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Determination of the antibiotic drug pefloxacin in bulk form, tablets and human serum using square wave cathodic adsorptive stripping voltammetry

A.M. Beltagi

Department of Chemistry and Physics, Faculty of Education, Tanta University, 33516 Kafr El-Sheikh, Egypt

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Abstract

A simple, rapid, reliable and fully validated square wave cathodic adsorptive stripping voltammetric procedure has been developed for the determination of the antibiotic pefloxacin drug in bulk form, tablets and human serum, based on its electrochemical reduction at a hanging mercury drop electrode. The Britton–Robinson buffer of pH 7.0 was found to be reasonable as a supporting electrolyte for assay of the drug. Pefloxacin drug, at the optimized conditions, showed a single 2-electron well-defined peak at -1.07 V (versus Ag/AgCl/KCl_s) using an accumulation potential of -0.40 V. This peak may be attributed to the reduction of the C=O group. A mean recovery of 99.54% $\pm$0.23 and a detection limit of 1.65×10^{-10} M pefloxacin were achieved. After being validated, the proposed procedure was successfully applied for the determination of the drug in tablets and human serum with mean recoveries of 99.57$\pm$0.48 and 98.55$\pm$0.78%, respectively. A detection limit of 4.50×10^{-10} M was achieved for the determination of the drug in human serum. Results of the proposed procedure were comparable with those obtained by reported methods.
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Keywords: Pefloxacin; Square wave cathodic adsorptive stripping voltammetry; Assay of tablets and human serum

1. Introduction

Fluoroquinolones are important antibacterials developed in recent years, which have wide applications in veterinary and human medicine. A broad spectrum of activity and good oral absorption has led to extensive clinical use of the newer fluoroquinolones [1]. They are effective against most gram-negative and gram-positive aerobic bacteria [2]. Pefloxacin mesylate (PE), 1-ethyl-6-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxoquinolone-3-carboxylic acid (Scheme 1) is a fluoroquinolone antibacterial agent. PE has a plasma half-life of about 8–13 h and is also extensively metabolized, the principle metabolite being *N*-demethyl pefloxacin, otherwise known as norfloxacin (NOR). PE is used for the treatment of diseases of the skin and various kinds of urinary tract infections. It is given by mouth or by intravenous infusion in the treatment of susceptible infections. Doses are expressed in terms of the

E-mail address: ambeltagi@hotmail.com (A.M. Beltagi).

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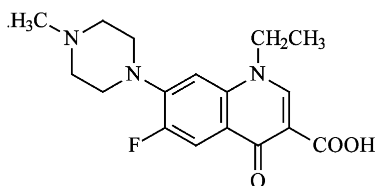

Scheme 1.

equivalent amount of PE and are usually 400 mg twice daily by mouth or by intravenous infusion. PE has bacterial activity against enterobacteriaceae, pseudomonas aeruginosa, haemophilus, neisseria, staphylococci, and mycobacterium leprea and has been tried in the treatment of leprosy [2].

The drug has been determined in pharmaceutical preparations, human serum and urine with several methods such as spectrophotometry and spectrofluorimetry [3–8], capillary electrophoresis [9,10] and high-performance liquid chromatography [11–17]. Electroanalytical techniques have been used for determination of other fluoroquinolones of the first and second generations such as NOR [18–20], ciprofloxacin [21], enrofloxacin [22], enoxacin [23], cinoxacin [24], ofloxacin [25] and lomefloxacin [26]. However no electrochemical procedure for the assay of pefloxacin is reported to date.

In this work, adsorptive stripping voltammetry in the square wave mode was used to provide a fully validated sensitive procedure for the determination of PE drug at a hanging mercury drop electrode in bulk, tablets and human serum. The procedure did not require sample pre-treatment or any time-consuming extraction steps other than centrifugal separation of the precipitated protein from serum solution prior to the drug assay.

2. Experimental

2.1. Instrumentation

The Electrochemical Analyzers Models 394 and 273A (PAR) were used for the present voltammetric measurements. The electrode assembly (Model 303A-PAR), with a dark micro-electrolysis cell of three electrode system comprises of a hanging mercury drop working electrode (area: 0.026 cm^2), a Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode, was used. Stirring of the solution in the micro-electrolysis cell was performed using a magnetic stirrer (Model 305-PAR) with a star-shaped magnet to provide the convective transport during the pre-concentration step. The whole measurements were automated and controlled through the programming capacity of the apparatus. Quantification was performed by means of calibration curve and standard addition methods. The data were treated through a PC-computer connected to the potentiostat and loaded with the 394 Analytical voltammetry software version 2.01©-1994 (from EG & G).

The de-ionized water was supplied from a Purite-Still Plus Deionizer connected to a Hamilton-AquaMatic bidistilled water system.

A Mettler balance (Toledo-AB104) was used for weighing the solid materials. An Orion SA-720 pH-meter served to carry out the pH measurements. A centrifuge instrument (Model Eppendorf-5417 C) was used to separate out the precipitated protein before analysis of the human serum. A micopipetter (Eppendorf-Multipette® plus) was used for transfer of the reactant solution to the micro-electrolysis cell throughout the experimental work. All data were obtained at 25 °C. The working solutions are stable and do not change with time.

2.2. Materials and reagents

All chemicals and reagents were of analytical grade quality. PE was supplied from Amriya Pharmaceutical Industries Co., Alexandria, Egypt. A stock solution of 1.0×10^{-3} M pefloxacin was prepared in deionised water and stored in the dark at 4 °C. More dilute solutions were prepared daily with deionised water just before use. The Britton–Robinson (pH 2–11), nitrate and phosphate buffers were prepared using analytical grade reagents and were used as supporting electrolytes.

2.3. Pharmaceutical dosage form

Peflacin tablet (Amriya Pharmaceutical Industries Co.) labeled to contain 400 mg pefloxacin drug and some inactive excipients.

2.4. Procedures

2.4.1. General analytical procedure

A 10 ml volume of the supporting electrolyte solution was introduced into a dark micro-electrolysis cell, then de-aerated with pure nitrogen for 10 min. A selected accumulation potential was then applied to a mercury drop for a selected time period, while the solution was stirred at 400 rpm. The stirring was then stopped, and after 15 s rest period, the voltammogram was recorded by applying a negative-going scan. After the background voltammogram had been recorded, aliquot of the reactant solution was introduced into the micro-electrolysis cell and the voltammogram was then recorded at a new mercury drop.

2.4.2. Procedure for peflacin tablets

The drug content of five tablets was weighed, finely powdered and mixed. The average mass per tablet was determined. A quantity of the powder equivalent to 400 mg of PE was transferred accurately into a 100-ml calibrated dark flask contains 70 ml deionized water. The content of the flask was shaken for about 15 min and diluted to volume with deionized water. The solution was then filtrated through a 0.45-μm milli-pore filter (Gelman, Germany), that to separate out the insoluble excipients, rejecting the first portion of the filtrate. The desired concentration for the drug was obtained by accurate dilution with deionized water and the analysis was followed up as in the general analytical procedure.

2.4.3. Procedure for human serum

Serum samples, obtained from healthy volunteers, were collected and stored frozen until assay. An aliquot serum sample was fortified with PE to achieve final concentration of 1×10^{-3} M, and treated with 0.2 ml methanol as serum protein precipitating agent, then the volume was completed to 1.0 ml with the same serum sample. After vortexing for 30 s, the precipitated protein was separated out by centrifugation for 3 min at 14 000 r.p.m. The clear supernatant layer was filtrated through a 0.45-μm milli-pore filter to produce a protein-free human serum. The analysis was followed up as in the general analytical procedure.

3. Results and discussion

3.1. Effect of pH

PE gave rise to a single well-defined reduction peak using adsorptive stripping square wave voltammetry at a HMDE in Britton–Robinson buffers of pH values 2–11. This peak may be attributed to the saturation of C=O double bond through a 2-electron process. As shown in Fig. 1, the peak current, i_p, increased gradually with the increase of pH of the solution till reach the maximum value at pH 7.0. A shift of the peak potential towards more negative value was observed as the pH increased, indicated the existence of a protonation reaction coupled with the PE reduction process. The effect of other supporting electrolytes (e.g. phosphate and nitrate buffers) of

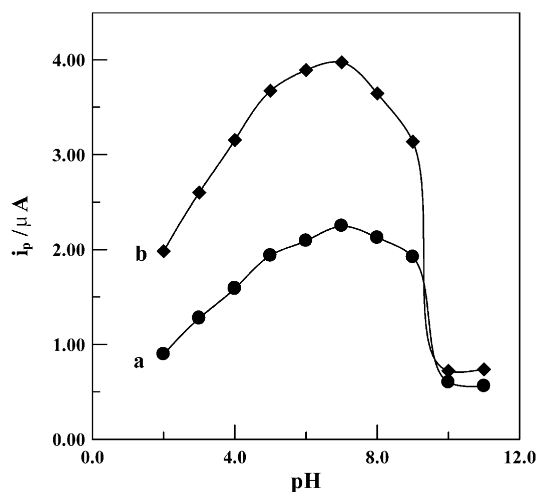

Fig. 1. Influence of pH on the SWCAdS peak current response for 1×10^{-7} M pefloxacin in B.R. buffers (pH 2–11) after (a) 30 s and (b) 60 s pre-concentration time; frequency (f) = 120 Hz, scan increment (Δs) = 10 mV and pulse height (E_{sw}) = 25 mV at E_{acc} = −0.50.

the same pH value, 7.0, on the PE peak current was examined. Fig. 2 showed that in Britton–Robinson buffer of pH 7.0, a higher peak current was achieved. Although when the ionic strength increased the stripping peak current decreased, no ionic strength adjustment was used because the ionic strength conditions were already imposed by the buffer solution needed to raise the optimized 7.0 pH value (10 ml of 0.04 M Britton–Robinson buffer solution).

Taking into account these results, the medium chosen to carry out further studies was the 0.04 M Britton–Robinson buffer solution of pH 7.0, where the PE peak potential value was at −1.27 V.

3.2. Cyclic voltammetry

The interfacial accumulation of the drug was indicated from repetitive cyclic voltammograms for 4×10^{-6} M PE in 0.04 M Britton–Robinson buffer (pH 7.0) recorded following stirring for 10 s at −0.40 V (Fig. 3). After a short pre-concentration period, a well-defined peak at −1.17 V was observed (scan 1). A substantial decrease of the cathodic peak current was observed in subsequent scans (scan 2). Such behavior indicated rapid

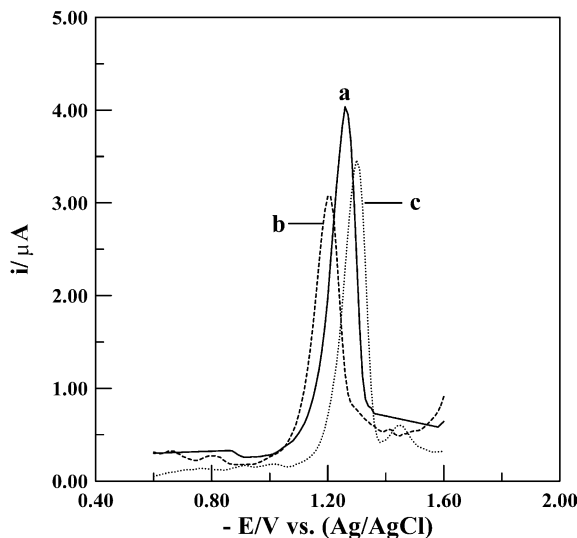

Fig. 2. SWCAdS voltammograms for 1×10^{-7} M pefloxacin in (a) B.R. buffer; (b) phosphate buffer and (c) nitrate buffer of pH 7.0; other parameters as indicated in Fig. 1.

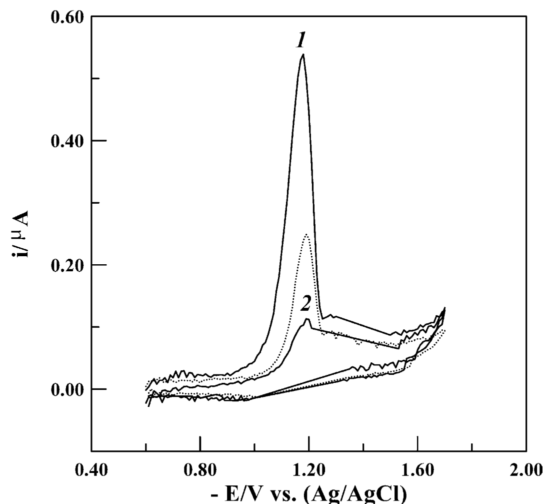

Fig. 3. Cyclic voltammograms for 4×10^{-6} M pefloxacin drug in B.R. buffer (pH 7.0); scan rate $v = 100$ mV s^{-1} and $E_{acc} = -0.40$ V at $t_{acc} = 10$ s (scan 1), and its second cycle at the same mercury drop (scan 2). Dashed scan represents the voltammogram without pre-concentration.

desorption of PE from the electrode surface. No peaks were observed on the reverse scan, indicated the irreversible nature of the electrode process. The voltammogram represented the analogous response without pre-concentration (dashed curve) showed that the peak current was substantially smaller than that obtained following pre-concentration. The cycles carried out for increasing values of the scan rate (v) between 0.025 and 0.2 V s^{-1}, under the above conditions, gave rise to a reduction peak with increased intensities. On plotting $\log i_p$ versus $\log v$ over the entire pH range of B.R. buffer (2–11), straight lines with slope values of 0.832–1.023 were obtained (Table 1). The slope value of 1.0 was as that expected for an adsorption-controlled process [27]. The peak potential shifted to a more negative value on the increase of the scan rate confirmed the irreversible nature of the reduction process. For a totally irreversible electrode reaction, the relationship between the peak potential (E_p) and scan rate (v) was expressed as [28]:

$$E_p = (2.303RT/\alpha n_a F)\log(RTk_f/\alpha n_a F) - (2.303RT/\alpha n_a F)\log v$$

The dependence of the peak potential of PE on

Table 1
Calculation of the transfer coefficient, α, obtained from both cyclic voltammetry and square wave measurements

pH	Cyclic voltammetry measurements					Square wave measurements		
	Slope log i_p/log v	r ($n=7$)	Slope E_p/log v	r ($n=7$)	αn_a	Slope E_p/log f	r ($n=6$)	αn_a
2	0.832	0.997	0.161	0.992	0.37	0.097	0.998	0.26
3	0.845	0.995	0.156	0.991	0.38	0.098	0.994	0.26
4	0.855	0.998	0.152	0.995	0.39	0.095	0.995	0.27
5	0.851	0.994	0.155	0.998	0.38	0.093	0.999	0.28
6	0.883	0.998	0.148	0.994	0.40	0.093	0.997	0.28
7	0.891	0.996	0.158	0.993	0.37	0.088	0.995	0.29
8	1.023	0.997	0.188	0.995	0.31	0.068	0.996	0.38
9	1.005	0.996	0.140	0.997	0.42	0.050	0.997	0.51
10	1.020	0.995	0.148	0.998	0.40	0.077	0.998	0.33
11	1.009	0.998	0.156	0.996	0.38	0.055	0.996	0.46

the decimal logarithm of the scan rate over the entire B.R. buffer pH range (2–11) are shown in Table 1. The slope values of the E_p/log v plots at different pH values were 0.140–0.188 over the entire pH range. Accordingly, the number of electrons, n_a, transferred in the rate-determining step should equal one ($n_a = 1$). Hence, the transfer coefficient values, α, were calculated at the different pH values as indicated in Table 1.

Using a 4×10^{-6} M PE solution, the adsorptive saturation of the drug onto the mercury electrode surface was achieved after pre-concentration for 10 s. The response of surface-adsorbed PE at saturation was used to determine the surface coverage (Γ) that can be evaluated as [29]:

$$\Gamma = Q/nFA$$

where (Q) is the charge in microcoulomb consumed by the surface process as calculated by the integration of the area under the peak corrected for residual current, n is the total number of electrons transferred in the reactant electrode reaction, A is the mercury electrode area (0.026 cm^2) and F is the Faraday charge (96 485 C). Since the reduction of PE molecule was attributed to the hydrogenation of its C=O double bond, the total number of electrons transferred should be two ($n = 2$) as confirmed from a complete electrolysis experiment of the drug at a constant potential. The electrode coverage was obtained as 5.7402×10^{-11} mol cm^{-2}. Each adsorbed PE molecule therefore occupied an area of 2.796 nm^2.

3.3. Optimization of the procedure-conditions

The optimum instrumental conditions (e.g. frequency f, scan increment Δs and pulse amplitude E_{sw}) were examined. The variation of the peak current for 1.0×10^{-7} M PE over the frequency range of 20–120 Hz was linear in Britton–Robinson buffer (pH 7.0) at accumulation time of 30 s, scan increment of 10 mV and pulse amplitude of 25 mV.

Moreover, the peak potential (E_p) shifted to a more negative value with the increase of frequency. On plotting the peak potential (E_p) versus log f over the entire pH range, straight lines were obtained. Values of the slope of these lines were 0.050–0.098 (Table 1). Using these slope values, the transfer coefficient, α, for PE electrode reaction was calculated using the equation [30]:

$$\Delta E_p/\Delta \log f = RT/\alpha n_a F$$

Since $n_a = 1$, transfer coefficient (α) values at different pH's were found close to those values obtained by cyclic voltammetry (Table 1).

At a frequency of 120 Hz, the peak current increased linearly with the scan increment up to 10 mV. The optimal pulse amplitude was examined at $f = 120$ Hz and $\Delta s = 10$ mV. Although the current increased linearly with the increase of the square wave amplitude from 25 to 100 mV, peak distortion resulted in a poorer resolution was observed only over 25 mV. Thus, pulse amplitude of 25 mV was applied, as it was better for analytical

purposes. Therefore, the square wave optimal conditions of frequency $f = 120$ Hz, scan increment $\Delta s = 10$ mV and pulse amplitude $E_{sw} = 25$ mV, were used over the rest of the present investigation.

The dependence of the stripping peak currents on the accumulation potential was examined over the range 0 to −1.0 V for 1×10^{-7} M PE in Britton–Robinson buffer (pH 7.0) and an accumulation period of 30 s. The maximum developed peak current was achieved over the potential range of −0.2 to −0.6 V (Fig. 4). The observed gradual decrease in peak intensity may be attributed to the consequence desorption of the drug at much higher or lower potential values than the zero charge potential, where a maximum adsorption of uncharged drug molecules can be expected. Hence, a pre-concentration potential of −0.40 V was used throughout the present study.

The adsorption of PE at the hanging mercury drop electrode can be used as an effective pre-concentration step, prior to the stripping voltammetric measurement. The longer the pre-concentration time, the more pefloxacin was adsorbed onto the mercury surface and the larger was the peak current. Fig. 5 shows the dependence of the peak current on the pre-concentration period for two PE concentration levels. For 5×10^{-8} M of PE (curve a), the peak current increased linearly with the increase of the pre-concentration period. In contrast, as expected for a process limited by adsorption, a 1×10^{-7} M of PE showed a curvature for a pre-concentration period longer than 300 s, indicated that full surface coverage was approached. Obviously, the choice of pre-concentration period required compromise between sensitivity and speed.

Several instrumental parameters, those directly affect the voltammetric response, were optimized, e.g. mercury drop size, stirring rate and the rest period. The working conditions decided upon were: large drop size and 400 rpm. The stripping current was not significantly affected when varying the rest period, since it was found that 15 s was sufficient for the formation of a uniform concentration of the reactant onto the mercury drop. All experimental conditions were summarized in Table 2.

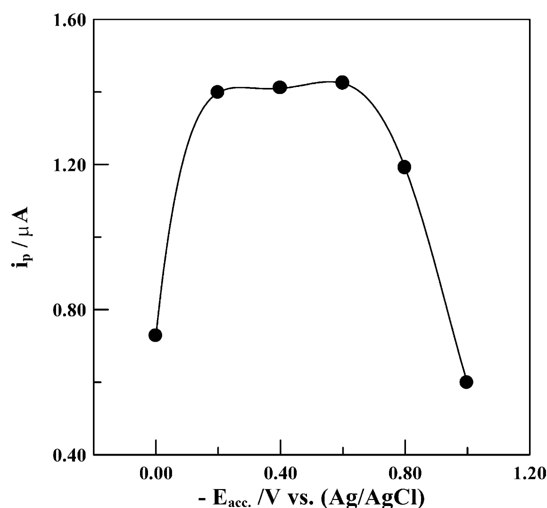

Fig. 4. Effect of accumulation potential (E_{acc}) on the SWCAdS peak current response (i_p) for 1×10^{-7} M pefloxacin after 30 s pre-concentration time. Other square wave parameters as indicated in Fig. 1.

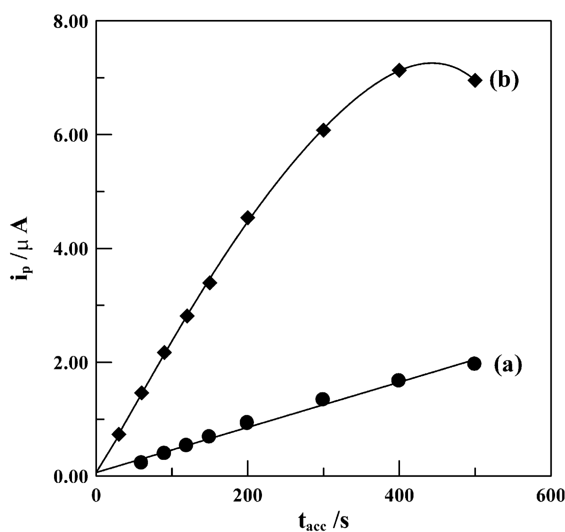

Fig. 5. Effect of the accumulation time (t_{acc}) on the SWCAdS peak current response for; (a) 5×10^{-8} and (b) 1×10^{-7} M pefloxacin, in B.R. buffer (pH 7.0) at $E_{acc} = -0.40$ V. Other parameters as indicated in Fig. 1.

Table 2
The optimized experimental conditions of the proposed procedure for the determination of pefloxacin

Variable	Optimized value
pH	7.0
Buffer type	B.R.
Strength of the buffer (M)	0.04
Temperature (°C)	25
Purge time (s)	240
Pre-concentration potential (V)	−0.4
Rest period (s)	15
Mercury drop size (cm^2)	Large (0.026)
Stirring rate (rpm)	400
Frequency (Hz)	120
Scan increment (mV)	10
Pulse amplitude (mV)	25

3.4. Analytical applications

3.4.1. Validation of the procedure

Using the optimal conditions of the proposed procedure, calibration curves within the concentration levels of 10^{-9} and 10^{-7} M PE were attempted followed different accumulation periods (0, 30, 150, 300 s). Linear range and regression equations using the least square method listed in Table 3 indicated the higher sensitivity of the proposed method. The limits of detection (LOD) and quantitation (LOQ) were calculated using the relation $k\text{S.D.}_{a}/b$ [31], where $k=3$ for LOD and 10 for LOQ, S.D._{a} is the standard deviation of the intercept and b is the slope of the calibration curve. Both LOD and LOQ values, shown in Table 3, confirmed the sensitivity of the proposed procedure.

Repeatability was examined by performing seven replicate measurements for 1×10^{-8} M PE after 30 s pre-concentration. A mean recovery of 99.54% ± 0.23 was achieved. This level of precision is suitable for the routine quality control analysis of the drug in pharmaceutical dosage form and biological fluids.

In order to access the possible analytical applications of the proposed procedure, the effect of some common excipients used in pharmaceutical preparations (e.g. starch, gelatin, talc and magnesium stearate) was studied by analyzing a sample solution containing 1×10^{-8} M PE mixed with

Table 3
Quantitative parameters of the proposed voltammetric procedure for assay of pefloxacin

Pre-concentration time (s)	Linearity range (M)	Slope[a] (μA nM^{-1})	S.D. of slope	Intercept[a] (μA)	S.D. of intercept	r ($n=12$)	LOD (M)	LOQ (M)
0	5×10^{-8}–1×10^{-6}	0.0113	0.0013	0.0170	0.0055	0.998	1.46×10^{-9}	4.87×10^{-9}
30	1×10^{-8}–5×10^{-7}	0.0231	0.0015	0.0167	0.0058	0.996	7.53×10^{-10}	2.51×10^{-9}
150	1×10^{-9}–5×10^{-7}	0.0437	0.0019	0.0211	0.0061	0.997	4.19×10^{-10}	1.40×10^{-9}
300	1×10^{-9}–1×10^{-7}	0.0966	0.0044	0.0376	0.0053	0.997	1.65×10^{-10}	5.50×10^{-10}

[a] Average of three determinations.

these excipients (added in the same ratio as in tablet) under the optimized conditions. The results indicated excellent percentage recoveries of PE as 99.54% ± 0.23 and 98.67 ± 088 for bulk drug and drug mixed with excipients, respectively. This means that the inactive excipients present in the pharmaceutical formulations should not interfere with the PE drug during its assay using the proposed procedure. Accordingly, the proposed procedure can be considered specific.

Robustness [31] was examined by studying the effect of small variation of some important procedure conditions (pH 7 ± 1.0 and accumulation potential $E_{acc} = -0.40$ V ± 0.10). As shown in Table 4, the recovery values were not significantly affected and consequently the optimized procedure was reliable for assay of PE drug and it could be considered robust [31].

Ruggedness [31] was examined by applying the proposed procedure to assay of PE under the same experimental conditions using two potentiostats, PAR-394 (Lab. 1) and 273A (Lab. 2), at different elapsed time. The results obtained due to lab. to lab. (Lab. 1: 99.54 ± 0.23; and Lab. 2: 97.11 ± 0.73) and even day to day were found reproducible, since there was no significant difference between the recovery and S.D. values.

Table 4
Influence of small variation in some of the assay conditions of the proposed procedure on the suitability and sensitivity of the procedure

Variable	Experimental conditions	%R ± S.D. ($n = 3$)
pH[a]		
6.0	$E_{acc} = -0.40$ V	101.03 ± 0.77
7.0	$t_{acc} = 30$ s	99.54 ± 0.23
8.0		98.17 ± 1.07
Pre-concentration potential (E_{acc})[a]		
−0.30 V	pH 7.0	99.25 ± 0.11
−0.40 V	$t_{acc} = 30$ s	99.54 ± 0.23
−0.50 V		101.32 ± 0.41
Potentiostat	pH 7.0	
Lab (1): model 394 (PAR)	$E_{acc} = -0.40$ V	99.54 ± 0.23
Lab (2): model 273 A (PAR)	$t_{acc} = 30$ s	97.11 ± 0.73

[a] Potentiostat Model 394 (PAR).

3.4.2. Analysis of tablets

The optimized procedure was successfully applied for determination of PE drug in tablets (Peflacin-400 mg). The percentage recovery of PE, based on the average of five replicate measurements was found as 99.57 ± 0.48 (Table 5). The obtained results were compared with those obtained by the reported method [9]. The accuracy of the proposed procedure was also judged by applying the standard addition method [32] as excellent percentage recovery (99.75 ± 0.51) of added PE was achieved. This means that the proposed procedure should be applicable to the analysis of this and other similar formulation products containing PE.

3.4.3. Analysis of human serum

Fig. 6 illustrates the square wave cathodic adsorptive stripping voltammograms of successive additions of PE-serum; each addition effected a 1×10^{-9} M PE; 300 s pre-concentration period was employed. The variation of the peak current versus the drug concentration was represented by a straight line followed the equation; i_p (μA) = 0.2001 C (nM) + 0.0381, with a correlation coefficient of 0.996. The percentage recovery of PE in human serum, based on the average of five replicate measurements, was found to equal 98.55 ± 0.78. The percentage recovery of PE was determined by comparing the peak currents of known drug concentrations in serum with their equivalents on the calibration curve. The obtained LOD and LOQ values (4.50×10^{-10} and 1.50×10^{-9} M PE, respectively) using the proposed procedure were compared with those obtained by reported HPLC method [17] (3.41×10^{-10} and 1.14×10^{-9} M, respectively). Moreover, analysis of serum sample using the proposed procedure required only separation of the precipitated protein with centrifugation, no time-consuming extraction and/or evaporation steps were required.

4. Conclusions

A fully validated electroanalytical procedure for the assay of antibiotic pefloxacin drug in bulk, tablets and human serum is described for the first

Table 5
Assay of PE drug in pharmaceutical formulation by the proposed and reported methods

Sample	Claimed value (mg)	Found by proposed method (%±S.D.[a])	Found by reported[b] method (%±S.D.[a])	Recovery[c] by proposed method (%±S.D.[a])
Peflacin tablet	400/tablet	99.57±0.48	103.15±0.87	99.75±0.51

[a] Average of five determinations.
[b] Reference [9].
[c] Recovery of 1.0 mg of PE added to different amounts of Peflacin-400 tablet using standard addition method [32].

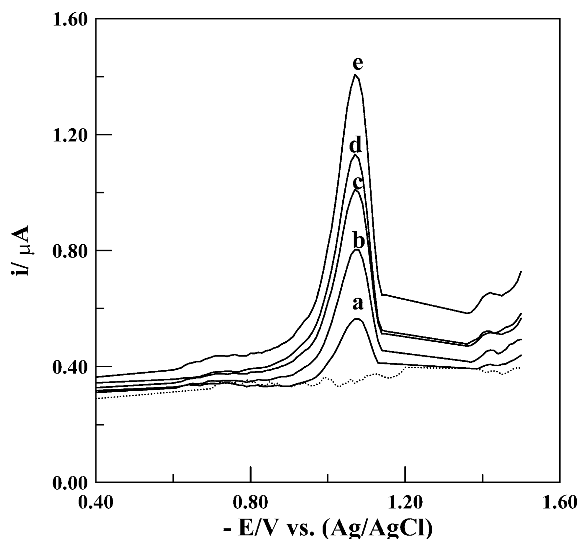

Fig. 6. SWCAdS voltammograms for different concentrations of pefloxacin in human serum samples recorded at $E_{acc} = -0.40$ V and $t_{acc} = 300$ s. The dotted line represents the background: (a) 1×10^{-9}; (b) 2×10^{-9}; (c) 3×10^{-9}; (d) 4×10^{-9}; and (e) 5×10^{-9} M pefloxacin. Other parameters as indicated in Fig. 1.

time. Hence, it can be recommended for the routine quality control of this antibiotic drug. Moreover, compared to the reported spectrophotometric and spectrofluorometric methods [3–8] for the pefloxacin assay, the proposed procedure is more sensitive and accurate. From the economical point of view, the proposed procedure is simple, rapid and low cost, so it is a good alternative to reported spectrophotometric, spectrofluorimetric and capillary electrophoresis methods [3–10] and to the high cost and time-consuming reported HPLC methods [11–17] with equivalent sensitivity.

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