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Original Research Article

Adsorptive Cathodic Stripping Voltammetric Determination of Ciprofloxacin in Bulk Powder, Pharmaceutical Dosage Forms and Urine

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Abstract

Purpose*: To investigate the electro-reduction behaviour and determination of ciprofloxacin using a hanging mercury drop electrode.*
Methods*: Cyclic voltammograms of ciprofloxacin recorded in Britton – Robinson buffers pH 2 – 5 exhibit a single irreversible cathodic peak. The dependence of the peak current and peak potential values on buffer (nature, pH and concentration) and accumulation conditions (time and potential) were examined and used for the quantitative analysis of ciprofloxacin in dosage forms and urine. Acetate buffer (0.08 M, pH 3.6) was selected as a supporting electrolyte for quantitative purposes by differential pulse and square wave adsorptive cathodic stripping voltammetry.*
Results*: A reduction wave was seen in the range of – 1.3 to – 1.5 V. These techniques were successfully validated as per ICH guidelines and comparable to US Pharmacopoeia HPLC method. The techniques was applied to the determination of ciprofloxacin in pharmaceutical formulations (i.e. precision (RSD < 1%) and accuracy (99 – 101%)), and was further extended to determine ciprofloxacin in spiked human urine with no matrix effect (i.e. LLOQ 0.01 μg/ml, precision (RSD < 15%) and accuracy (85 – 115%)).*
Conclusion*: Validated adsorptive cathodic stripping voltammetric technique can be recommended for use in quality control and pharmacokinetics studies.*

Keywords*: Ciprofloxacin, Mercury electrode, Adsorptive cathodic stripping voltammetry, Pharmaceutical formulation, Urine.*

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INTRODUCTION

Ciprofloxacin is a second-generation synthetic fluoroquinolone antibiotic. Chemically, it is described as 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid. Since being first patented by Bayer AG and subsequently approved by US Food and Drug Administration in the 1980s, it has been commonly used for urinary tract and intestinal infections [1]. It is also one of the antibiotics recommended by FDA for inhalational anthrax post-exposure [2]. This drug is bactericidal over a wide range of Gram-positive and Gram-negative bacteria via the blockage of cell growth and division by interfering with two essential bacterial enzymes DNA gyrase and DNA topoisomerase IV, which probably leads to the lethal release of double-strand DNA breaks [3]. The bioavailable salt forms of ciprofloxacin in the market are hydrochloride (tablets and eye/ear drops) and lactate (intravenous infusion solutions).

In literature, the determination of ciprofloxacin in pharmaceutical dosage forms is achieved by high performance liquid chromatography (HPLC) [4], high performance thin layer chromatography (HPTLC) [5], spectrophotometry and spectrofluorimetry [6,7], potentiometry [8], atomic absorption spectrometry [9], Rayleigh light scattering technique [10] as well as voltammetry [11–14].

The purpose of this work was to electroanalytically investigate the reduction mechanism and voltammetric behavior of ciprofloxacin at the hanging mercury drop electrode, which has not been reported so far, using cyclic, differential pulse and square wave voltammetry for its quantification in pharmaceuticals and spiked human urine.

EXPERIMENTAL

Reagents and apparatuses

Ciprofloxacin hydrochloride standard was kindly supplied by National Institute of Drug Quality Control (Vietnam) and used without further purification. A standard stock solution of 500 μg/ml ciprofloxacin was prepared in water and protected from light in a refrigerator. This solution was stable for at least one week as voltammetrically tested. Working standards of ciprofloxacin were freshly prepared just before the assay by adding appropriate amounts of stock solution with concentrated supporting electrolytes and water to the mark in 50 ml volumetric flasks. Other chemicals were of analytical grade and purchased from Merck (Germany). Concentrated supporting electrolytes were prepared on the day the experiment was performed. Urine was collected from healthy human was used for ciprofloxacin – spiked samples. Freshly prepared de-ionized doubly distilled water (Maxima Ultra. Pure Water, Elga-Prima Corp, UK) was used throughout this study. pH measurements were performed on a CyberScan pH 510 (Eutech Instruments Pte Ltd., Singapore). All voltammetric measurements were carried out at 25°C with a 797 VA Computrace (Metrohm AG, Switzerland) in connection with a Dell computer using Microsoft Windows XP and controlled by VA Computrace Software 1.3. The three – electrode system consisted of a hanging mercury drop electrode (HMDE) as working electrode, a platinum wire as auxiliary electrode and an Ag|AgCl|KCl(3 M) as reference electrode. A medium size mercury drop was employed. Oxygen – free nitrogen gas was used for the removal of dissolved oxygen from the measured solutions for 100 s with a stirring rate of 2000 rpm. After a pre-concentration step, the solutions were left quiescent for 15 s equilibrium before measurement.

To elucidate the characteristics of electrode process, the cyclic voltammetric behaviour of 0.05 μg/ml ciprofloxacin in the Britton – Robinson universal buffers at the HMDE was studied. The experimental parameters were accumulation potential – 1.1 V, accumulation time 30 s, scan rate 0.1 V/s, pulse step 0.005 V and potential range from – 1.1 to – 1.6 V. In this study, differential pulse and square wave adsorptive cathodic stripping voltammetry (DP-AdCSV and SW-AdCSV as respectively abbreviated) were taken into account for quantitative purposes. The optimization process started with the selection of buffer type (i.e. acetate, phosphate and citrate buffers pH 3.5) with voltammetric measurements of 0.1 μg/ml ciprofloxacin. Unless stated otherwise, the voltammograms were recorded in triplicate for each automatic run. Optimization process was carried out using Modde 9.1 software (Umetrics, Sweden). All experiments were performed in a randomized order as proposed by the software so as to minimize the effect of uncontrolled factors that may introduce a bias on the response. To determine the optimal factors, the optimizer function of Modde software i.e. Nelder Mead simplex method was used.

Sample preparation

Twenty tablets (Ciprobay 500 mg, Bayer AG, Germany) were finely pulverized in a mortar. A quantity equivalent to 400 mg ciprofloxacin was accurately weighed and dissolved in about 25 mL of water in a 50 mL volumetric flask by sonication for 20 min, subsequently diluted to the mark with the same solvent. For intravenous infusion solution 2 mg/mL (Pharbaco Central Pharmaceutical Joint Stock Company, Vietnam) and eye drops 0.3% (Quindrops, Altomega Drugs Pvt., Ltd., India), the content of five bottles or vials were well mixed. Appropriate dilutions were then made in 50 mL volumetric flasks to obtain ca. 0.08 μg/mL ciprofloxacin test solution.

Analysis of samples

The general procedure adopted for obtaining adsorptive stripping voltammograms was as follows. For pharmaceutical formulations, 0.25 mL of test solution was diluted with 0.1 mL concentrated supporting electrolyte solution (i.e. 0.08 M acetate buffer pH 3.6) and water to the mark in a 25 mL volumetric flask. For spiked human urine, accurately measured aliquots of 5 μg/mL ml ciprofloxacin were pipetted into 25 mL volumetric flasks, subsequently added with 0.1 mL concentrated supporting electrolyte solution

and human urine diluted with water to 1250 times to the mark. The content of these flasks was completely transferred into the voltammetric cell for measurement after shaking for 5 min mechanical shaking.

RESULTS

Characteristics of the electrode process

A well-defined single peak was yielded in the cathodic direction in the pH range 2 – 5 (Figure 1). The peak current obtained without accumulation was substantially smaller than that obtained after a pre-concentration step (data not shown). On the other hand, the peak potential and current were not appreciably influenced by varying the scan rate from 0.03 – 0.1 V/s at different pH values. No oxidation peak was observed in the positive scanning half-cycle. This irreversible cathodic peak disappeared at pH > 5 (data not shown).

A linear relationship of E_p vs. pH following the regression equation: E_p (V) = – 0.067 pH – 1.237 (R^2 = 0.998 and n = 4) was obtained. Its slope value equals 67 mV = {(59/α)×(m/n_a)}, where n_a and m are the numbers of electrons and protons involved in the rate determining step, respectively, and α is the symmetry transfer coefficient.

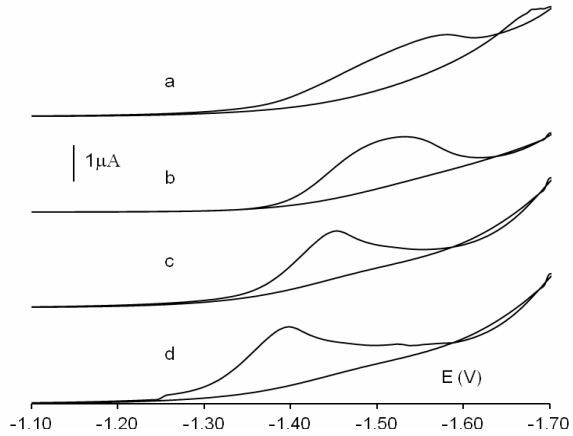


Figure 1: Cyclic voltammograms of 0.05 μg/ml ciprofloxacin in Britton – Robinson buffers pH 5.0 (a), 4.1(b), 3.4 (c), 2.5 (d)

Optimal parameters and experimental conditions

DP-AdCSV and SW-AdCSV measurements of 0.1 μg/ml ciprofloxacin recorded in acetate, phosphate and citrate buffers pH 3.5 only show an askew well-defined sharp peak in acetate medium. Accordingly, this type of buffer was chosen as a supporting electrolyte for further investigation on its concentration and pH in parallel with accumulation parameters (potential and time), which influenced the peak current magnitude most on preliminary measurements.

Table 1 presents a Central Composite Face experiment design with these 4 factors. While implementing this experiment design, other voltammetric parameters were kept constant. In Figures 2 and 3, response surface plots for the influence of the interaction of buffer pH with concentration, accumulation potential with time on the magnitude of the peak current measured are shown for DP-AdCSV and SW-AdCSV techniques, respectively.

The optimal parameters generated for the minimum current peak recorded with 0.1 μg/ml ciprofloxacin are summarized in Table 2 for both techniques.

Validation and application

A linear correlation between the voltammetric peak intensity and drug concentration was obtained over the range 0.05 – 0.15 μg/ml (Figures 4 A and B, and Table 3).

Repeatability and intermediate precision of the described stripping voltammetric techniques were examined by performing six replicate measurements of 0.08 μg/ml ciprofloxacin over one day (intraday assay) and for three successive days (interday assay), then calculating the relative standard deviation (RSD < 2%) for the concentrations obtained. The statistical comparison of voltammetric and liquid chromatographic techniques was done by the Student's t-test and Snedecor's F-test at a 95% confidence level (Table 4). No marked changes was observed under the influence of small variation (5%) of the most important variables i.e. buffer pH and concentration, accumulation time and potential.

For the determination of ciprofloxacin in spiked human urine, the above-mentioned optimal experiment conditions were also applied. No reduction wave was seen in the range of from – 1.3 to – 1.5 V with pure urine water-diluted by 1250 times (blank) (Figures 5 A and B). The calibration curve for this analysis was constructed in the range 0.01 – 0.20 μg/ml (Table 2). Analyte peak was identifiable, discrete and reproducible at this concentration (RSD < 20 % and recovered within 80 – 120 %). The precision (RSD < 15%) and accuracy (85 - 115%).

Table 1: A Central Composite Face experiment design for voltammetric measurements of 0.1 µg/mL ciprofloxacin

No.	Buffer		Accumulation		Current (µA)	
	pH	Concentration (M)	Potential (V)	Time (s)	DP-AdCSV	SW-AdCSV
1	3	0.06	– 1.3	50	– 5.102	– 4.230
2	4	0.06	– 1.3	50	– 5.203	– 4.010
3	3	0.1	– 1.3	50	– 4.987	– 4.020
4	4	0.1	– 1.3	50	– 5.121	– 4.010
5	3	0.06	– 0.9	50	– 4.890	– 3.950
6	4	0.06	– 0.9	50	– 5.090	– 4.150
7	3	0.1	– 0.9	50	– 4.923	– 3.450
8	4	0.1	– 0.9	50	– 4.914	– 3.700
9	3	0.06	– 1.3	100	– 4.201	– 5.102
10	4	0.06	– 1.3	100	– 3.462	– 5.203
11	3	0.1	– 1.3	100	– 3.382	– 4.987
12	4	0.1	– 1.3	100	– 3.402	– 5.121
13	3	0.06	– 0.9	100	– 4.103	– 4.890
14	4	0.06	– 0.9	100	– 3.301	– 5.090
15	3	0.1	– 0.9	100	– 3.421	– 4.923
16	4	0.1	– 0.9	100	– 3.918	– 4.870
17	3	0.06	– 1.1	75	– 4.563	– 4.570
18	4	0.06	– 1.1	75	– 4.569	– 4.630
19	3.5	0.06	– 1.1	75	– 4.890	– 4.890
20	3.5	0.1	– 1.1	75	– 4.872	– 4.872
21	3.5	0.08	– 1.3	75	– 4.456	– 4.456
22	3.5	0.08	– 0.9	75	– 4.472	– 4.502
23	3.5	0.08	– 1.1	50	– 6.102	– 6.000
24	3.5	0.08	– 1.1	100	– 4.113	– 4.210
25	3.8	0.08	– 1.1	75	– 5.324	– 5.324
26	3.8	0.08	– 1.1	75	– 5.432	– 5.320
27	3.8	0.08	– 1.1	75	– 5.351	– 5.351

Table 2: Optimal parameters for voltammetric measurements of 0.1 µg/ml ciprofloxacin

Parameter	DP-AdCSV	SW-AdCSV
Buffer pH	3.6	3.6
Buffer concentration	0.08 M	0.08 M
Accumulation potential	– 1.1 V	– 1.1 V
Accumulation time	50 s	100 s
Pulse amplitude	0.1 V	0.1 V
Frequency	50 Hz	20 Hz
Pulse step	0.005 V	0.005 V
Potential range	– 1.1 → – 1.6 V	– 1.1 → – 1.6 V

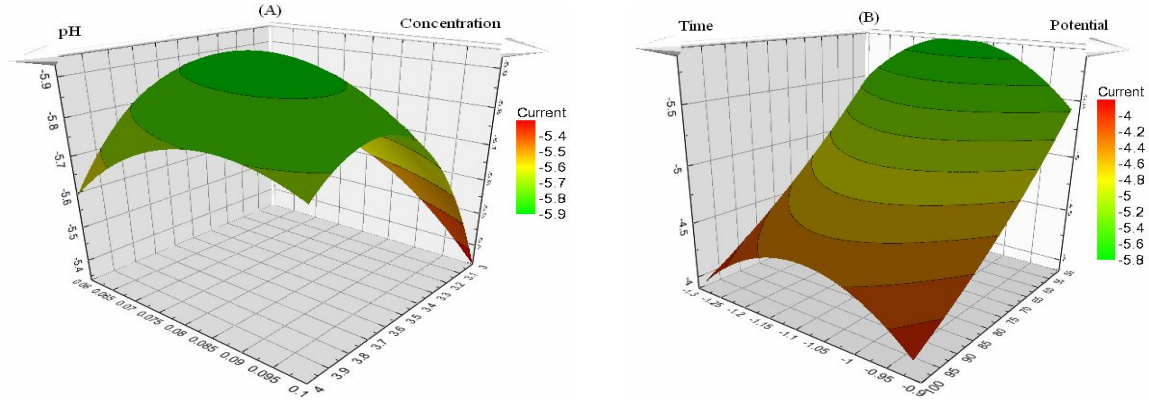


Figure 2: Response surface plots for the influence of buffer pH and concentration (accumulation potential – 1.1 V and accumulation time 50 s) (A), and accumulation potential and time (buffer pH 3.6 and concentration 0.08 M) (B) on DP-AdCSV peak current of 0.1 µg/mL ciprofloxacin

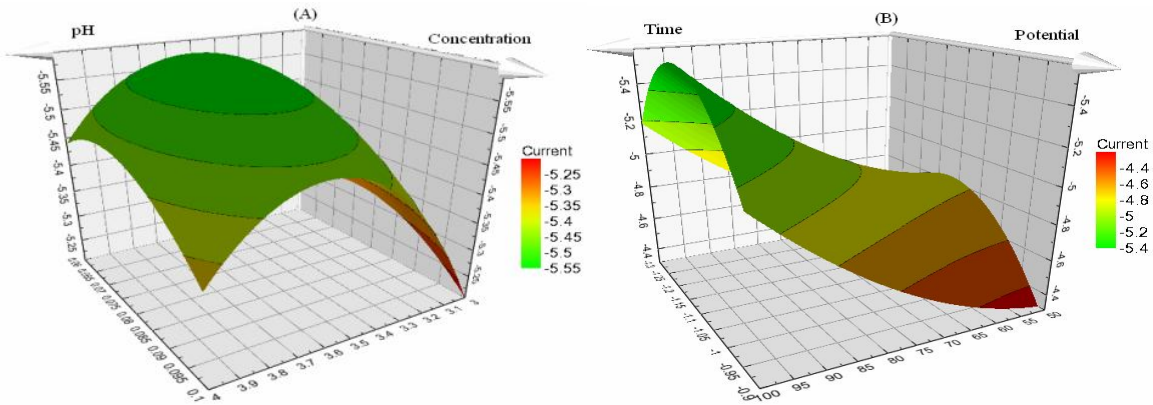


Figure 3: Response surface plots for the influence of buffer pH and concentration (accumulation potential – 1.1 V and accumulation time 100 s) (A), and accumulation potential and time (buffer pH 3.6 and concentration 0.08 M) (B) on SW-AdCSV peak current of 0.1 μg/ml ciprofloxacin

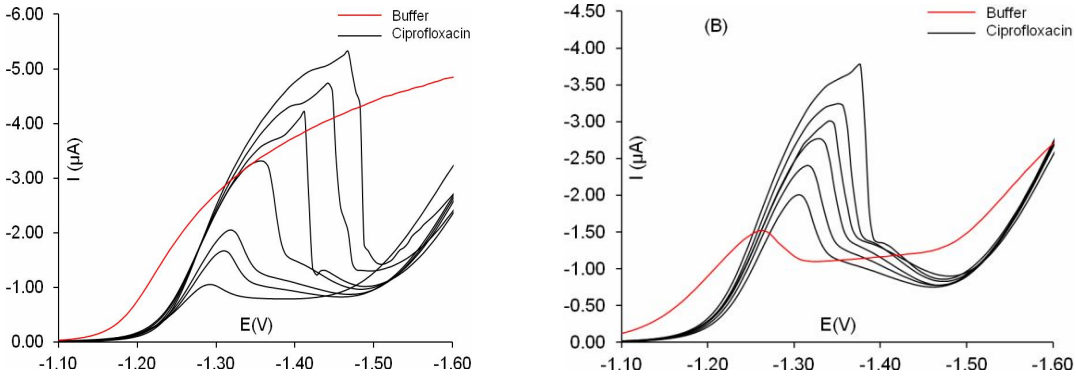


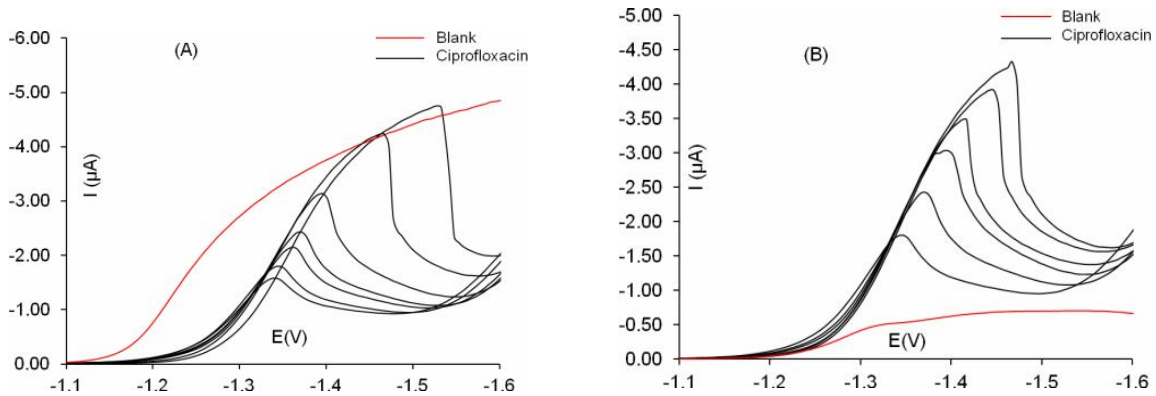
Figure 4: DP-AdCSV (A) and SW-AdCSV (B) measurements of 0.05 – 0.15 μg/ml ciprofloxacin in 0.08 M acetate buffer pH 3.6

Table 3: Linearity statistical analysis for the proposed voltammetric techniques

Parameter	Pharmaceutical formulation		Spiked human urine	
	DP-AdCSV	***SW-AdCSV***	***DP-AdCSV***	***SW-AdCSV***
Concentration range (μg/mL)	0.05 – 0.15	0.05 – 0.15	0.01 – 0.20	0.01 – 0.20
Number of data points, n	6	6	7	7
Coefficient of determination, R^2	0.9964	0.9906	0.9984	0.9977
Slope	–15.700	– 24.271	– 18.282	– 15.877
Intercept	– 1.258	– 0.758	– 1.159	– 1.661
SD of the residuals, $S_{y/x}$	0.0395	0.0990	0.05793	0.0603
SD of the slope, S_a	0.4726	1.1840	0.32590	0.3394
SD of the intercept, S_b	0.0499	0.1251	0.03404	0.0355

Table 4: Assay results for the determination of ciprofloxacin in pharmaceutical formulations by the proposed voltammetric techniques and USP-HPLC

Pharmaceutical formulation	Recovery ± SD % (n = 6) ($t_{(0.05, 10)}$ = 2.228, $F_{(0.05, 5, 5)}$ = 5.050)		
	USP-HPLC	***DP-AdCSV***	***SW-AdCSV***
Ciprobay 500 mg/tablet	101.3 ± 1.1	102.1 ± 0.9 t = 1.379 F = 1.494	101.5 ± 1.2 t = 0.301 F = 1.190
Quindrops 0.3%	101.9 ± 1.5	101.3 ± 1.0 t = 0.815 F = 2.250	100.9 ± 1.1 t = 1.317 F = 1.860
Intravenous infusion 2 mg/ml	101.1 ± 1.6	101.9 ± 0.9 t = 1.067 F = 3.160	101.5 ± 1.0 t = 0.519 F = 2.560



Figures 5: DP-AdCSV (A) and SW-AdCSV (B) measurements of 0.01 – 0.20 µg/ml ciprofloxacin in spiked human urine

were obtained from the back calculation of other calibration standards.

This analysis was also intraday and interday validated with four spiked quality control (QC) samples (Table 5). The relative analytical recovery (comparing the measured concentration with actual added ones) of these techniques was within the range 85 – 115% with RSD < 15%. Interestingly, the percentage absolute analytical recovery of the proposed DP-AdCSV and SW-AdCSV techniques (comparing the absolute peak intensity of ciprofloxacin from a spiked urine sample to the absolute peak intensity of an equivalent aqueous standard) was within the range 95 – 100%.

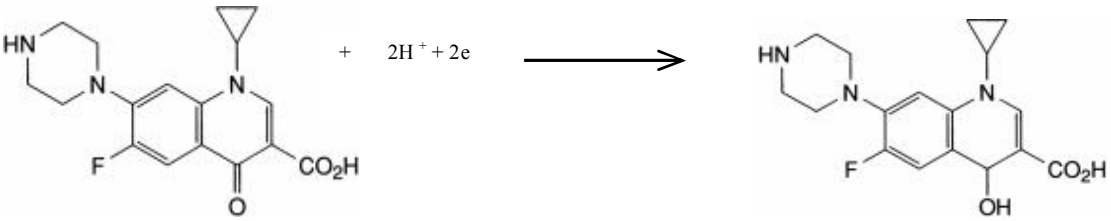
Table 5: Accuracy and precision data for the determination of ciprofloxacin in spiked with Human urine by the proposed voltammetric techniques (n = 3)

	Actual conc. (µg/mL)	Experimental conc. (µg/mL)	Precision as RSD (%)	Accuracy (%)
		DP-AdCSV		
Intraday	0.01	0.011 ± 0.004	3.7	107.4
	0.025	0.024 ± 0.001	1.0	96.6
	0.10	0.097 ± 0.001	1.0	96.7
	0.16	0.167 ± 0.001	1.9	104.4
Interday	0.01	0.011 ± 0.002	1.0	106.7
	0.025	0.024 ± 0.008	2.0	97.8
	0.10	0.095 ± 0.009	4.5	95.4
	0.16	0.016 ± 0.008	1.0	98.9
		SW-AdCSV		
Intraday	0.01	0.009 ± 0,001	4.4	90.0
	0.025	0.025 ± 0,001	2.4	98.8
	0.10	0.100 ± 0,002	2.0	99.8
	0.16	0.160 ± 0.003	1.9	100.4
Interday	0.01	0.009 ± 0,001	0.1	90.0
	0.025	0.025 ± 0,001	0.8	98.9
	0.10	0.100 ± 0,001	1.0	99.7
	0.16	0.016 ± 0.004	2.5	100.0

DISCUSSION

Cyclic voltammetric data strongly indicates that ciprofloxacin adsorbs readily onto the surface of HMDE, and a considerable increase in sensitivity can be gained by adsorptive stripping voltammetry. The peak potential (E_p) shifted to more negative values and broadened upon the increase of medium pH revealing the involvement of protons in the electrode reaction, and that the proton-transfer reaction precedes the electron transfer [15]. The irreversible nature of the electrode process is confirmed as no oxidation peak observed. In the acidic medium, ciprofloxacin must be in protonated forms and its reduction pathway at the mercury electrode could be postulated according to the following reaction (Scheme 1).

Given the fact that two electrons (n_a = 2) and two proton (m = 2) are involved in the rate-determining step of the electro-reduction process of ciprofloxacin as explained above, α- value of



Scheme 1: Proposed mechanism of ciprofloxacin reduction at the mercury electrode

0.88 was estimated from the slope value (67 mV / pH) of the (E_p) vs. (pH) plot.

In our study, the selection of acetate buffer as a supporting electrolyte is in an agreement with previously reported data [13]. Optimization data show that a minimum current peak of 0.1 µg/ml ciprofloxacin reached when the values of buffer pH, concentration and accumulation potential were the middle points of the ranges investigated. In contrast, 50 and 100 s were the ideal choice of accumulation time to DP-AdCSV and SW-AdCSV techniques for optimal sensitivity, respectively. This could be attributed to the difference in frequencies used in these techniques.

Under the optimized conditions presented in Table 2, the proposed voltammetric techniques (DP-AdCSV and SW-AdCSV) were validated according to ICH guidelines [16]. RSD < 2 % means that the proposed voltammetric techniques were precise. Accuracy was assessed by comparing the voltammetric results with US pharmacopoeia HPLC [17] considered as a well-characterized procedure [16] when quantitatively analyzing ciprofloxacin in various pharmaceutical formulations. No statistically significant differences was observed between these techniques suggesting that DP-AdCSV and SW-AdCSV could be used for the routine analysis of ciprofloxacin pharmaceutical formulations in place of HPLC. In addition, these voltammetric techniques were fairly robust as reflected by its capacity to remain unaffected by small variation of the most important variables. The determination of ciprofloxacin in spiked human urine was specific as the matrix influence of diluted human urine to its voltammetric measurement was not considerable. For this analysis, the construction of the calibration curve was based on the fact that urine concentrations of unchanged ciprofloxacin are approximately 30 – 200 µg/ml within 2 – 10 h after a 250 mg oral dose [18]. As a consequence of this linear range, the sensitivity (Lower Limit Of Quantification – LLOQ) was 0.01 µg/ml for both DP-AdCSV and SW-AdCSV techniques. Clearly, the validation data support that DP-AdCSV and SW-AdCSV determination of ciprofloxacin in human urine satisfactorily meets the requirement for assay methods for biological samples [19].

CONCLUSION

The mechanism of electro-reduction of ciprofloxacin at the HMDE was elucidated. Using 0.08 M acetate buffer pH 3.6 as a supporting electrolyte, DP-AdCSV and SW-AdCSV techniques were developed for the determination of ciprofloxacin in bulk powder, pharmaceutical dosage forms and spiked human urine without any prerequisite extraction, separation and adsorption steps. Votammetric data were statistically comparable to pharmacopoeia HPLC suggesting that the determination of ciprofloxacin was accurate, precise and without interferences from excipients. Moreover, the sensitivity and reliability of the determination of ciprofloxacin in spiked human urine sample was also acceptable. Hence, the validated techniques could be recommended for use in quality control and pharmacokinetics studies.

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