

Cathodic Stripping Voltammetry of Pipemidic Acid and Ofloxacin in Pharmaceutical Dosages and Human Urine

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Sensitive cathodic stripping voltammetric methods have been developed for two quinolone antibacterial drugs, pipemidic acid (PIP) and ofloxacin (OFL) using hanging mercury drop electrode as working electrode vs. Ag/AgCl reference electrode. The methods were developed for the determination of drugs individually as well as simultaneously. 0.1 M and 0.01 M hydrochloric acid was used as medium for PIP and OFL, respectively, 0.1 M potassium chloride was used as base electrolyte. Reduction waves were observed for PIP within -700 mV to -800 mV and for OFL within -1100 mV to -1200 mV. Linear calibration ranges for PIP and OFL were observed within 10-100 $\mu\text{g ml}^{-1}$ with detection limits of 50 ng ml^{-1} and 1 $\mu\text{g ml}^{-1}$, respectively. Relative standard deviations (RSD) for the analysis of 10 $\mu\text{g ml}^{-1}$ of PIP and OFL ($n = 6$) were 0.5% and 1.4%, respectively. The presence of glucose, lactose, sorbitol, gum arabic, starch, magnesium stearate, methylparaben and propylparaben did not affect the determinations of both PIP and OFL. The methods were used for the analysis of pharmaceutical preparations and the results indicated relative deviation of 0.5-5.5% from labeled values with RSD within 0.49-2.5%. PIP and OFL could also be determined simultaneously, and were determined from spiked human urine.

Keywords: Cathodic stripping voltammetry, Quinolones, Pipemidic acid and ofloxacin, Determination

INTRODUCTION

Pipemidic acid (PIP), 8-ethyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)-pyrido[2,3-*d*]pyrimidine-6-carboxylic acid, and ofloxacin (OFL), 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid (Fig. 1) are antibacterial agents of a group of synthetic compounds quinolone used for the treatment of infections in both humans [1] and in animals [2]. OFL is a synthetic fluorinated quinolone derivative having activity against both Gram-negative and Gram-positive bacteria through inhibition of their DNA gyres [1]. PIP belongs to first generation and OFL to second generation

quinolones.

A number of methods are reported for the determination of PIP and OFL. The analytical procedures include atomic absorption spectrometry [3], colorimetry [4], flow injection spectrophotometry [5], spectrophotometry [6-8], microbiological assay [9], spectrofluorimetry [10-12], liquid chromatography [13-17], chemiluminescence [18-22] and electroanalytical techniques [23-29].

Electroanalytical methods are sensitive, selective and suitable for biological active compounds. The presence of carbonyl and carboxylic acid groups within the molecule has initiated several polarographic studies. Tamer [23] applied differential pulse polarography (DPP) to the determination of PIP at pH 1.7. PIP was also determined by concentrating it on carbon fiber indicator electrode at 100 mV for 30 s and

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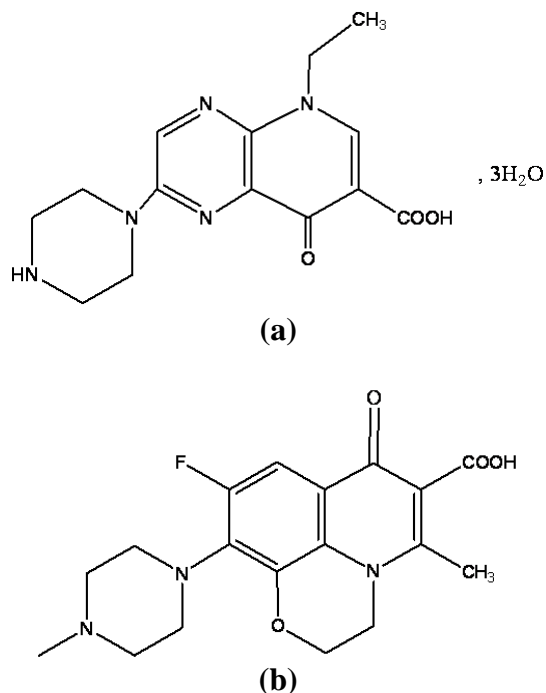


Fig. 1. Structure of Pipemidic acid (a) and Ofloxacin (b).

applying anodic stripping voltammetry between 0 and 1.3 V [24]. Telting-Diaz *et al.* determined PIP by linear sweep differential pulse and square wave voltammetry at hanging mercury drop electrode. The adsorbed species were measured voltammetrically by using a cathodic process appearing at -0.76 V in 0.1 M HClO₄ [25]. OFL was determined by single sweep polarography by measuring reduction peak at 1.55 V *vs.* SCE in buffer of pH-6 [26]. OFL was also determined polarographically in buffer (pH 4) [27], by DPP in buffer (pH 8.36) at 1.4 V *vs.* Ag/AgCl over the range 0.01-0.1 mM [28] and by DPP through its complex with copper(II) [29].

In the present work, new cathodic stripping voltammetric methods have been developed for PIP and OFL in hydrochloric acid at pH 2-2.4 within -700 to -800 mV and -1100 to -1200 mV separately and in mixtures.

EXPERIMENTAL

Chemicals and Reagents

Reagent grade chemicals, hydrochloric acid (37%) and base electrolytes potassium chloride, potassium nitrate, lithium chloride, sodium acetate, potassium dihydrogen phosphate, potassium citrate, sodium carbonate, sodium borate,

ammonium chloride and sodium hydroxide (E-Merck, Germany) were used. Freshly prepared doubly distilled deionized water was used throughout the study. PIP was obtained from Abbott Laboratories, Karachi, Pakistan and OFL was purchased from Sigma, Switzerland. Stock solutions of PIP and OFL were prepared in 0.1 M and 0.01 M HCl, respectively. The solutions were further diluted in the same solution. Potassium chloride solution (0.1 M) was prepared in distilled deionized water.

Instrumentation

Voltammetric studies were performed with an automatic 746-VA Trace analyzer equipped with 747-VA stand (Metrohm, Switzerland). The 747 VA stand includes a three electrode system consisting of a Ag/AgCl reference electrode, a platinum wire as auxiliary electrode and a hanging mercury drop (HMDE) as working electrode was used. A printer attached with the instrument was used for printing purpose. A medium size mercury drop was employed as the HMDE. The solutions were stirred by a PTFE-coated stirring bar rotated by a magnetic stirrer during the preconcentration step.

Determination of Pipemidic Acid and Ofloxacin (Analytical Procedure)

An aliquot of solution of PIP (in 0.1 M HCl) or OFL (in 0.01 M HCl) containing 100-1000 µg of the drugs was adjusted to pH 2 or 2.4, respectively, and volume was made up to 9 ml. 1 ml of 0.1 M potassium chloride was then added and contents were transferred into polarographic vessel. The sample was purged for 200 s with oxygen free nitrogen (British Oxygen Company, Karachi, Pakistan). The preconcentration potential -775 mV or -1150 mV, measured against Ag/AgCl reference electrode, was applied to the fresh mercury drop for 60 s ($t_{acc} = 60$ s) while the solution was stirred. The stirring was then stopped for a period of 10 s (equilibration time = 10 s). The voltammogram was then recorded by applying a cathodic differential pulse scan with pulse amplitude of -50 mV. The voltammograms were recorded in triplicate for each run automatically by the instrument. The peak heights were assessed on the basis of difference between the peak height of the analyte and that of base electrolyte alone recorded under the same conditions. The quantitation was carried out by calibration curve and standard

addition technique. Voltammogram recorded for PIP or OFL using standard addition method are given in Figs. 2 and 3.

Determination of Pipemidic Acid and Ofloxacin in Pharmaceutical Preparations

Eight tablets of Urixin (Abbott Lab., Karachi, Pak) for the analysis of PIP and Oflobid (Hilton, Karachi, Pak), Curitol (Standpharm Pvt., Ltd., Karachi, Pak), Tarivid (Avantis, Karachi, Pak) and Zoflox (Pharmatec Pvt., Ltd., Karachi, Pak) for the analysis of OFL were ground to a fine powder. An average quantity equivalent to one tablet containing 400 mg of PIP or 200 mg of OFL was weighed, dissolved in HCl (0.1 M or 0.01 M), and volume was adjusted to 100 ml with HCl. This solution was slightly turbid but no further treatment was made. Again dilution was made to $50 \mu\text{g ml}^{-1}$ of the drugs. An aliquot from each of the pharmaceutical preparation was taken and the recommended procedure was followed.

Simultaneous Determination of Pipemidic Acid and Ofloxacin

An aliquot of solution (10 ml) of HCl (0.01 M) plus 1 ml potassium chloride containing PIP ($10\text{-}50 \mu\text{g ml}^{-1}$) and OFL ($10\text{-}50 \mu\text{g ml}^{-1}$) adjusted to pH 2.2 and was transferred to the polarographic vessel. The recommended analytical procedure was followed. The voltammograms were monitored with accumulation time 60 s at -755 nm with peak potentials within -700 to -1400 mV . The voltammetric quantitation of PIP and OFL was carried out using standard addition method.

Simultaneous Determination of Pipemidic Acid and Ofloxacin in Urine Samples

Urine samples, 3 ml each were spiked with varying amounts of PIP and OFL ($10, 20, 50 \mu\text{g ml}^{-1}$) and treated with 2 ml methanol as urine protein precipitating agent. After vortex mixing during 30 s the precipitated protein was separated out by centrifugation for 20 min at 4000 rpm. The filtrate was collected and added 0.01 M HCl and pH adjusted to 2.2 and the volume was adjusted to 9 ml and the recommended analytical procedure was followed.

RESULTS AND DISCUSSION

The antimicrobial agents PIP and OFL are reported to

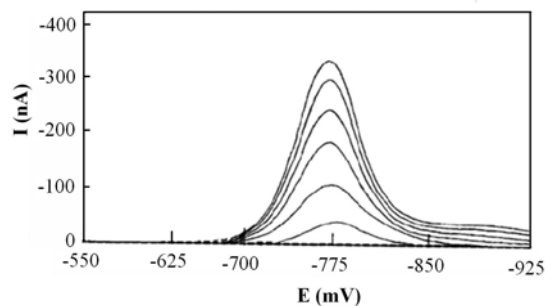


Fig. 2. Cathodic stripping voltammogram for PIP indicating potential (mV) vs. current (nA) in 0.1 M HCl plus 0.1 M KCl, pH 2.0, peak potential -775 mV and CSV response of PIP with 10, 20, 40, 60, 80, 100 $\mu\text{g ml}^{-1}$.

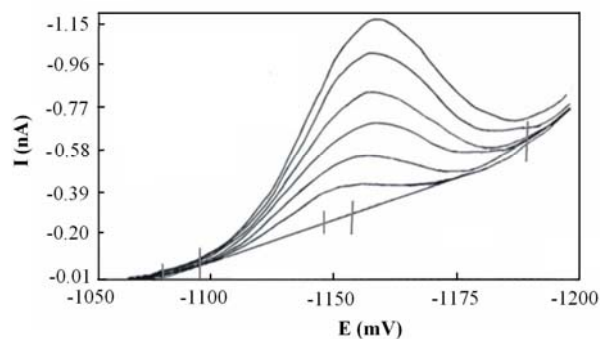


Fig. 3. Cathodic stripping voltammogram for ofloxacin indicating potential (mV) vs. current (nA), 0.01 M HCl, plus 0.1 M KCl, pH 2.4, peak potential -1150 mV and CSV response of OFL with 10, 20, 40, 60, 80 and 100 $\mu\text{g ml}^{-1}$.

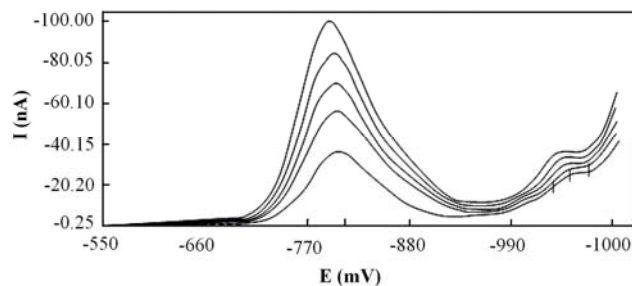


Fig. 4. Simultaneous determination of PIP and OFL in 0.01 M HCl with 0.1 M KCl at pH 2.2 with concentrations, 10, 20, 30, 40 and 50 $\mu\text{g ml}^{-1}$.

indicate adsorptive behavior on the electrode during voltammetry [24,25,28]. Therefore, for the sensitive determination of PIP and OFL, a preconcentration step at hanging mercury drop electrode prior to cathodic stripping voltammetry (CSV) was examined. The effects of pH, electrolyte, accumulation time and peak potential on CSV were investigated. The effect of pH was examined in different buffers varying from 1-5 at 0.5 pH unit interval first and then 0.1 unit interval. It was observed that current was highest at pH 2.0 for PIP. OFL showed highest current at pH 4.7 but linearity of response with variation in concentration was poor. However, when pH was adjusted to 2.4, a stable response was observed and was selected. Effect of different electrolytes added on the peak potential (mV) and the current (nA) were examined.

In the case of PIP, among different base electrolytes potassium chloride, lithium chloride, potassium dihydrogen phosphate, sodium oxalate, potassium citrate, sodium carbonate, sodium acetate and sodium hydroxide, the use of potassium chloride resulted in maximum response at peak potential (-775 mV) and was selected for further studies. However, in the case of OFL, the trend was not that smooth and high peak currents with a shift in peak potentials to more negative side were observed when sodium acetate, ammonium acetate and potassium citrate were used, possibly due to the buffering action of the salts and observation of higher peak currents at pH 4.7. Meanwhile, a stable and reproducible response was observed at -1150 mV as peak potential when potassium chloride was used.

The accumulation time (t_{acc}) was examined for 30 s to 60 s and a better response was observed with 60 s and was selected. Linear calibration curves for PIP and OFL were obtained in the range of 10-100 $\mu\text{g ml}^{-1}$ with regression equations $y = 3.7087x + 11.978$ ($R^2 = 0.9995$) and $y = 1.1216x + 9.7151$ ($R^2 = 0.997$), respectively. Precision measured from repeatability of the determination in terms of peak potential and peak current for PIP and OFL at 10 $\mu\text{g ml}^{-1}$ ($n = 6$) indicated relative standard deviations of 0.5-1.1% and 1.4-1.8%, respectively. The detection limits, measured as three times the background noise, were obtained as 50 ng ml^{-1} for PIP and 1 $\mu\text{g ml}^{-1}$ for OFL. The method indicated comparable detection limit for PIP as reported [24] and better detection limit than that reported by Zhou for OFL [26].

In order to apply the method for determination of PIP and OFL in pharmaceutical preparations, the interfering effects of the common additives glucose, lactose, sorbitol, gum arabica, magnesium stearate, methylparaben and propylparaben on the determination were examined at least twice the concentration of PIP or OFL. It was observed that glucose, lactose, sorbitol, gum arabica slightly decrease the peak current for OFL with relative error within -3% but did not have any effect on PIP.

The method was used for the determination of OFL and PIP from pharmaceutical preparations. The results of analysis (summarized in Table 1) agreed well with the labeled values, with relative standard deviation of 0.496-2.5%.

The method was examined for the simultaneous determination of PIP and OFL in the solution following the same analytical procedure. The PIP and OFL indicated the corresponding responses independently and did not interfere with each other. The peak potential for simultaneous determination of PIP and OFL were slightly shifted from individual measurements. Peak potentials were observed within the range of -790 to -820 mV for PIP and -1035 to -1045 for OFL. The linear calibration curves for simultaneous determinations were observed in the concentration range of 10-50 $\mu\text{g ml}^{-1}$ for PIP or OFL. The method was examined for analysis of test solutions containing mixtures of different concentrations of PIP and OFL ($n = 4$). The relative percent error was obtained within $\pm 1.2\%$.

For the precision and accuracy check, the method was examined for the analysis of both drugs in human urine samples. The urine samples were collected from two healthy volunteers and the samples were spiked with different amounts of OFL and PIP. A shift in peak potential towards more negative side was observed. Fresh calibration curves were prepared for the simultaneous determination of OFL and PIP from urine matrix and percent recovery from the spiked urine was within 100.2-100.9% with RSD of 0.9-2.1% ($n = 3$) for PIP and within 100-101% with RSD of 0.5-1.3% ($n = 3$) for OFL (Table 2).

CONCLUSIONS

Simple cathodic stripping voltammetric methods are described for the analysis of PIP and OFL separately and in their mixture. The method was used for the determination of

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Table 1. Results Obtained from the Analysis of Pharmaceutical Products Containing Pipemidic Acid and Ofloxacin

Samples	Amount labeled (mg/Tablet)	Amount found (mg/Tablet)	RSD (%) n = 3
Urixin (Abbott, Karachi)	400 mg PIP	403 ± 2	0.49
Oflobid (Hilton Pharma, Pvt., Ltd., Karachi)	200 mg OFL	205 ± 2	0.97
Curitol (Standpharm, Pvt., Ltd., Karachi)	200 mg OFL	189 ± 1	0.52
Tarivid (Avantis)	200 mg OFL	201 ± 3	1.49
Zoflox (Pharmatec, Pvt., Ltd., Karachi)	200 mg OFL	195 ± 5	2.50

Table 2. Results of Recovery Assay to Accuracy and Precision of the Proposed Method for Human Urine Samples

Samples	Spiked PIP and OFL (µg ml ⁻¹)	PIP found (µg ml ⁻¹)	Recovery (%)	RSD (%) n = 3	OFL found (µg ml ⁻¹)	Recovery (%)	RSD (%)
1	10	10.05	100.5	1.1	10.10	101.0	0.54
	20	20.11	100.5	0.9	20.15	100.7	1.20
	50	50.18	100.4	1.3	50.10	100.2	0.86
2	10	10.09	100.9	2.0	10.00	100.0	1.10
	20	20.01	100.1	2.1	20.08	100.4	1.00
	50	50.10	100.2	1.9	50.20	100.4	1.30

PIP and OFL from pharmaceutical preparations and spiked urine samples. The relative standard deviations (RSD) for the analysis of 10 µg ml⁻¹ PIP and OFL (n = 6) were found to be 0.5% and 1.4%, respectively. Limit of detection for PIP and OFL were 50 ng ml⁻¹ and 1 µg ml⁻¹, respectively.

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