

Voltammetric Studies for Analytical Determination of Antibacterial Danofloxacin and Orbifloxacin

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Abstract

The voltammetric behaviour of antibacterial danofloxacin (DANO) and orbifloxacin (ORBX) was studied in a wide pH range from 5 to 12 using phase sensitive alternating current, cyclic voltammetry and differential pulse cathodic adsorptive stripping voltammetric (DPCASV). The adsorption behaviour of both biological compounds at the hanging mercury drop electrode was investigated in order to achieve an increase in sensitivity and a possibility of the antibacterial DANO and ORBX determination by applying the adsorptive stripping voltammetric method. Adsorptive preconcentration followed by differential pulse cathodic stripping showed one wave at -1.27 V (DANO) and -1.37 V (ORBX) being the most sensitive for analytical determination of the investigated compounds. The limits of detection of 2.93×10^{-9} M and 5.30×10^{-9} M, and quantitation 9.77×10^{-9} M and 1.77×10^{-8} M for DANO and ORBX, respectively, were achieved. The degree of interference from coexisting metal ions and some organic compounds on the DPCASV signal for DANO and ORBX was evaluated. The proposed method was successfully applied to the determination of the studied fluoroquinolones in human urine and serum, obtained from healthy volunteers, without the necessity for sample pretreatment or time-consuming extraction steps prior to the analyses.

Keywords:

Danofloxacin and orbifloxacin; determination; stripping voltammetry; human urine; human serum

1. Introduction

Fluoroquinolones display a wide antibacterial spectrum and act directly on bacterial DNA gyrase inhibiting cell reproduction that leads to cell death [1]. They have a broad range of action against both gram-negative and gram-positive bacteria [2] and also have some activity against mycobacteria and rickettsias [3]. The widespread use of these compounds and the need for clinical and pharmacological study require fast and sensitive analytical techniques to determine the drug in several biological fluids. In this context several methods have been reported for the determination of fluoroquinolones such as spectrophotometry and spectrofluorimetry [4-8], fluorescence [9-12] capillary electrophoresis [13-16] and high-performance liquid chromatography [17-21]. Electroanalytical techniques have been used for determination of some fluoroquinolones of the first and second generations [22-34]. Review of the literature revealed that, up to the present time, nothing has been published concerning adsorptive stripping voltammetry of antibacterial danofloxacin and orbifloxacin (Fig.1) using a hanging mercury drop electrode.

In this work, adsorptive stripping voltammetry in the differential pulse mode was applied to provide a fully validated sensitive procedure by statistical analysis for the

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determination of DANO and ORBX compounds at HMDE. The adsorption and interfacial behaviour of the studied compounds at HMDE initiated the present study and this phenomenon was put to analytical advantage in the design of an adsorptive stripping method for the determination of DANO and ORBX in bulk and human urine and serum.

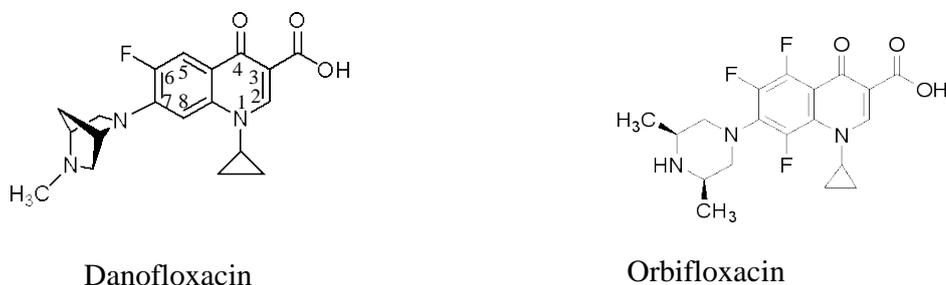


Fig.1. The structure of Danofloxacin and Orbifloxacin

2. Experimental

2.1. Instrumentation

2.1.1 A C measurements

A Princeton Applied Research (PAR) Model 173, coupled with universal programmer Model 175, lock-in amplifier/phase detector Mode 15210 and a PAR 303A hanging mercury drop electrode (Princeton Applied Research, New Jersey, U.S.A) were employed for a.c voltammetric measurement. Phase sensitive a.c voltammograms were recorded with phase angle adjusted to 90° corresponding to the out-of-phase component of the total a.c current. The amplitude of the a.c current was 10 mV, the scan rate of the d.c ramp of the negative electrode potential was 5 mVs^{-1} and the frequency was 330 Hz.

2.1.2 Cyclic voltammetry and DPCASV

Cyclic and DPCASV measurements were carried out with a PAR Model 264 A polarographic analyzer/stripping voltammetry in conjunction with a PAR Model 303A HMDE and a PAR Model 305 magnetic stirred (Princeton Applied Research, New Jersey, U.S.A). The voltammetric cell was maintained at $22 \pm 0.5^\circ \text{C}$. A Ag/AgCl (saturated KCL) electrode and Pt wire electrode were used as reference and auxiliary electrode.

2.2. Regents and procedures

DANO and ORBX were obtained from Sigma, Deisenhofen, Germany and were used without further purification. Stock Solutions of each of DANO and ORBX ($1.0 \times 10^{-3} \text{M}$) were prepared by dissolving the exact weighing of the investigated compounds in twice distilled water and stored in the dark at 4°C . More dilute solutions were prepared dialy with twice distilled water just before use. As a supporting electrolyte a series of Britton- Robinson (B.R) buffer of pH 2 – 11 (a mixture of 0.4 M of each acetic, orthophosphoric and boric acids, adjusted to the required pH with 0.2M sodium hydroxide) was prepared [35]. The pHs of the buffer solution were measured with a digital radiometer pH-meter, Jenway 3310, accurate to ± 0.05 unit. All metal salts were in the nitrate form. The probe solution (5mL) was added to the cell and purged with oxygen-free nitrogen gas for 15 min. When preconcentration was done in a stirred solution, a quiescent period of 15 s was allowed before the potential scan started.

2.2.1. Procedure for human urine and serum

The cells and fibrinogen were separated from the blood of healthy volunteers by centrifugation for 4 min at 14000 rev./min. The clear supernatant layer was filtered through a 0.45 μm mili-pore filter to produce the human serum sample. Urine and serum samples were collected and stored frozen until assay. Different volumes of urine or serum were added to B.R buffer to give a total volume of 10.0 mL. When the volume of urine or serum increased, the background current increased rapidly, so to avoid the sample interferences dilution was carried out (9:1 buffer/sample mixture). The influence of the concentration of the investigated compounds to urine or serum samples was studied by standard addition method.

3. Results and Discussion

3.1. A C Voltammetric studies

The out-of-phase a.c current of danofloxacin (DANO) and orbifloxacin (ORBX) recorded as a function of the mean electrode potential in solutions of varying pH was studied and represented in Fig 2. In this context the dependence of the out-of-phase a.c current on prior adsorption time t_s was obtained by adjusting the adsorption potential i.e the mean electrode potential, to a predetermined value, extruding the mercury drop and recording after the respective t_s had elapsed the out-of-phase a.c current with a suitable time base [36]. The a.c voltammograms indicate that adsorption of both DANO and ORBX occurs across the whole potential range from the positive potentials adjustable up to the more negative potential region. The depression of a.c current in the potential range -0.2 V to -1.5 V corresponds to the adsorption of the investigated biological compounds.

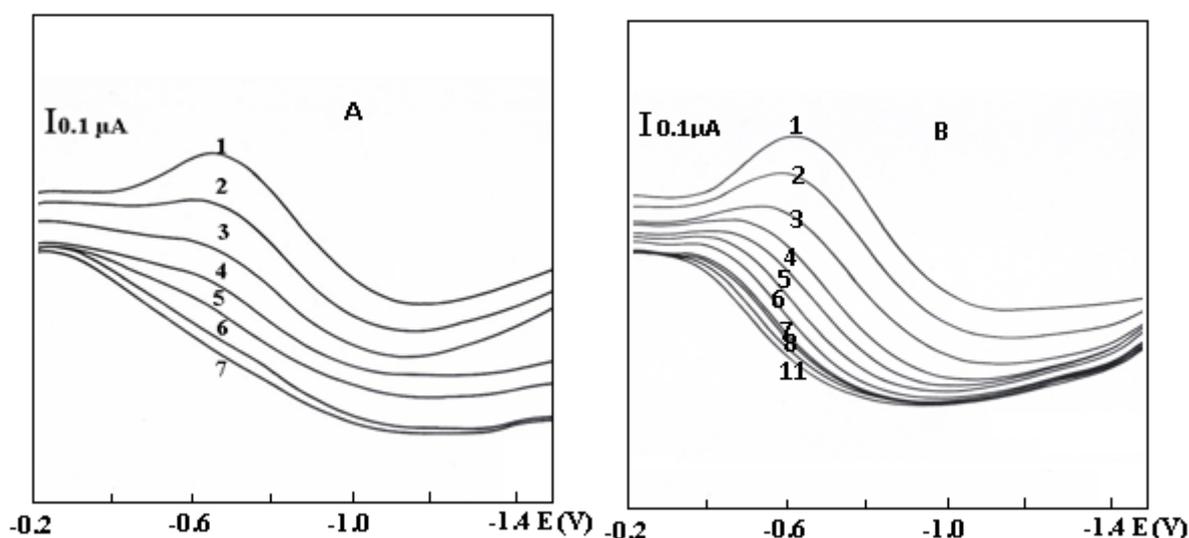


Fig.2. A.C. capacitive currents curves of DANO (A) and ORBX (B) at pH 7, scan rate 5 mVs^{-1} , amplitude 10 mV peak-to-peak, phase angle 90° , frequency 330Hz, adsorption time 60 s. (A) (1) 0.0, (2) 0.79×10^{-6} M, (3) 1.76×10^{-6} M, (4) 2.72×10^{-6} M, (5) 4.58×10^{-6} M, (6) 6.36×10^{-6} M, (7) 8.04×10^{-6} M DANO.

(B) (1) 0.0, (2) 0.99×10^{-6} M, (3) 1.96×10^{-6} M, (4) 2.91×10^{-6} M, (5) 3.80×10^{-6} M, (6) 4.76×10^{-6} M, (7) 5.66×10^{-6} M, (8) 6.54×10^{-6} M, (9) 7.40×10^{-6} M, (10) 10.7×10^{-6} M, (11) 19.4×10^{-6} M ORBX

The interfacial behaviour of DANO and ORBX shows around the electrocapillary zero of the blank supporting electrolyte a progressive suppression of the capacitive a.c signal as increasing the bulk concentration of the investigated fluoroquinolones. This suppression of a.c

current corresponds to a progressive coverage of the electrode surface by the dilute adsorption layer.

The dependence of $\Delta i_{a.c}$ (the decrease of the capacitive a.c current with respect to the i_{ac} value of the blank supporting for a given bulk concentration) on the bulk concentration of DANO or ORBX at different pH values has the form of one isotherm. The isotherm corresponds to one adsorption stage reflects the dilute adsorption layer. It is concluded that at maximal adsorption potential DANO and ORBX are oriented in a dilute adsorption layer planar to the electrode surface where the interaction of π electrons with the surface favors the adsorption. It has been found that the experimental data fit well a Frumkin adsorption isotherm given by Equation (1)

$$\beta C = [\theta/(1-\theta)] \exp -2a\theta \quad (1)$$

Where θ is the degree of coverage, a the interaction coefficient, β the adsorption coefficient and C the bulk concentration of DANO and ORBX.

The interaction coefficient a was determined from the slope of the logarithmic plot of the Frumkin isotherm and the adsorption coefficient β from the value at half coverage. The Gibbs energy of adsorption ($-\Delta G^\circ$) was then calculated from the adsorption coefficient β using Equation (2).

$$\beta = (1/55.5) \exp (-\Delta G^\circ / RT) \quad (2)$$

The calculated values of the adsorption parameters of DANO and ORBX at various pH values are given in Table 1. The adsorption parameters of DANO are rather similar to that of ORBX. This indicates that the structure of the adsorption layer similar in all cases and corresponds to a flat orientation of the adsorbed species at the electrode surface.

3.2. Cyclic voltammetric studies

The surface activity and the redox behaviour of biological compounds under investigation were studied at different pH values using cyclic voltammetry (CV) at HMDE (Fig. 3). Over the investigated pH range the surface redox reaction shows one cathodic peak at pHs 10 and 7 for DANO and ORBX respectively and the corresponding oxidation process. Closer inspection, the time concentration dependence of the cyclic voltammetric behaviour of DANO and ORBX compounds reveals that the redox peak is mainly faradiac peaks and the phenomenon is not capacitive current. The comparison of CV behaviour of DANO and ORBX both compounds exhibit a cathodic peak at potential close to -1.27 V and -1.37 V respectively. The cathodic peak of the investigated fluoroquinolones at various pH values is mainly due to the reduction of the carbonyl group ($>C=O$) on C_4 to the 4-hydroxyl product [37]. The peak potential separation $\Delta E_p = E_{pc} - E_{pa}$ is beyond 140 mV and 280 mV for DANO and ORBX respectively indicating a irreversible redox process. On plotting $\log i_p$ versus $\log v$ at different pH values straight lines with slope values of 0.920 – 0.983 were obtained. This confirmed the irreversible nature of the adsorption process. The slope values of the $E_p / \ln v$ plots at pH 7 and pH 10 were 0.0144 and 0.0171 respectively. Accordingly, the number of electrons, n_a transferred in the rate determining step should equal one ($n_a=1$), the transfer coefficient values, α , were 0.881 (pH 7) and 0.743 (pH 10).

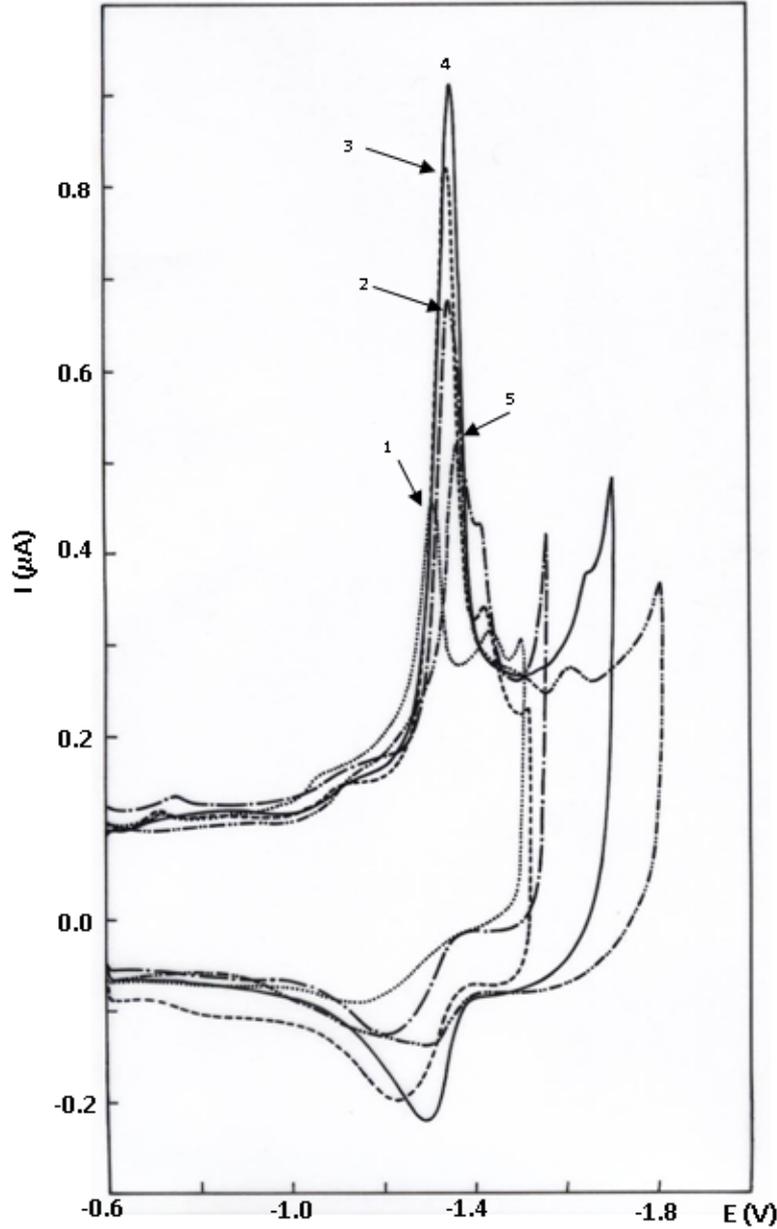


Fig.3. Cyclic voltammograms of 9.9×10^{-6} M DANO at different pH values. $t_s = 30$ s, $E_s = -0.6$ V and scan rate 100 mVs^{-1} . (1) pH 7.0, (2) pH 8.5, (3) pH 9.0, (4) pH 10.0, (5) pH 11.0.

An additional information about the course of the adsorption of DANO and ORBX at a charged interface offers the time dependence of cyclic voltammograms. The CV peak height of the adsorbed molecules increases with increasing the adsorption time in the form of an adsorption isotherm. At adsorption time 40 sec an equilibrium surface concentration is reached and the CV peak height became then constant. This reflects the adsorption character of DANO and ORBX compounds on the electrode surface and the CV peak current depends on the amount adsorbed. The response of surface adsorbed molecules at saturation was used to determine the surface excess concentration (Γ) that can be evaluated as Korytys Equation.

$$\Gamma_m = 0.736 \times 10^{-3} D^{1/2} C t^{1/2} \quad (3)$$

Where C is the bulk concentration of DANO or ORBX (mol.cm^{-3}) and D is the diffusion coefficient (cm^2s^{-1}). The value of the maximum surface concentration Γ_m was obtained from equation (3) by taking the adsorption time (t) at which an equilibrium surface concentration is

reached i.e full coverage. The values of Γ_m were 5.23×10^{-10} and 7.75×10^{-10} mol cm² for DANO and ORBX respectively.

3.3. Stripping voltammetry

The aforementioned results of a.c. and cyclic voltammetry supported the adsorption behaviour and the surface activity of DANO and ORBX compounds at the Hg surface. Stripping analysis employing adsorption preconcentration has been applied for the ultratrace determination of DANO and ORBX compounds at the HMDE with the aid of differential pulse cathodic adsorptive stripping voltammetry (DPCASV). The DPCASV of 9.9×10^{-6} M DANO and ORBX was recorded in a solution of varying pH (5-11) and represented in Figs. 4 and 5. The observed voltammetric peaks are mainly due to the reduction of C=O group of the adsorbed DANO or ORBX on the electrode surface. The more sensitive peaks were observed at pH 10.0 for DANO and pH 7.0 for ORBX. This peak corresponds to the adsorption and reduction species of DANO or ORBX according to their pK_a values (pK_{a1} = 6.07, pK_{a2} = 8.56 for DANO and pK_{a1} = 5.95, pK_{a2} = 9.01 for ORBX).

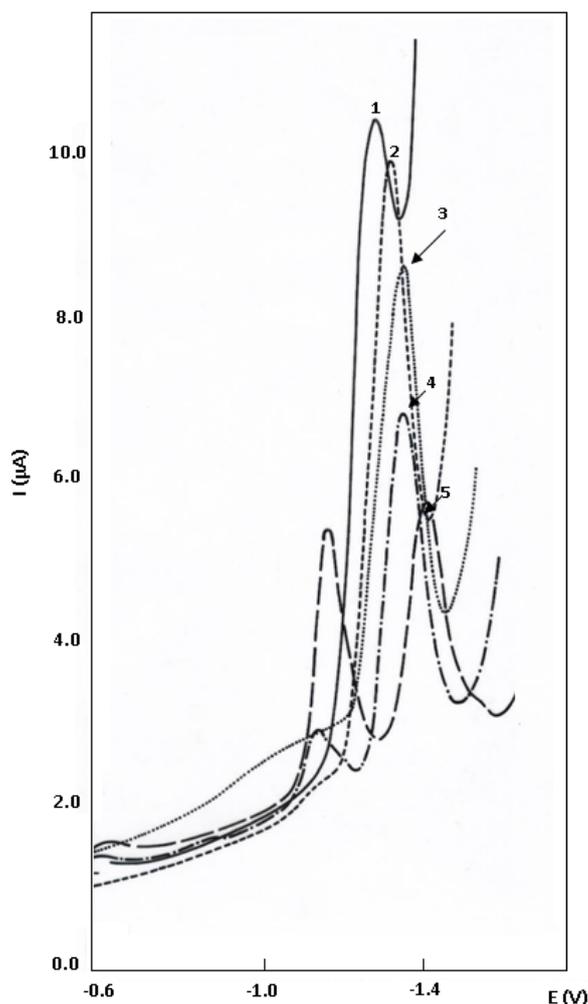


Fig.4. Effect of pH on the DPCASV peak of 9.9×10^{-6} M ORBX. Scan rate 10 mVs^{-1} , $t_s = 30 \text{ s}$, $E_s = -0.6 \text{ V}$ and Pulse Amplitude 100 mV peak-to-peak. (1) pH 5.0, (2) pH 6.0, (3) pH 7.0, (4) pH 8.0, (5) pH 9.0.

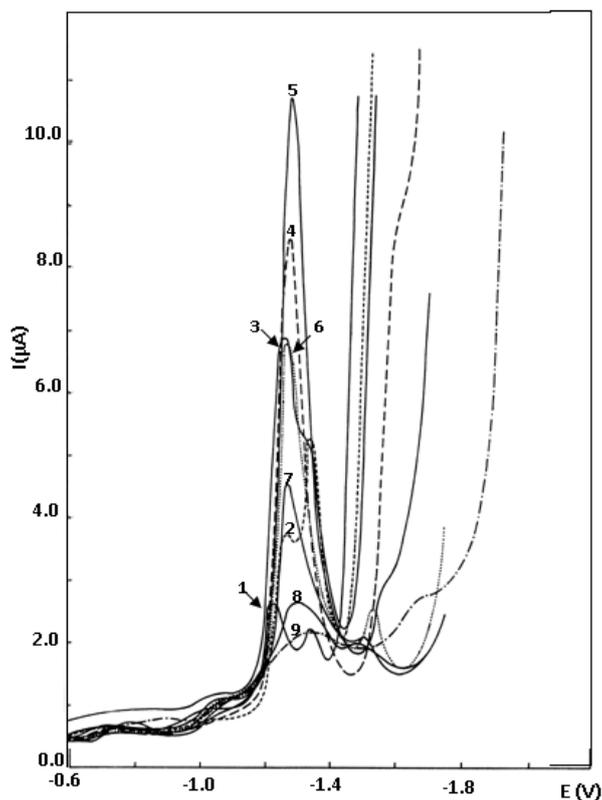


Fig.5. Effect of pH on the DPCASV peak of 9.9×10^{-6} M DANO. Scan rate 20 mVs^{-1} , $t_s = 30 \text{ s}$, $E_s = -0.6 \text{ V}$ and Pulse Amplitude 100 mV peak-to-peak. (1) pH 8.0, (2) pH 8.5, (3) pH 9.0, (4) pH 9.5, (5) pH 10.0, (6) pH 10.5, (7) pH 11.0, (8) pH 11.5, (9) pH 12.0.

The effect of the pulse amplitude on the height of the DPCASV of the investigated compounds was studied. The plot of peak height against pulse amplitude is linear up to amplitude of 100 mV peak-to-peak. A sharp and sensitive peak was found of 100 mV peak-to-peak. The result indicates that the selected adjustment for the pulse amplitude is an important parameter to be considered in electroanalytical application of cathodic stripping voltammetry.

The dependence of the adsorptive stripping peak current on the adsorption time of DANO and ORBX was studied. The peak height increases with adsorption time in the form of an adsorption isotherm. At adsorption time (t_a) 50 sec the equilibrium surface concentration is reached and the peak height becomes then constant. Therefore a pre-electrolysis time of 50 sec was arbitrary adopted for the cathodic stripping analysis of the investigated compounds.

The effect of adsorption potential of the stripping analysis of DANO and ORBX was examined at the optimum pH in the potential range -0.4 to -0.9 V . No great effect of adsorption potential on the peak intensity was observed when E is shifted to more negative potentials than -0.4 V . The adsorption potential was adopted at -0.6 V for the stripping analysis experiments.

The scan rate dependence of the DPCASV peak height of the adsorbed danofloxacin is studied at pH 10. At higher scan rate than 10 mV s^{-1} the width of the peak increases markedly and its height decreases. The dependence of the reduction peak on scan rate shows that a scan rate of 2 mVs^{-1} and 5 mVs^{-1} gave a maximum response compare to that 20 mVs^{-1} , hence a scan rate of 5 mVs^{-1} is chosen for the stripping analysis experiments.

The applicability of the DPCASV technique as an analytical method for trace determination of DANO and ORBX was studied as a function of the depolarizer concentration. Under the optimum conditions and over a concentration range of 9.9×10^{-9} – 6.54×10^{-8} M for DANO and 1.99×10^{-8} – 1.67×10^{-7} M for ORBX, the DPCASV peak height varied linearly with the concentration of the investigated compounds. The variation of $i_p(\mu\text{A})$ with the concentration of both biological compounds (C, μM) is represented by the straight line equation $i_p = ac + b$ where a and b are the slope and the intercept of straight line respectively (Table 2). The data for three to five replicated measurements are subject to a least square refinement and the value of the regression coefficient (R) are computed and assembled together with the straight line constant. The limits of detection (LOD) and quantitation (LOQ) of both biological compounds were calculated using the relation $K S.D/b$ (where $K=3$ for LOD and 10 for LOQ, S.D is the standard deviation of the intercept and b is the slope of the calibration curve) [38]. The LOD and LOQ for the investigated compounds were estimated and reported in Table 3; their values confirming the sensitivity of the proposed procedure for trace determination of the DANO and ORBX compounds.

The selectivity of the optimized procedure for the assay of DANO and ORBX was examined in the presence of several types of inorganic and organic compounds (Table 4). Metal ions tested at the micromole concentration (1.36×10^{-5} M) of some metal ions e.g Fe(II), Ba(II), Cu(II), Pb(II), Ni(II), and Zn(II), the degree of recovery of ORBX was lowered by 6.67%, 23.34%, 6.67%, 3.34%, 3.34% and 6.60% respectively. This indicates that these metal ions affect, to some extent as a result of their tendency for complexation with the investigated compounds under these conditions. The interfering effects of some organic compounds on DANO or ORBX determination were tested. Many organic molecules may interfere with the determination of DANO if they adsorb on the mercury drop electrode or if they are electroactive by themselves with a reduction potential close to that of DANO. Organic additives such as glutaric acid, DL- alanine, oxalic acid and ascorbic acid indicate that the degree of recovery of the DANO was lowered by 8.44, 7.70, 0.00 and 0.80 % respectively. Addition of 1.99×10^{-5} M EDTA for instance as an organic chelating agent caused the peak height of DANO to diminish. In presence a surface active substance e.g of 20 mg l^{-1} Triton x-100 the sensitivity of the peak current is diminished.

3.4. Assay of DANO and ORBX in human urine

The proposed method was successfully applied to the determination of DANO or ORBX in human urine samples without any sample pretreatment or any time-consuming extraction or evaporation steps prior to analysis. Adsorptive stripping voltammogram of diluted urine sample (9:1 B.R buffer /urine mixture) is shown in Fig. 6. However, when the investigated compound is added to urine, a concentration-sensitive peak height was observed at -1.23 and -1.37 V for DANO and ORBX respectively. The peak height under these conditions is linearly with the concentration of DANO or ORBX and values of the regression coefficient (R) were computed and assembled together with the straight line constant, LOD and LOQ values (Tables 2 and 3).

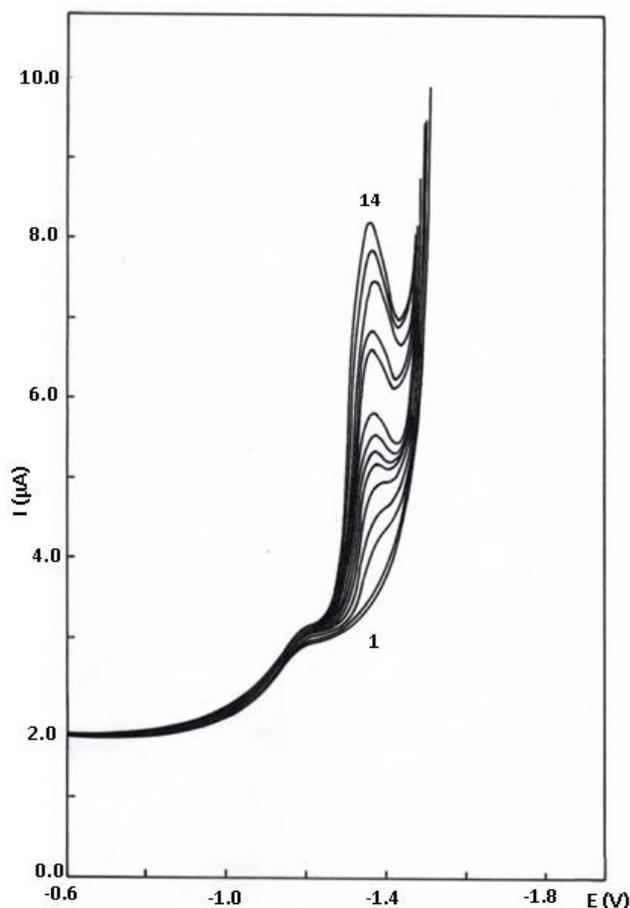


Fig.6. Concentration dependence of DPCASV peak of ORBX in human urine at pH 7, Scan rate 20 mVs^{-1} , $t_s = 35 \text{ sec}$, $E_s = -0.6 \text{ V}$ and Pulse Amplitude 100 mV peak-to-peak. (1) 0.0 , (2) $0.5 \times 10^{-6} \text{ M}$, (3) $1.47 \times 10^{-6} \text{ M}$, (4) $2.4 \times 10^{-6} \text{ M}$, (5) $3.38 \times 10^{-6} \text{ M}$, (6) $4.30 \times 10^{-6} \text{ M}$, (7) $5.20 \times 10^{-6} \text{ M}$, (8) $6.54 \times 10^{-6} \text{ M}$, (9) $8.25 \times 10^{-6} \text{ M}$, (10) $9.90 \times 10^{-6} \text{ M}$, (11) $12.20 \times 10^{-6} \text{ M}$, (12) $14.00 \times 10^{-6} \text{ M}$, (13) $18.00 \times 10^{-6} \text{ M}$, (14) $21.00 \times 10^{-6} \text{ M}$ ORBX.

3.5. Assay of orbifloxacin in human serum

Fig.7 illustrates the DPCASV of successive additions of orbifloxacin-serum at pH 7. The variation of the peak current versus ORBX concentration was represented by a straight line followed the equation $i_p(\mu\text{A}) = -0.17 + 5.64 \times 10^4 C(\text{M})$, with a regression coefficient of 0.996. The achieved LOD and LOQ values of ORBX drug in human serum samples are $1.82 \times 10^{-6} \text{ M}$ and $4.28 \times 10^{-6} \text{ M}$ using the proposed procedure. The mean percentage recoveries, based on five replicate measurements were achieved for determination of DANO and ORBX in urine and serum samples (Table 3). These data indicate high precision of the proposed procedure for the assay of both drugs.

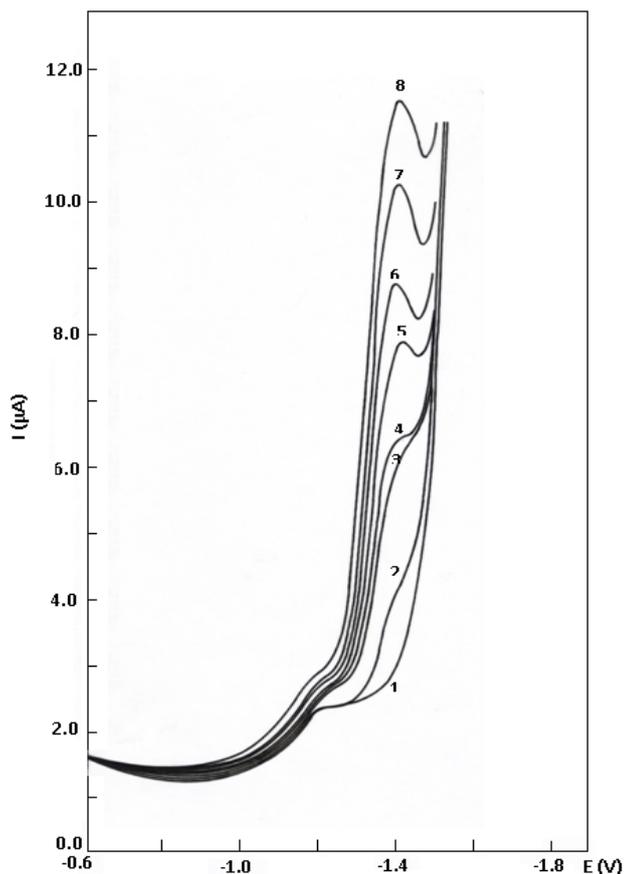


Fig.7. Concentration dependence of DPCASV peak of ORBX in serum sample at pH 7, Scan rate 20 mVs^{-1} , $t_s = 35 \text{ sec}$, $E_s = -0.6 \text{ V}$ and Pulse Amplitude 100 mV peak-to-peak. 1) 0.0, (2) $1.18 \times 10^{-5} \text{ M}$, (3) $2.15 \times 10^{-5} \text{ M}$, (4) $3.10 \times 10^{-5} \text{ M}$, (5) $4.03 \times 10^{-5} \text{ M}$, (6) $4.94 \times 10^{-5} \text{ M}$, (7) $5.80 \times 10^{-5} \text{ M}$, (8) $6.70 \times 10^{-5} \text{ M}$ ORBX.

4. Conclusion

A sensitive and practical differential pulse cathodic adsorptive stripping voltammetric method for the determination of the antibacterial danofloxacin and orbifloxacin is presented. The detection limit obtained, $2.93 \times 10^{-9} \text{ M}$ (DANO) and $5.30 \times 10^{-9} \text{ M}$ (ORBX), was the lowest reported up to date. It was successfully applied to the determination of the studied fluoroquinolones in to human urine and serum samples. From the economical point of view, the proposed procedure is simple, rapid and low cost.

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