovery test reveal that this method can be used for the determination of cefotaxime (Table 3).

5. Conclusion

(1) This paper reveals that the anodic voltammetry using CP and activated GC electrodes can be used for the determination of cefotaxime. But the concentration range in the case of activated GC electrode is wider.

(2) The voltammograms showed a good repeatability only in strong acidic media; the most suitable electrolyte was 0.2 M H_3PO_4.

(3) The voltammetric method described in this work has the advantage of being rapid simple and inexpensive.

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