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Study on electrooxidation of cefadroxil monohydrate and its determination by differential pulse voltammetry

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Abstract

In this work electrooxidation of cefadroxil monohydrate was investigated using a glassy carbon electrode depending on pH and supporting electrolyte. It was shown that the direct determination of the substance from capsules and in oral suspension could be made by differential pulse voltammetry (DPV). UV and first derivative UV spectrophotometric methods are also proposed as comparative methods.

Keywords: Cefadroxil monohydrate; Electrooxidation; Differential pulse voltammetry (DPV); UV spectrophotometry; Determination

1. Introduction

Cephalosporins are β-lactam antibiotics and they are closely related in structure and in antibactericidal action mechanism to penicillins and cephamicins which are also β-lactam antibiotics. The main nucleus of cephalosporins is 7-amino cephalosporanic acid (7-ACA) which is a cephem derivative and is obtained from cephalosporin C which is formed as a fermentation product of *cephalosporium acremonium* type of fungus. Cephalosporins which are used for therapeutic purposes are semisynthetic products.

Electroreduction of cephalosporins has been intensively investigated by polarography [1–7], but only a few papers were found in the literature concerning oxidative voltammetry [7,8].

Cefadroxil is a first class cephalosporin and is effective against gram-positive cocci.

In the present study electrooxidation of cefadroxil was investigated using a glassy carbon electrode (GCE) by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in order to throw light on the reaction mechanism and to propose a method for the determination of this substance.

2. Experimental

2.1. Apparatus

The measurements were taken and curves were obtained using a BAS 100 W/B electrochemical

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analyser and an HP 5L printer. Working and counter electrodes were a BAS MF 2012 glassy carbon disk and a BAS MV 1032 platinum, respectively. A BAS MF 1063 type silver/silver chloride electrode was used as reference. In the text all potentials are given versus silver/silver chloride electrode.

A double beam, Shimadzu 1601 spectro photometer model with a fixed slit width (2 nm) connected to an IBM PC computer with a Lexmark printer was used for all the absorbance signals and treatment of data.

2.2. Reagents

Cefadroxil monohydrate, kindly provided by Eczacibasi Drug (Istanbul, Turkey) was used without further purification. All other reagents were of analytical grade. A stock solution of 10^{-3}

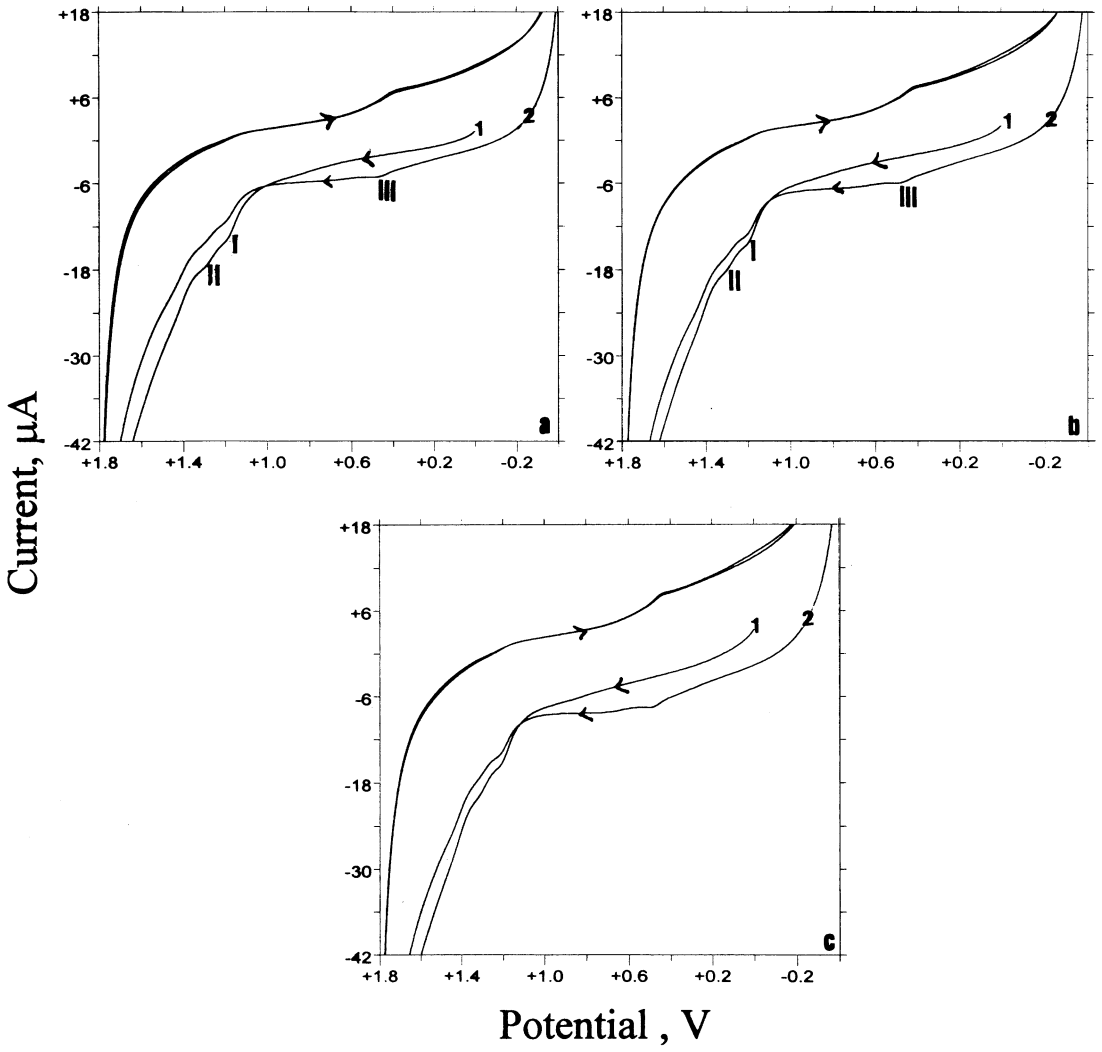

Fig. 1. Cyclic voltammograms obtained in (a) 0.1-M H_2SO_4; (b) 0.2-M H_2SO_4; (c) 0.5-M H_2SO_4 solutions containing 2×10^{-4} M cefadroxil: (1) first scan; (2) second scan.

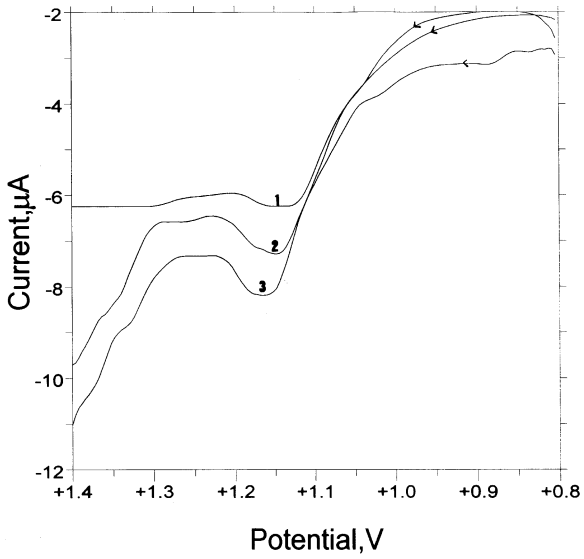

Fig. 2. Differential pulse voltammograms of 2×10^{-4} M cefadroxil obtained in (1) 0.1-M H_2SO_4, (2) 0.2-M H_2SO_4, and (3) 0.5-M H_2SO_4 solutions. Scan rate: 20 mV s^{-1}; pulse amplitude: 50 mV; sample width: 17 ms; pulse width: 50 ms; pulse period: 200 ms.

M cefadroxil was prepared in MeOH. Standard solutions were prepared using this stock solution and contained 20% MeOH and 80% buffer. Britton-Robinson (BR) buffers were prepared using 0.04 M phosphoric, acetic and boric acids. pH was adjusted by the addition of 6 M NaOH solution. All solutions were prepared using doubly distilled water.

2.3. Pretreatment of the working electrode

In order to obtain a clean surface the electrode was polished using alumina ($\Phi = 0.01$ μm) on a polishing pad and then carefully washed with bidistilled water and dried on a filter paper.

2.4. Analysis of pharmaceutical dosage forms

2.4.1. Capsules

In the case of capsule form, the capsule contents were taken and by weighing the proper amount of this substance a stock solution (the concentration of which was assumed to be 10^{-3} M) was prepared by dissolving and diluting it with phosphate buffer of pH 7 in case of DPV and with methanol in case of spectrophotometry.

2.4.2. Oral suspension

A stock solution $\sim 10^{-3}$ M for oral suspension of cefadroxil was prepared by taking proper volume of suspension and diluting it with phosphate buffer of pH 7 for DPV and with methanol for spectrophotometry in a volumetric flask.

By diluting this stock, 2×10^{-4} M cefadroxil solutions were prepared. Measurements were made in these solutions and the amount of cefadroxil was calculated.

3. Results and discussion

Tests were performed using glassy carbon electrode at different scan rates in various supporting electrolyte solutions, namely 0.1-, 0.2- and 0.5-M H_2SO_4 and phosphate buffers of pH 5.5, 6.5 and 7.0, and Britton-Robinson (BR) buffers in the pH range covering 2, 3, 4, 6, 7, 8 and 10.4. In 0.1- and 0.2-M H_2SO_4 supporting electrolytes, cyclic voltammograms of 2×10^{-4} M cefadroxil showed two ill-defined anodic peaks at ~ 1.150 V (peak I) and 1.300 V (peak II) and a cathodic peak at 0.375 V at the first scan. In 0.5-M H_2SO_4 solution peak II became broader (Fig. 1). At the second scan peak currents of both of the oxidation peaks decreased and peak II nearly disappeared, and a new anodic peak at ~ 0.450 V (peak III) appeared. This points to an intermediate species more easily oxidised than starting material. Differential pulse voltammograms (DPV) of cefadroxil obtained in H_2SO_4 solutions are seen in Fig. 2. In 0.5-M H_2SO_4 solution a well-defined peak at ~ 1.16 V (peak I) is seen. When H_2SO_4 concentration decreased this peak was split and shifted to less positive potentials. In BR buffer solution of pH 2 cyclic voltammograms were recorded by different scan rates: on the CV curves obtained by 25 mV s^{-1}, two anodic peaks at ~ 0.95 and 1.15 V and a cathodic peak at 0.300 V were obtained similar to those obtained in H_2SO_4 solutions. When scan rate increased anodic peaks slightly shifted to more positive potentials as ex-

pected from a non-reversible reaction. With scan rates of 200 mV s^{-1} and higher, all the peaks became ill-defined. At the second and following scans a new anodic peak (peak III) was observed as in the case of H_2SO_4 solutions.

In H_2SO_4 solutions peak I nearly disappeared and peak II became ill-defined and split into two peaks in 2×10^{-4} M and higher concentrations with increased scan rate and number of scans.

In lower concentrations two anodic peaks (peak I, peak II) could be observed at second and following scans at lower scan rates (e.g. 50–100 mV s^{-1}). Only peak I appeared when scan rate exceeded 100 mV s^{-1}. This peak could barely be observed in 10^{-4} M and more dilute solutions. This points to adsorption of the substance at the electrode surface. At lower scan rates after the first scan there was enough time for cefadroxil

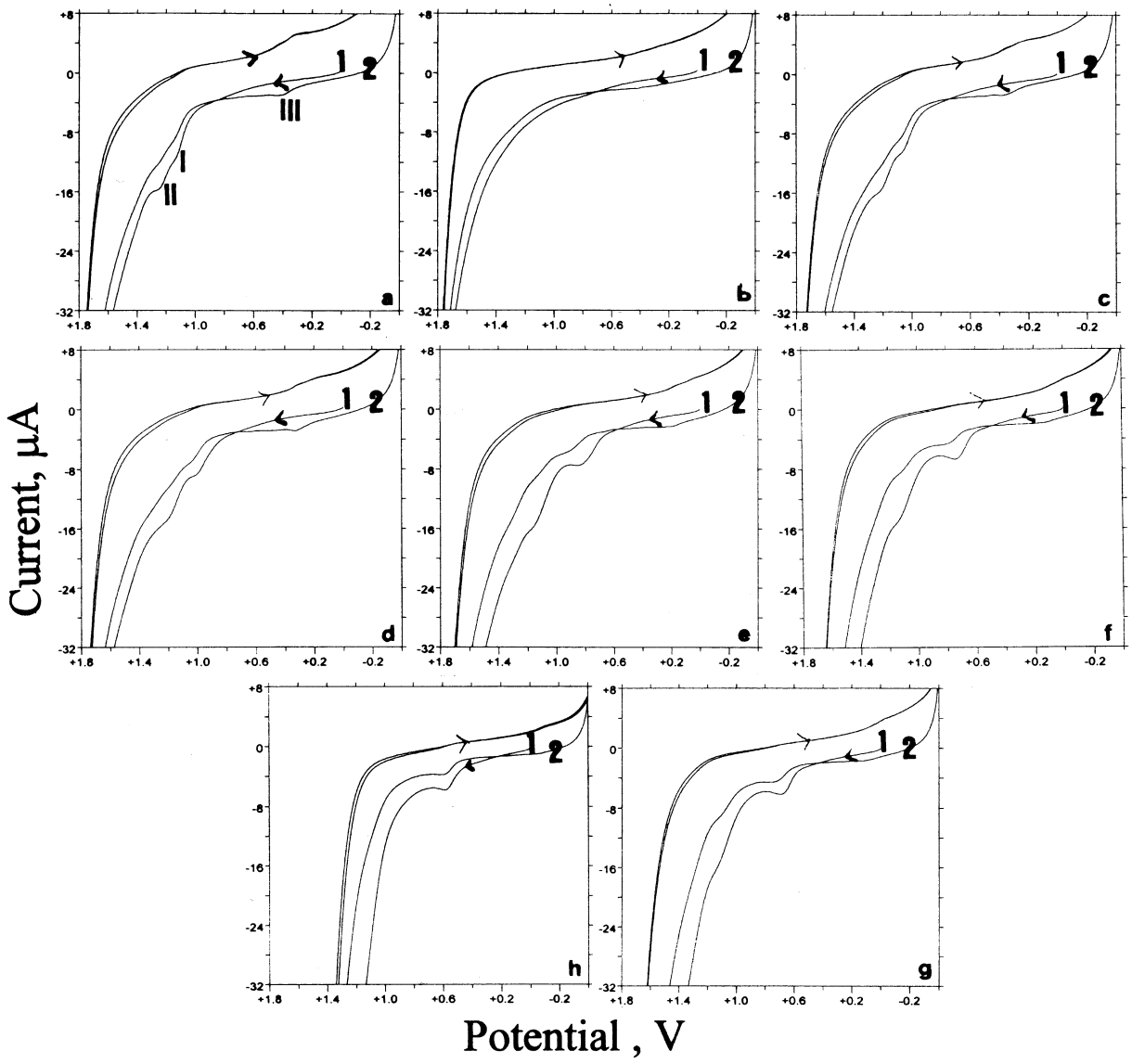

Fig. 3. Cyclic voltammograms of 2×10^{-4} M cefadroxil recorded in BR buffer of various pH: (a) 2.0; (b) 3.0 BR buffer supporting electrolyte only; (c) 3.0; (d) 4.0; (e) 6.0; (f) 7.0; (g) 8.0; (h) 10.4. Scan rate: 100 mV s^{-1}.

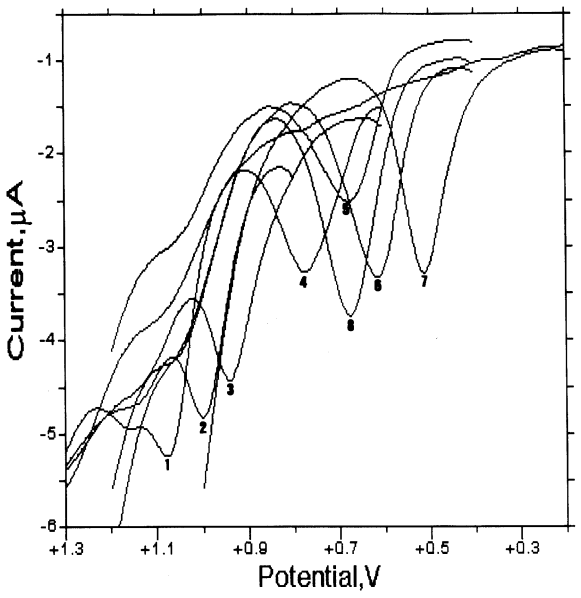

Fig. 4. Differential pulse voltammograms of 2×10^{-4} M cefadroxil recorded in different electrolytes: (1) BR buffer pH 2.0; (2) BR buffer pH 3.0; (3) BR buffer pH 4.0; (4) BR buffer pH 6.0; (5) BR buffer pH 7.0; (6) BR buffer pH 8.0; (7) BR buffer pH 10.4; (8) phosphate pH 7.0. Scan rate: 20 mV s^{-1}; pulse amplitude: 50 mV; sample width: 17 ms; pulse width: 50 ms; pulse period: 200 ms.

molecules to reach the electrode by diffusion and these peaks could be observed. When scan rate increased at first scan, mainly adsorbed molecules took part in the electrode reaction but at the following scans, as the time for a new adsorbtion equilibrium decreased, the peaks could not be seen clearly at the scan rate of 100 mV s^{-1}. Instead of the new peak at 0.300 V which appeared beginning from the second scan, two peaks were recorded at 0.15 and -0.05 V. This may be because of the oxidation of products of cefadroxil took place in a chemical step to produce a substance which was reduced at ~ 0.3 V but when the concentration decreased the rate of chemical step also decreased and the oxidation product itself reduced in two steps at 0.15 and -0.05V.

In BR buffer solutions when pH increased, at the first scan the peak potentials of peak I and peak II became less positive but not to the same degree. The shift of the first peak potential is more pronounced (Fig. 3). When pH increased the effect of adsorption gradually decreased and at the second and following scans the anodic peaks still could be observed. At pH 10 only the first peak could be seen even at first scan. At cathodic branch in the pH interval of 2–6, two peaks were observed. But in neutral and basic solutions only one cathodic peak was observed (Fig. 3 a–h). The slope of log scan rate versus log peak current graph for BR buffer of pH 2 was found as 0.7, while a value of 0.5 for phosphate buffer of pH 7 was obtained. Slopes of 1.00 and 0.5 are the values for ideal reactions of surface and solution, respectively, so the effect of adsorption in the acidic region is clearly seen.

Voltammograms recorded in phosphate buffers of various pH are similar to those obtained in BR buffer solutions of corresponding pH in shape and in peak potential. In Fig. 4 differential pulse voltammograms which were obtained in BR buffer and phosphate buffers are given. Evaluation of these curves revealed that quantitative determination of cefadroxil could be made by differential pulse voltammetry and the optimum conditions were found as 20 mV s^{-1} scan rate, 50-mV pulse amplitude, 17-ms sample width, 50-ms pulse width and 200-ms pulse period.

Under these conditions peak current of DPV curves are linearly dependent on concentration. Statistical treatment of this dependence is seen in Table 1. The detection limit was found as 8×10^{-6} M. Reproducibility for DPV peak current and peak potentials was tested by repeating ten experiments in 1×10^{-4} M cefadroxil. The relative standard deviation was calculated to be 0.99% with a standard deviation of 1.45×10^{-2} for peak current, and 0.81% with a standard deviation of 5.48 for peak potential. The peak potentials of the CV curves obtained in BR and phosphate buffers linearly change with the pH of the solutions, the slopes of which were 0.075 and 0.08 V/pH, respectively. Peak currents of CV and DPV are also linearly dependent on pH (Fig. 5).

CV curves were obtained for different inversion potentials in BR buffer solution of pH 2 and phosphate buffer of pH 7 (Fig. 6). According to these curves, with BR buffer solution the cathodic peak at 0.3 V is related to the anodic peak II (at 1.3 V). In phosphate buffer of pH 7 the anodic

Table 1
Characteristics of cefadroxil monohydrate calibration plot

Method	Medium	Concentration range (M)	Slope (μA/M)	Intercept (μA)	Correlation coefficient	S.E. of slope (μA/M)	S.E. of intercept (μA)
DPV	Phosphate buffer of pH 7	1×10^{-5}–4×10^{-4}	1.45×10^{4}	4.32×10^{-2}	0.997	4.44×10^{2}	3.41×10^{-2}
Zero order spectrophotometry (265.3 nm)	Methanol	2.5×10^{-5}–1.5×10^{-4}	5.07×10^{3}	4.67×10^{-3}	0.999	1.30×10^{2}	1.27×10^{-2}
First derivative spectrophotometry (277.4 nm)	Methanol	2.5×10^{-5}–1.5×10^{-4}	1.98×10^{2}	4.87×10^{-3}	0.999	5.40	5.26×10^{-4}

peak at 0.8 V is related to the cathodic peak at 0.05 V. When the peak current of this anodic peak was plotted against the square root of scan rate a line was obtained (r: 0.998, n: 0.29, m: 0.28) indicating that the reaction is diffusion controlled. Tafel plot was obtained with a scan rate of 10 mV s^{-1} beginning from a steady-state potential in phosphate buffer of pH 7 and from the slope of the linear part αn was found to be 0.5 (Fig. 7).

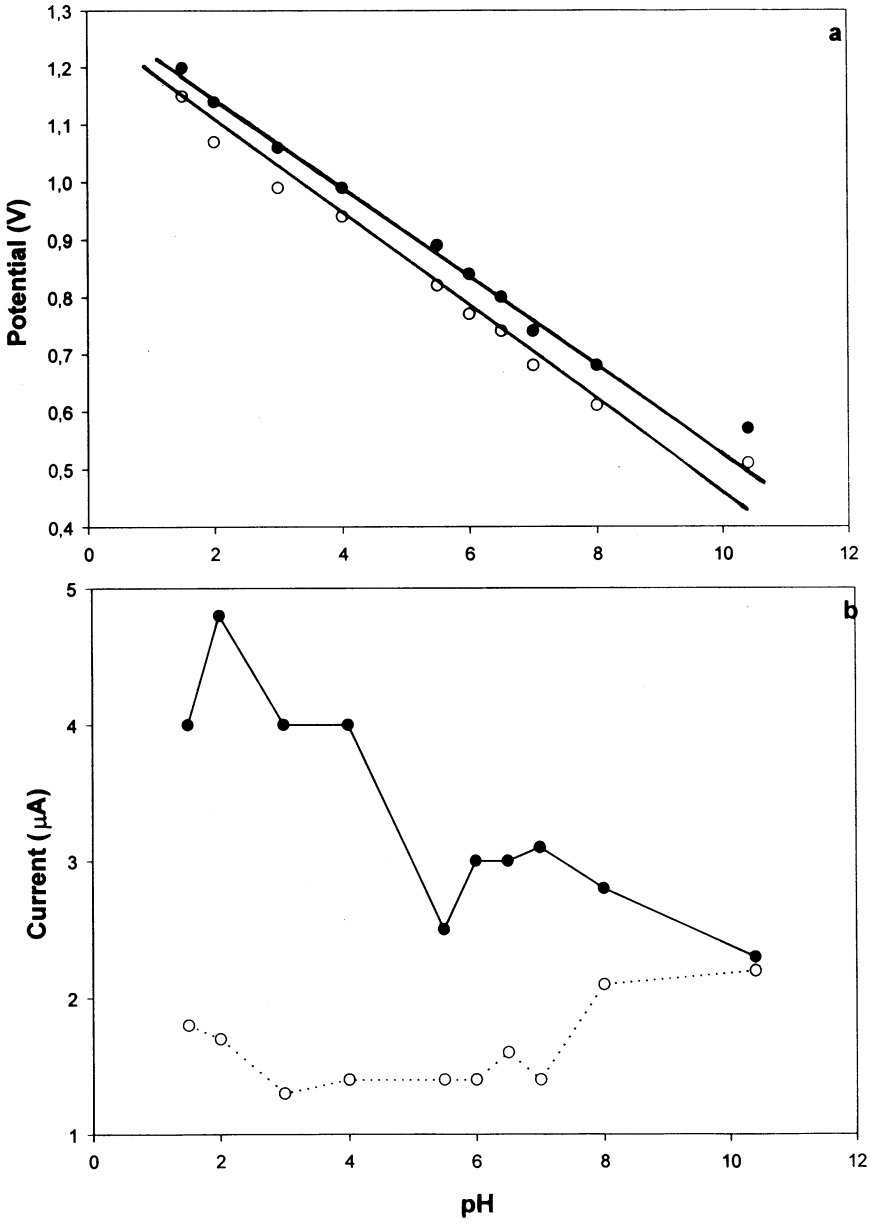

Fig. 5. Effect of the pH on cefadroxil peak potential (a) and peak current (b); (●) cyclic voltammograms; (○) differential pulse voltammograms.

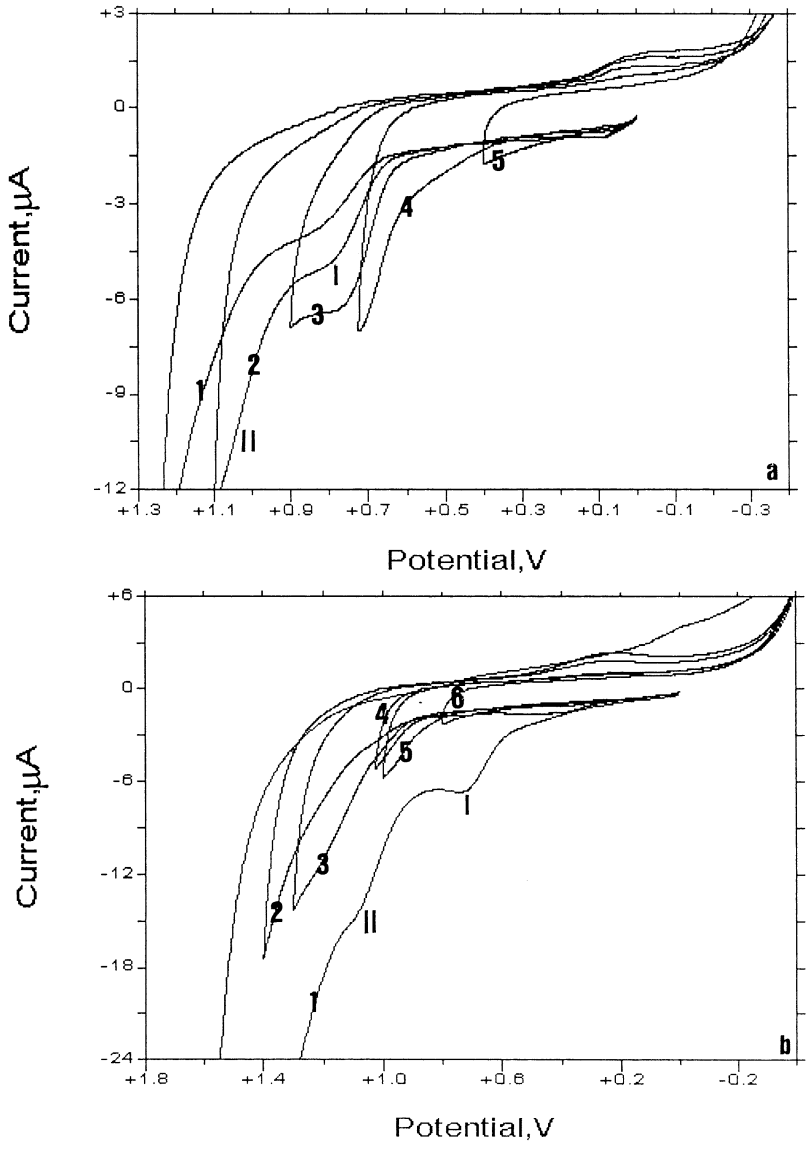

Fig. 6. Cyclic voltammograms obtained for different inversion potentials in (a) BR buffer of pH 2.0, (b) phosphate buffer of pH 7.0. Scan rate: 20 mV s^{-1}.

Some papers have been published related to the electrooxidation of some cephalosporins [9,10] having aminothiazole ring at the side chain: the electrooxidation of these substances can take place via this group. In the case of cefadroxil as it has no aminothiazole group, probably the product of amide hydrolyses or/and phenolic hydroxide at the side chain must be electrooxidized depending on the solution conditions. In this study the solution conditions support phenolic hydroxide oxidation.

The proposed DPV method was compared with spectrophotometric and derivative spectrophotometric methods which were also developed in our

Table 2
Results of cefadroxil monohydrate pharmaceutical dosage forms

Methods	Formulations					
	Capsules (500 mg)			Oral suspensions (250 mg/5 ml)		
	Amount found (mg)[a]	RSD% amount found	Calculated t-value	Amount found (mg/5 ml)[a]	RSD% amount found	Calculated t-value
Official HPLC method	500.2	0.49	$t_{theoretical}$: 2.306	250.2	0.34	$t_{theoretical}$: 2.306
DPV	500	0.71	0.021 (NS[b])	250.8	0.35	1.145 (NS)
Zero order spectrophotometry	499.9	0.35	0.222 (NS)	250.0	0.30	0.358 (NS)
First derivative spectrophotometry	499.9	0.47	0.184 (NS)	249.7	0.30	0.905 (NS)

[a] Each value is the mean of five experiments
[b] NS, not significant.

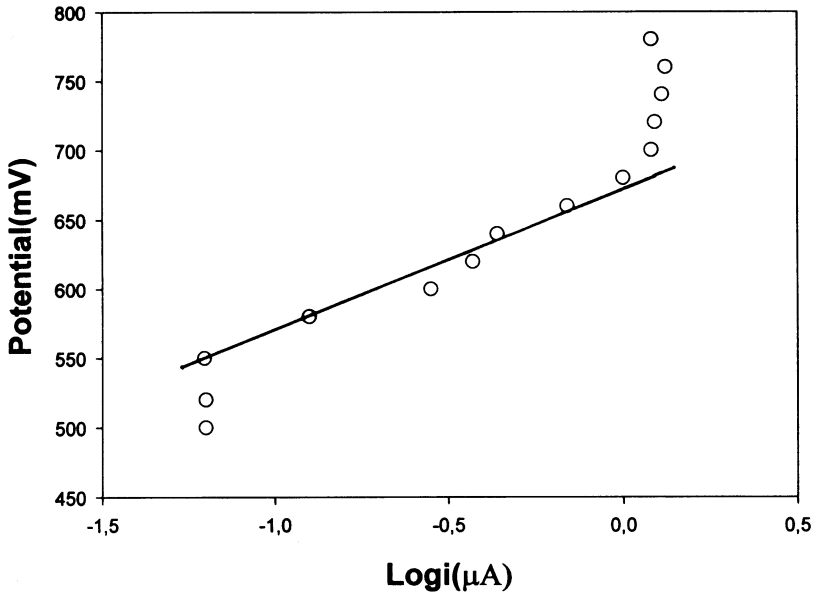

Fig. 7. Tafel plot obtained in phosphate buffer pH 7.0. Scan rate: 10 mV s^{-1}.

laboratory. A 1×10^{-3} M stock solution of cefadroxil was prepared in methanol and UV spectrum was recorded in the range of 205–300 nm. As can be seen in Fig. 8, the normal UV peaks are broad. When first derivative UV spectrum was recorded sharp peaks could be obtained. It was observed that the absorbances at 265.3 nm UV spectra and at 277.4 nm for first derivative UV

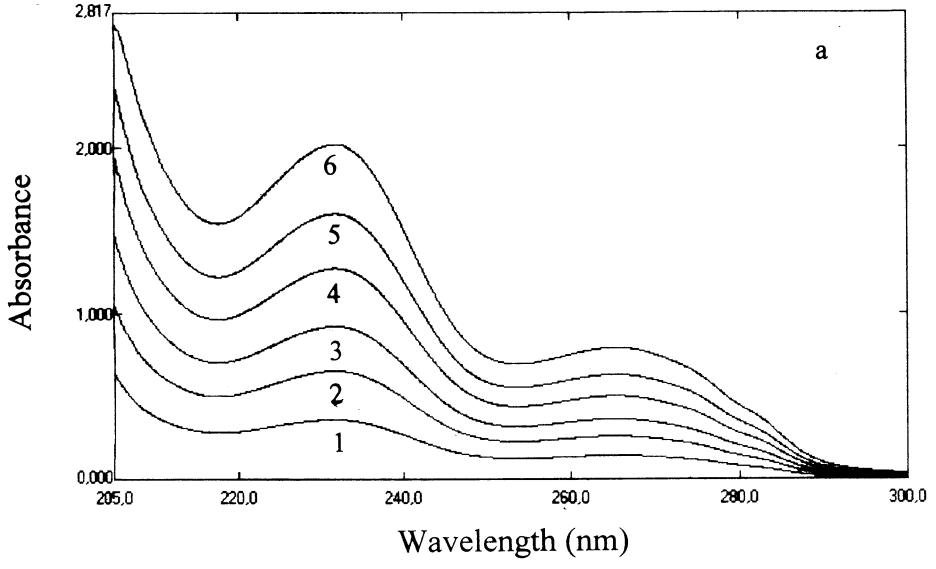

Fig. 8. Zero order (a) and first derivative (b) spectra of cefadroxil depending on the concentration. (1) 2.5×10^{-5} M cefadroxil; (2) 5×10^{-5} M cefadroxil; (3) 7.5×10^{-5} M cefadroxil; (4) 1.0×10^{-4} M cefadroxil; (5) 1.25×10^{-4} M cefadroxil; (6) 1.50×10^{-4} M cefadroxil.

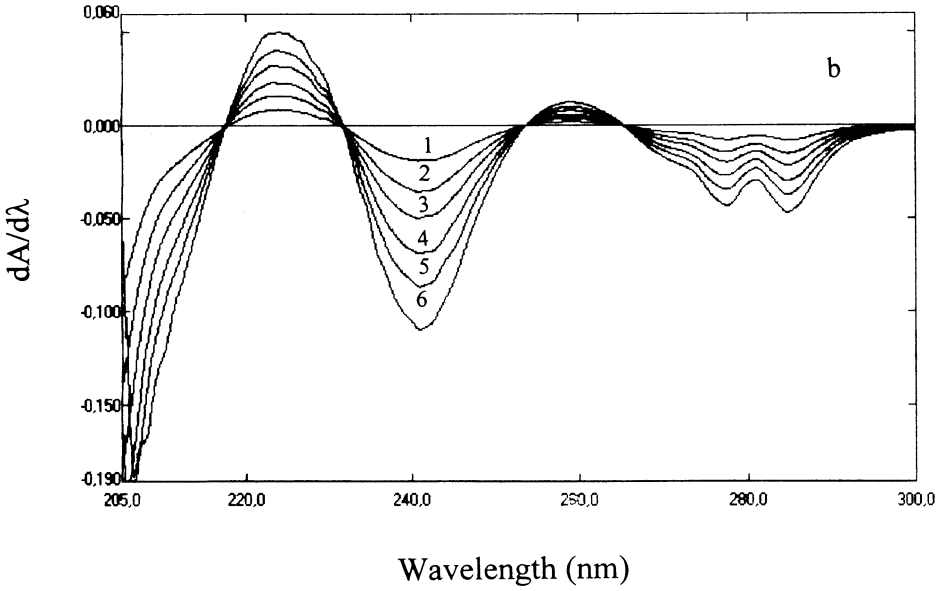

Fig. 8. (*Continued*)

spectra were linearly dependent on cefadroxil concentration (Table 1), so it was confirmed that both methods could be applied to the cefadroxil monohydrate analysis.

The applicability of the DPV method for the assay of a simple dosage form was examined by analysing capsules and oral suspensions.

The results confirm the suitability of the proposed method for the accurate and sensitive analysis of cefadroxil monohydrate. The DPV and spectrophotometric results were compared to those of official high performance liquid chromatographic (HPLC) methods [11] by means of Student's *t*-test at the 95% confidence level, and no significant difference was found between them (Table 2).

It is concluded that the proposed DPV and spectrophotometric methods have the advantages of being rapid, simple, directly applicable to dosage forms and inexpensive when compared to official HPLC method, and hence are suitable for the routine quality control of this drug.

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