ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
22 (2000) 925–932

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Voltammetric methods for analytical determination of fleroxacin in Quinodis® tablets

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Received 24 August 1999; received in revised form 16 December 1999; accepted 19 December 1999

Abstract

The direct current (dc) and differential pulse (dp) polarographic reduction of fleroxacin was done in a wide pH range from 2.48 to 13.00. The appropriate buffer choice was made for its dp polarographic determination in a range from 1.845 to 16.926 μg/ml, at pH 8.50. The adsorptive properties of fleroxacin were investigated in order to achieve an increase in sensitivity and a possibility of fleroxacin determination by applying the adsorptive stripping voltammetric method. The adsorptive processes at the hanging mercury drop electrode were investigated in Britton–Robinson and borate buffers. Adsorptive preconcentration followed by differential-pulse cathodic stripping showed one wave at ~ -1.1 V being the most sensitive for analytical determination of fleroxacin. Two linear ranges were obtained, the first one from 18.465 to 258.51 ng/ml, and the second one from 3.693 to 18.465 ng/ml.

Keywords: Fleroxacin; dpp Polarography; Adsorptive stripping voltammetry; Determination; Tablets

1. Introduction

Fleroxacin belongs to the third generation of multiple fluorinated antibacterial quinolone derivatives widely used in the treatment of urinary infections. This generation members are of a broader spectrum and greater activity compared to nalidixic and oxolinic acid [1–3]. The mechanism of their action was extensively studied [4]. These agents are proved to prevent bacterial DNA biosynthesis by inhibiting the bacterial enzyme DNA gyrase.

The behavior of fluoroquinolone is significantly influenced by their physicochemical properties, particularly by their ionisation degree expressed by the pK value and partition coefficient [5,6]. These data are important for a thorough understanding of absorption transport and receptor binding of these drugs at the molecular level. Therefore, the acid–base equilibrium of fleroxacin has been reported in our previous paper [7].

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0731-7085/00/$ - see front matter
PII: S0731-7085(00)00289-2

Several modern instrumental methods, including high-performance liquid chromatography (HPLC) [8], gas chromatography followed by mass spectrometry [9] and fluorimetry [10] have been used for analysis of the similar quinolone antibiotics. Literature data related to fleroxacin determination showed that HPLC with spectrofluorimetric detection is the major analytical method for its determination [11–14] in biological fluids.

However, the presence of reducible groups makes these drugs useful for electroanalytical determination. In fact, polarography and adsorptive stripping voltammetry (AdSV) have already successfully been used for determining some quinolone derivatives [15–21]. The earlier papers related to polarographic investigation of nalidixic acid by applying dc polarography are given by Staroscik et al. [22]. The polarographic behavior of pipemidic acid has been study and applied to the analysis of a pharmaceutical formulations [23]. The more extensive electrochemical studies of nalidixic acid have been done by Van Oort [24] and coworkers and the mechanism of the reduction has been proposed. According to these authors polarographic activity of this compound is expected regarded to the presence of the azinone ring. The reduction occurs at dropping mercury electrode in two one-electron waves for protonated and uncharged form of nalidixic acid, whereas its anion is reduced in a single one-electron wave. Reduction of nalidixic acid results in hydrogenation of the ethylenic bond in the azinone ring.

The polarographic behavior of fleroxacin has not been studied so far. In this paper, the direct current (dc) and differential-pulse (dp) polarographic reduction of the fleroxacin has been studied in a wide pH range. Since the literature data showed the strong adsorptive properties of the quinolone antibiotics, fleroxacin was studied by adsorptive stripping voltammetry on a static mercury electrode as well. Two electrochemical methods based on these voltammetric studies were proposed for determination of fleroxacin and its determination in commercial tablets.

2. Experimental

2.1. Apparatus and reagents

Polarographic analyser PAR 174A connected with three electrodes cell (SCE, DME and Pt) was used. Dropping times of 2 s (dc mode) and 1 s (dp mode) were used. Scan rate was 2 mV/s (dc and dp mode), module amplitude was 25 and 50 mV (dpp), and the mercury column height was 82 cm.

Voltammetric measurements were carried out with a PAR 174A and PAR 170 Universal Programmer coupled with a PAR Model 3030 static mercury drop electrode (SMDE) serving as the working electrode. A saturated Ag/AgCl was used as the reference one and Pt/wire as auxiliary electrode. These electrodes were kept in linear configuration, which appeared to give somewhat higher sensitivity than the usual triangular arrangement. The initial potential of -0.3 V versus Ag/AgCl with negative scan direction and scan rate of 10 mV/s and a pulse amplitude of 25 and 50 mV were used.

All investigations were carried out with fleroxacin produced by Hoffmann La Roche (Basel, Switzerland) and the corresponding commercial tablets, Quinodis® -400 mg. The following buffer solutions were used: Britton–Robinson (0.04 M regarded to the acetic, phosphoric and boric acid), borate (0.1 M) and TRIS (0.1 M). Other reagents used were of analytical grade from 'Merck'. Double distilled water was used throughout.

2.2. Polarographic determination

2.2.1. Standard solutions

A stock fleroxacin solution 5×10^{-4} M (9.22 mg/50 ml), used in polarographic analysis was prepared by dissolving fleroxacin standard in bidistilled water with addition of 0.3 ml 0.1 M HCl. The spectrophotometric study of the stability of the fleroxacin solutions showed that no change of the absorption was found during 7 days.

2.2.2. Procedure for making calibration graphs

10.00 ml of buffer solution were transferred to the cell and deareated for 8 min. Prior to entering the voltammetric cell nitrogen was passed, successively, through a solution of chromium(II) ions in dilute hydrochloric acid containing heavily amalgamated zinc granules, distilled water and molecular sieves.

A stock solution of 5×10^{-4} M fleroxacin solution was spiked into the buffer solution (0.2–1.1 ml). The current–voltage curves were recorded after each addition. The limiting currents were measured and calibration curves were constructed.

2.2.3. Procedure of determination of fleroxacin in Quinodis® tablets

Five Quinodis® tablets were weighed and powdered (average weight of 0.5154 g); 0.064425 g of the powder was transferred quantitatively to 250 ml volumetric flask, added 0.3 ml 0.1 M HCl and diluted with ≈100 ml double distilled water, dissolved in ultra sonic bath 10 min and diluted with water to mark. The final solution was turbid but further treatment is not needed. Known volumes of 0.5 and 0.75 ml of this solutions were transferred into polarographic cell and made the final volume of 10 ml with adequate buffer solution (borate buffer pH 8.5). The solution was bubbled 10 min with nitrogen and the polarograms were scanned.

2.3. Adsorptive stripping determination of fleroxacin

2.3.1. Procedure of making calibration graphs

A 10 ml aliquot of the corresponding supporting electrolyte solution was placed in a voltammetric cell and deaerated for 12 min with high-purity nitrogen.

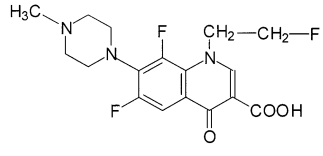

Fig. 1. Structural formula of fleroxacin.

The required amount (20 μl portions) of ten times diluted stock solution (5×10^{-5} M) of the investigated substance was added after recording the baseline. When a fresh mercury drop had formed, the voltammogram was recorded immediately or after a certain time of adsorptive accumulation at a selected potential in a stirred or quiscent solution.

Calibration graphs were constructed using data from three series of measurements and evaluated by the least-squares linear regression method. Measurements were performed at room temperature.

2.3.2. Procedure for determination of fleroxacin in Quinodis® tablets by AdSV)

Five Quinodis® tablets were weighed and powdered (average weight of 0.5154 g); 0.064425 g of the powder was transferred quantitatively to 250 ml volumetric flask, added 0.3 ml 0.1 M HCl and diluted with ≈100 ml double distilled water, dissolved in ultra sonic bath 10 min and diluted with water to mark. Five milliliters of this solution is diluted to 50 ml. A known volume of this solution was spiked into 10 ml aliquot of the base electrolyte followed by spikes of the standard fleroxacin solution. The corresponding voltammograms have been recorded before and after each addition.

3. Results and discussion

Fleroxacin is 6,8-difluoro-1-(2-fluoro ethyl)-1,4-dihydro-7-(methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, shown in Fig. 1. Fleroxacin shows the polarographic activity through the wide pH range from 2.48 to 10.00 (Britton–Robinson buffer), and pH 13.00 (0.1 M NaOH).

The one polarographic wave exists in pH range from 2.48 to 8.50, with $E_{\frac{1}{2}}$ from −1.1 to −1.4 V. At pH 9.5 and 10.00, two well defined polarographic waves are obtained, the first one at $E_{\frac{1}{2}}$ −1.47 V and the second one at more negative potential, $E_{\frac{1}{2}}$ −1.8 V, very close to the supporting electrolyte potential.

At pH > 12, only one polarographic wave is obtained. The dependence of $E_{\frac{1}{2}}$ and i on pH are

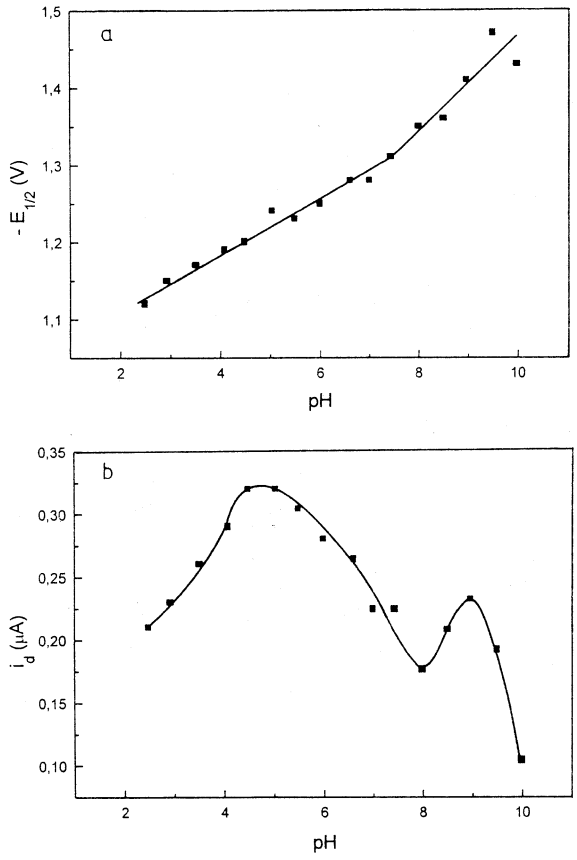

Fig. 2. The dependence of $E_{\frac{1}{2}}$ and i_d on pH, a and b, respectively.

shown in Fig. 2(a and b), respectively. The cross section point obtained at pH value being ≈ 8, corresponds to the second pKa value of fleroxacin [7].The first part of the curve shows a slope ($\Delta E/\Delta$pH) of 0.0348 V, and the second part of 0.074 V, indicating processes involving the protons in the electrode reaction. This is in accordance with literature data that in mechanistic scheme of quinolone antibiotic hydrogenation of the double bond of 2–3 azinone ring occurs [24].

From the i on pH dependence it is seen that two different ionic species, having different diffusion coefficients are present in the solution. The minimum of the curve is at pH ~ 8.

Since the dc polarographic waves were not well resolved from the supporting electrolyte line, especially in an acidic medium, dp mode was applied in pH range from 4 to 10, and the representative dp curves obtained in BR buffer (8.90), TRIS (8.60) and borate (8.50) buffer are shown in Fig. 3. The main peak (at ~ -1.40 V in all buffers) height on pH dependence is attached in the same figure. Further studies have been performed with borate buffer due to its accessibility.

Very good analytical response was obtained in BR, TRIS and borate buffers and the corresponding graphs were made at chosen pH. The regression equations obtained in all buffers investigated are presented in Table 1(A). According to the results presented in Table 1, the borate buffer was chosen as the best one for fleroxacin determination. The precision of the proposed method was checked in borate buffer for fleroxacin concentration of 10 μg/ml with relative standard deviation of 0.62% obtained for five determinations. The determination limit was calculated as ten times the standard deviation for ten determinations of

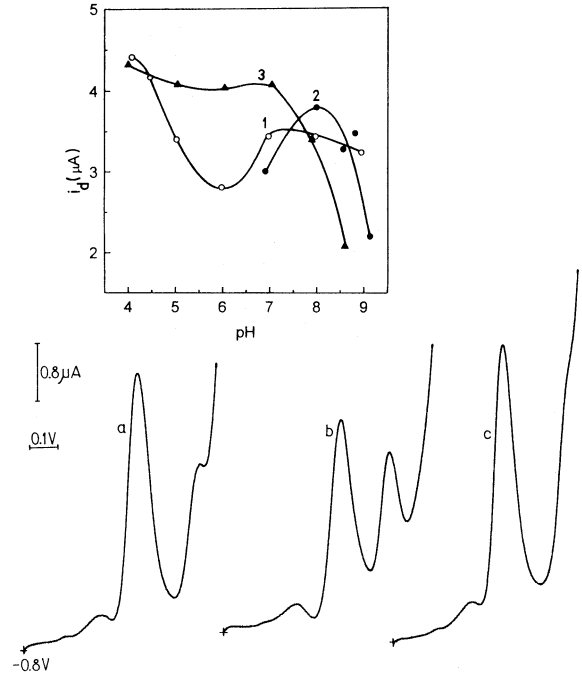

Fig. 3. Representative dp curves of fleroxacin 1×10^{-4} M obtained in different. Buffers: (a) BR pH 8.90; (b) TRIS pH 8.60; and (c) borate pH 8.50; the main peak height vs. pH (attached): (1) BR; (2) borate and (3) TRIS buffer.

Table 1
Statistical parameters for fleroxacin determination obtained in different buffers

	Buffer	Concentration range (μg/ml)	Regression equation	S[a]	S[b]	r
	BR	3.69–18.635	$y = -5.273 + 5.736x$	1.99	0.15	0.9979
A (dpp method)	Borate	1.845–16.926	$y = 5.428 + 4.530x$	0.78	0.07	0.9993
	TRIS	1.845–13.764	$y = 7.004 + 4.103x$	2.62	0.29	0.9898
		(ng/ml)				
B (AdSV method)	Borate	18.465–258.51	$y = -23.70 + 239.70x$	1.04	1.42	0.9998
	Borate	3.693–18.465	$y = -1.987 + 142.49x$	0.21	2.11	0.9996

[a] Standard deviation of the intercept.
[b] Standard deviation of the slope.

Table 2
Statistical parameters obtained for fleroxacin determination inQuinodis®

	Taken concentration[a] (μg/ml)	Found (mg per tablet)	RSD% ($n = 6$)	Percentage of label claim[b]
A (dpp method)	10.0	387.9	0.79	96.96
	15.0	388.5	1.30	97.14
B (AdSV method)	0.12	385.44	1.76	96.36
	0.16	387.48	1.46	96.87

[a] Fleroxacin concentration in the working solution calculated relative to the label claim.
[b] Label claim: fleroxacin 400 mg per tablet.

the analyte at a concentration corresponding to the lowest point of the appropriate calibration graph [25], and was found to be 1.43×10^{-6} M (0.526 μg/ml), with limit detection 4.27×10^{-7} M (0.158 μg/ml) The method proposed was applied for fleroxacin determination in Quinodis® tablets as shown in Table 2(A).

The sensitivity of the determination of fleroxacin can be further increased by adsorptive accumulation of the investigated substance on a hanging mercury drop electrode surface. The potential surface-active behavior of the investigated substance, and consequently, the possibility of applying the adsorptive stripping voltammetric technique were investigated by measuring electrocapillary curves [26]. The mercury dropping time at a determined potential was measured as the interval required for 50 mercury drops to spontaneously form at the outlet of the capillary, 0.04 mm diameter, and a mercury reservoir high of 82 cm. The concentration of the investigated substance was 1×10^{-4} M. Fig. 4(b) shows such curve for fleroxacin (curve 2), together with that for the supporting electrolyte (curve 1) at pH 9.0. Analysis of the electrocapillary curves showed the maximum decrease of electrocapillary maximum at -0.3 V, suggested this potential as the best for analytical purposes. This finding was proved by scanning the adsorptive differential pulse voltamogramms after deposition at different potentials (within the range -0.2– -0.6 V). The most regular peaks and highest AdSV determination were obtained at accumulation potential of -0.3 V. A procedure, recommended by Benadikova and Kalvoda [27] to investigate the accumulation time effect on the peak current at a determined potential was also used. The obtained adsorptive behavior data are elaborated and graphically presented in Fig. 4(a). The concentration of the investigated substance was 1×10^{-5} M, while the accumulation potential used was -0.3 V, at pH 9. The effect of accumulation time on the peak height, Fig. 4(a), shows 3 min as the optimum adsorption time. These, as well as the electrocapil-

lary measurements, have shown the surface-active behavior of the investigated substance with respect to mercury, as well as the possibility of using the AdSV technique for fleroxcin determination. In order to establish the optimum conditions for the AdSV determination of fleroxacin, the effect of the following parameters was also investigated: the scan rate, the modulation pulse amplitude, the mercury drop size and the temperature.

The peak current increases with increasing potential scan rate. However, due to the simultaneous increase in the peak width a rather low scan rate of 10 mV/s was chosen as being the most suitable. The medium drop size and an amplitude of 25 mV were used. From an analytical viewpoint, the optimal supporting electrolyte was established to be the buffer with pH 9.0, where the highest and more easily measured DPV peaks were obtained. Taking in mind that pK values of fleroxacin are 5.61 and 8.11, respectively, it can be concluded that predominately the anionic form of the fleroxacin is present in the solution at pH 7. The DPV determination with and without accumulation, curves presented in Fig. 5(b and a, respectively), was performed in concentration range from 5×10^{-8} to 1×10^{-6} M, but linear dependence was achieved from 5×10^{-8} to 1×10^{-7} M (18.465–258.51 ng/ml). A further increase in sensitivity was achieved through the adsorptive accumulation of the fleroxacin on the surface of a hanging mercury drop electrode. Three minutes accumulation in Britton–Robinson buffer solution permits the AdSV determination of fleroxacin. Using a tenfold diluted supporting electrolyte and the sensitivity of 0.5 μA the concentration range from 1×10^{-8} to 1×10^{-7} M was investigated, but the linear dependence was obtained from 1×10^{-8} to 5×10^{-8} M (3.693–18.465 ng/ml). Calibration graphs were measured triplicate and evaluated by the least-squares linear regression method. The regression line equations are shown in Table 1B.

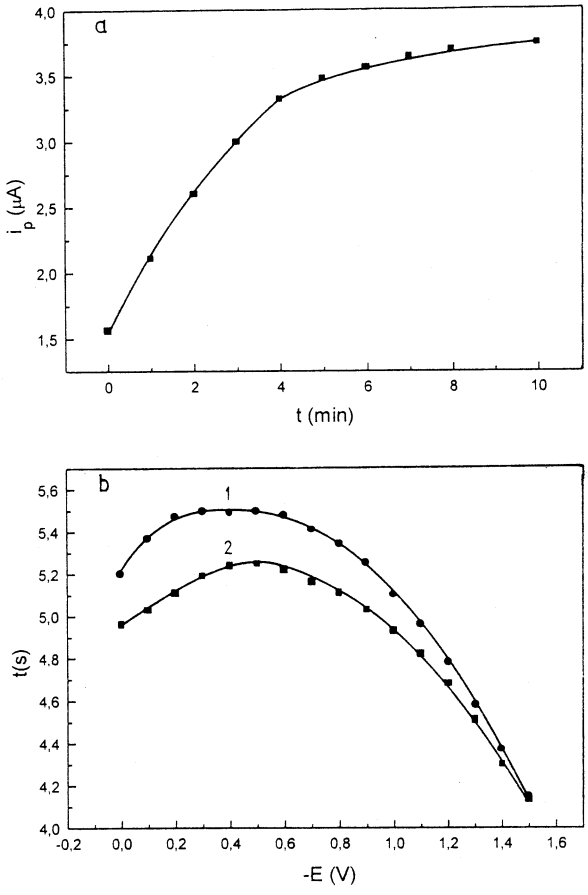

Fig. 4. (a) Effect of the accumulation time on the peak current of the differential-pulse adsorptive stripping voltammograms of fleroxacin (1×10^{-5} M). (b) Dependence of the mercury drop time on the dropping mercury electrode potential 1–supporting electrolyte (BR buffer pH 9.00), 2–1×10^{-4} M fleroxacin in supporting electrolyte.

The slopes and intercepts of the regression lines are given in ng/ml and μA. The determination limit was found to be 7.22×10^{-9} M (2.66 ng/ml), with limit detection of 3×10^{-9} M (1.1 ng/ml) for the higher concentration range, while for the lower concentration range was 2.33×10^{-9} M (0.86 ng/ml) and 9.34×10^{-10} M, (0.34 ng/ml), respectively. The precision of the AdSV method was checked in borate buffer for fleroxacin concentration of 0.12 μg/ml with relative standard deviation of 1.5% for five determinations.

The results obtained from the same tablets sample by dpp and AdSV technique, are presented in Table 1(A and B, respectively). Comparing the results obtained by dp polarography and AdSV method it can be concluded that the higher sensitivity method is AdSV, but the precision and accuracy of dpp method is better. Since the determination of fleroxacin was performed in tablets,

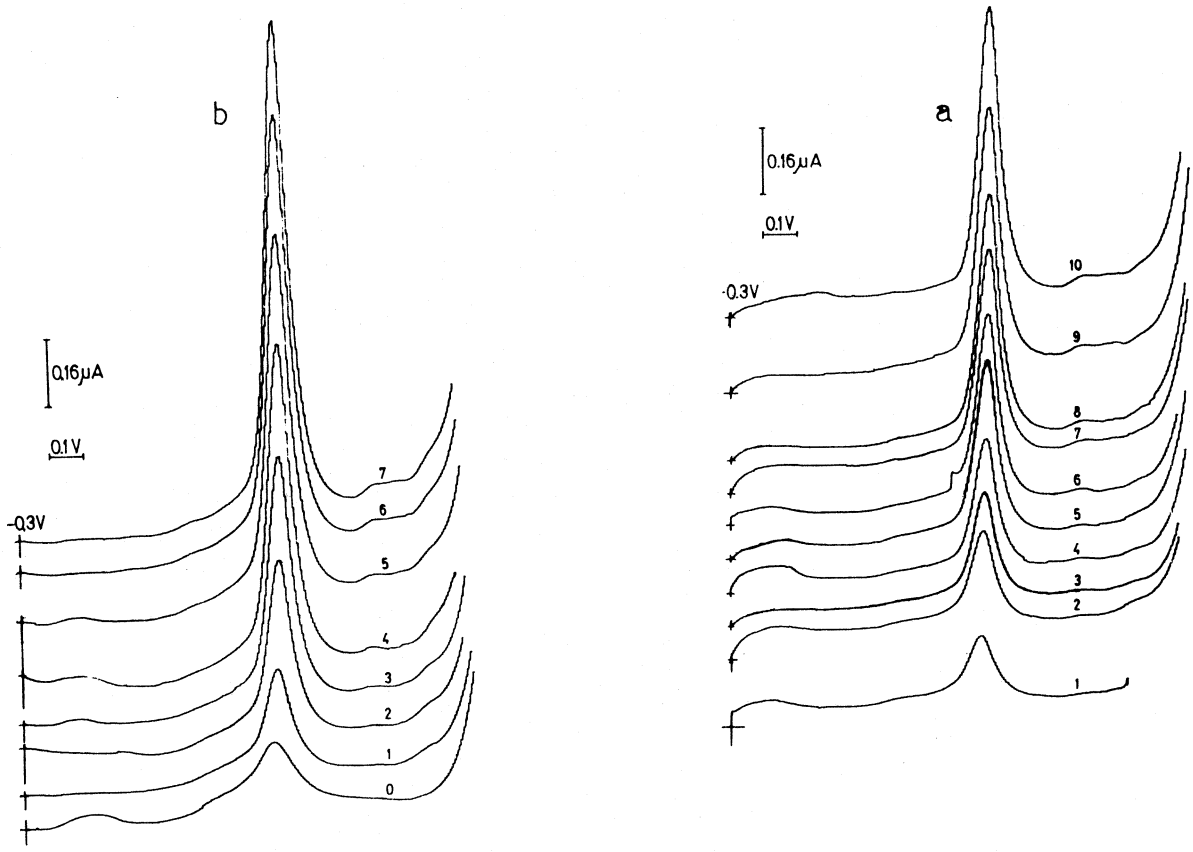

Fig. 5. Adsorptive stripping voltammograms of fleroxacin with accumulation (b) ($5 \times 10^{-8} - 1 \times 10^{-6}$ M) and without accumulation (a) ($5 \times 10^{-8} - 1 \times 10^{-7}$ M).

where the concentration of fleroxacin is not a critical value, the dpp method can be suggested as a method of choice due to its simplicity and accuracy. Both developed methods represent a good alternative for the quality control because the preparation of the sample is easy and the excipients do not interfere with the determination, and consequently, separations or extraction procedures are not needed.

4. Conclusions

Since the electrochemical methods for pharmaceutical analysis are proved to be fast, precise, simple to perform and produce low cost results with minimum interference from excipients of the drugs, the above methods can be suggested as the simple ones for fleroxacin determination.

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