

SQUARE-WAVE VOLTAMMETRY OF OFLOXACIN

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Adsorption phenomena of ofloxacin at hanging mercury drop electrode (HMDE) in Britton-Robinson buffer solution with pH of 8.36 were studied by means of square-wave voltammetry (SWV). The SWV response of ofloxacin is sensitive to pH, type of the supporting electrolyte, the ofloxacin concentration, the accumulation time and potential, as well as the exciting signal parameters such as frequency, amplitude and potential increment. The optimization of experimental conditions for quantitative determination of ofloxacin was achieved. A square-wave voltammetric method for quantitative determination of ofloxacin is developed. The detection limit of $4 \cdot 10^{-8}$ mol/l ofloxacin was found.

Key words: ofloxacin; adsorption; surface redox reaction; square-wave voltammetry

INTRODUCTION

The demand for detection of trace levels for substances of environmental, clinical and forensic importance is growing tremendously. Electrochemical techniques such as square-wave voltammetry (SWV), differential pulse voltammetry (DPP) and linear scan voltammetry (LSV) have been a powerful tool for analytical purposes. Among them, the SWV is the most advanced technique both for elucidation of electrode mechanisms as well as for analytical applications, especially when adsorption of reactant or/and product occur [1–5]. The unique advantages of square-wave voltammetry such as fast scan rate, large frequency, large amplitude, short time required for measurements as well as the specific approach in the current sampling procedure rank this technique as one of the most advanced among the family of pulse voltammetric techniques [1–7].

The compound studied in this paper, known in the pharmacy as ofloxacin (see. Fig. 1) is a synthetic fluorinated quinolone derivative having ac-

tivity against both gram negative and gram positive bacteria through inhibition of their DNA gyrase [8]. It is widely used in the treatment of respiratory and urinary tract infections [9]. Several polarographic and voltammetric techniques for its quantitative determination have been utilized [10–12]. No one of the published papers has been concerned to the adsorption properties of ofloxacin at a mercury electrode. Also, the effect of the methanol concentration added in the polarographic cell has not been discussed. There are also reports that H⁺ ions have been included in the electrochemical reaction of ofloxacin, however its number is not yet determined precisely [12].

This paper deals with the adsorption behavior of ofloxacin at a HMDE in order to elucidate some specific properties of its electrode reaction. The objective of this work is also to develop a stripping square-wave voltammetric method for quantitative determination of ofloxacin.

EXPERIMENTAL

All measurements were performed with a multimode polarographic analyzer Princeton Applied Research Model 384B connected with a static

mercury drop electrode PAR 303A. An Ag/AgCl (saturated KCl) was the reference and Pt wire was the counter electrode. The stock solution of oflox-

acin (purchased from MERCK) was prepared by dissolving an appropriate amount in 98 % methanol. All chemicals used such as methanol, boric acid, acetic acid, phosphoric acid and NaOH (all obtained from SIGMA) were of analytical grade and were used as received. 0.1 mol/l Britton-Robinson buffer solutions (mixture of 0.1 mol/l boric acid, 0.1 mol/l acetic acid and 0.1 mol/l phosphoric acid, adjusted to different pH values by

0.1 mol/l NaOH) were used as supporting electrolytes. All solutions were prepared with ultrapure water. Solutions were degassed with high-purity nitrogen for 8 min prior to the measurements and for an additional 20 s before each scan. A nitrogen atmosphere in the cell was maintained throughout experiments. Each scan was done on a separate mercury drop at room temperature.

RESULTS AND DISCUSSION

Ofloxacin (see Fig. 1) has been a subject of several electrochemical investigations. However, its electrochemical behavior at a hanging mercury drop electrode (HMDE) is not yet fully clear. Belal *et al.* [12] have recently studied ofloxacin electrochemical behavior by means of differential pulse polarography (DPP). They found that ofloxacin's electrochemical activity is due to the reduction of the carbonyl C=O group and it proceeds according to the following Scheme:

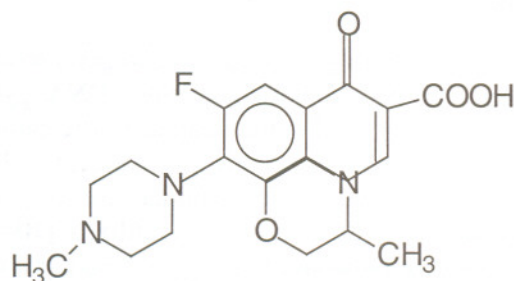
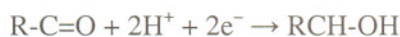


Fig. 1. Structure of ofloxacin

Obviously, the reduction of ofloxacin is an irreversible redox process followed by protonation. Rizk *et al.* [12] pointed out that the number of exchanged protons in the redox reaction of ofloxacin is 1.13 and they approximate it for 2. In the same paper, adsorption phenomena of ofloxacin molecules at a HMD electrode in Britton-Robinson buffer medium with pH of 8.36 are just noted, but not investigated in details. Keeping in mind the fact that the adsorption of electroactive compounds at a mercury electrode could be useful for developing of sensitive stripping voltammetric method for quantitative determination, adsorption phenomena of ofloxacin at a HMDE deserve serious attention. Aiming to inspect electrochemical behavior of ofloxacin at a HMD electrode as well as to develop a sensitive SW voltammetric method for its quanti-

tative determination, supplementary voltammetric investigations were carried out by means of square-wave voltammetry.

The SW voltammetric response of ofloxacin is sensitive to pH, accumulation time, accumulation potential, as well as the exciting signal parameters such as frequency amplitude and potential increment. All these dependencies were investigated further.

The pH of supporting electrolyte solutions influences both the peak potential and peak currents of the SW voltammetric responses of ofloxacin. Changing the pH from 4.0 to 11.0 causes the SW peak potential of ofloxacin to shift towards more negative values. For instance, where pH of the supporting electrolyte solution was changed from 4.0 to 11.0, the peak-potential E_p was shifted from -1.436 V to -1.780 V, respectively. The linear dependence between the peak potential and pH of supporting electrolyte solutions exist, represented by the following equation: $E_p / \text{V} = -0.057 \text{ V/pH} - 1.180$ with $R^2 = 0.986$. The slope of the linear line, which reads -57 mV/pH implicates that one H^+ ion is involved in the elementary act of the electrochemical reaction. This result differs from that presented by Belal *et al.* [12].

Aiming to select the best buffer solution for analytical purposes, several calibration curves of ofloxacin solutions were constructed in Britton-Robinson buffer solutions with different pH values (see Fig. 2). The slope of the linear line as well as the correlation coefficient of the linear regression line are ones of the major criteria to select an optimal electrolyte solution for analytical purposes. Regarding the constructed calibration curves depicted in the Fig. 2, one should observe that the greatest slope of the I_p vs. pH as well as the best linearity in Britton-Robinson buffer solution with pH of 8.36 exist. Thus, all additional voltammetric measurements of ofloxacin were performed in Brit-

ton-Robinson buffer solution with pH of 8.36. In the Fig. 3 is presented a square-wave voltammogram of ofloxacin solution recorded in Britton-Robinson buffer with pH of 8.36. A well defined

reduction peak of ofloxacin with peak potential of $E_p = -1.56$ V and half-peak width $\Delta E_{p/2}$ of 80 mV is observed.

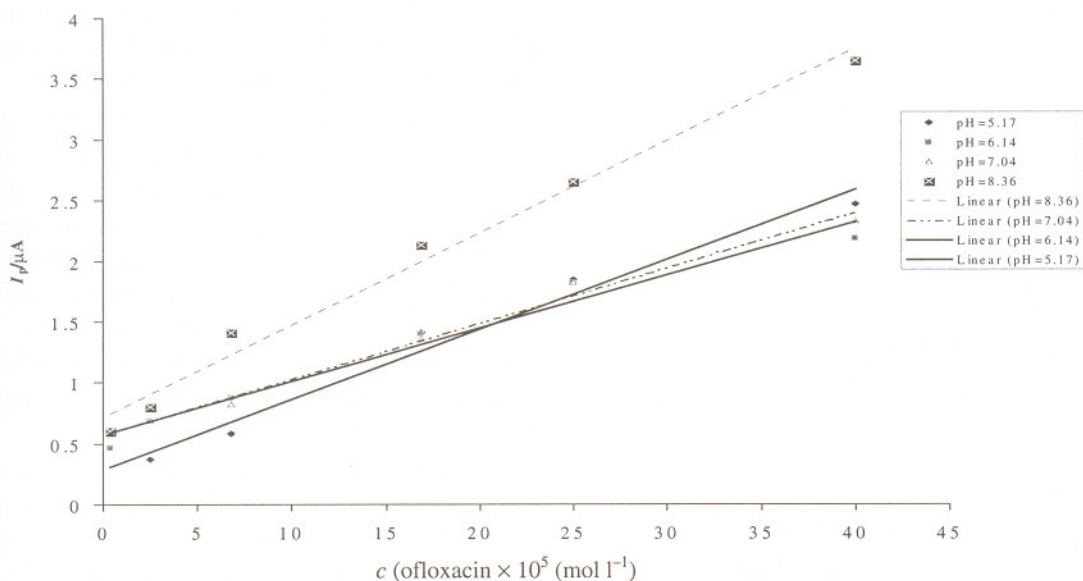


Fig. 2. The calibration plots of ofloxacin constructed in Britton-Robinson buffers with different pH values. The experimental conditions are: frequency $f = 120$ Hz, amplitude $E_{sw} = 20$ mV, scan increment $dE = 4$ mV

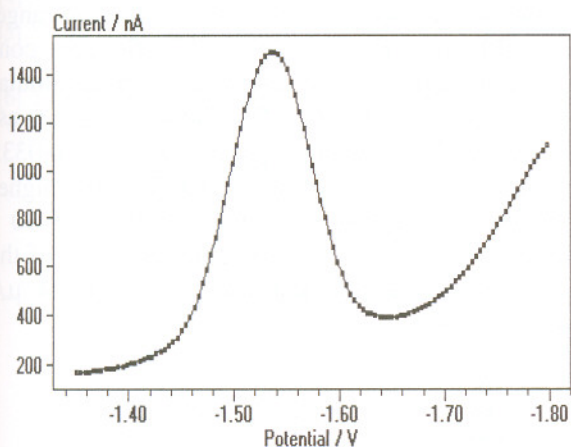


Fig. 3. Square-wave voltammogram of 5×10^{-5} mol/l ofloxacin recorded in Britton-Robinson buffer solution with pH of 8.36. All other conditions are the same as in the caption of the Fig. 2

Additional information about the SW voltammetric behavior of ofloxacin in 0.1 mol/l Britton-Robinson buffer solution with pH of 8.36 have been collected by varying the frequency of the signal. The peak potential is almost unaffected by the SW frequency. For instance it shifts for only 12

mV in negative direction changing the SW frequency from 30 to 120 Hz, respectively. However, the SW peak current is severely sensitive to the SW frequency. A linear relationship between the peak currents and the SW frequency was observed in 0.1 mol/l Britton-Robinson buffer solution with pH of 8.36. The linear line is represented by the following equation: $I_p/\mu A = 0.0069 \mu A/Hz + 0.008$. The linearity between the peak current and the SW frequency is characteristic properties for the redox processes controlled by adsorption. This property appears to be one of the most important discrepancies to the diffusion controlled redox processes in which the peak current increases in proportion to the square root of the frequency.

Intending to inspect the adsorption phenomena of ofloxacin molecules at a HMD electrode, additional experiments were carried out. Recording the SW voltammograms of ofloxacin after prolonged accumulation time, significant increase of the SW peak heights was observed. In the same time, the peak potential remained totally unaffected by the accumulation time. The function of SW peak currents of the accumulation time is depicted in the Fig. 4 A. Obviously, the relationship

has followed the Langmuire isotherm, reaching a limiting peak current value at accumulation time of 150 s. The linear part of the curve depicted in the Fig. 4 A suggests that a linear relationship between the surface and bulk concentration of ofloxacin exist in that region.

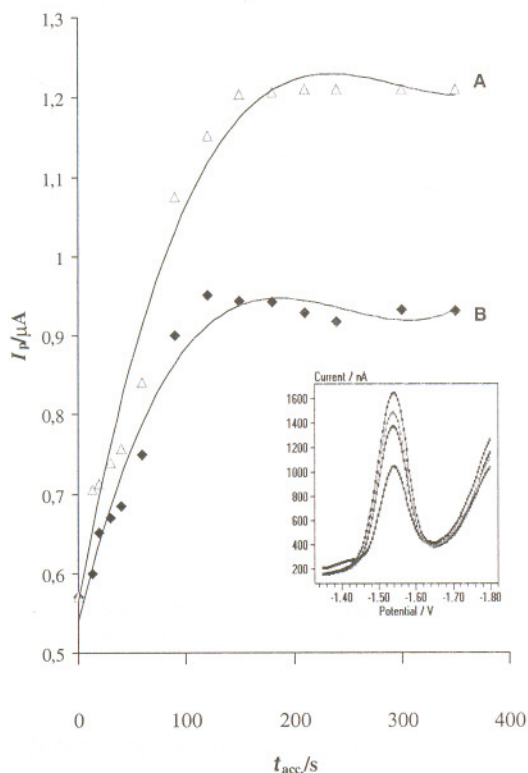


Fig. 4. The dependence of the SW peak current of ofloxacin on the accumulation time: without methanol (curve A) and with 10% v/v methanol (curve B). $E_{acc.} = -1.35$ V, pH = 8.36, c (ofloxacin) = 5×10^{-6} mol/l. All other conditions are the same as in the Fig. 2. In the inset are shown SW voltammograms of ofloxacin recorded at accumulation time of 0 s (curve on the bottom), 5, 10 and 15 s (towards top), respectively

The accumulation potential $E_{acc.}$ also affects the SW voltammetric response of ofloxacin. A parabolic dependency of the SW peak currents of ofloxacin on the accumulation potential was observed, with a maximal peak current located at $E_{acc.} = -1.35$ V.

The effect of the methanol added in the buffer solution was also studied. As it is shown in the Fig. 4 B, 1 ml methanol added in the supporting electrolyte solution caused a significant decrease of the peak heights of ofloxacin. This is for expecting knowing the fact that methanol is adsorbed itself at a HMD electrode and it shows concurrent adsorption to the ofloxacin molecules. Generally speaking, one should expect appreciable interference's

originated from concurrent adsorption if some surface active compounds are presented in the sample and the supporting electrolyte.

All these results indicate that the redox reaction of ofloxacin in Britton-Robinson buffer solution with pH of 8.36 occurs predominantly as a surface redox process in which the ofloxacin molecules are significantly confined to the electrode surface.

It should be pointed out that the SW voltammetric response of ofloxacin is particularly sensitive to the signal amplitude. If the amplitude was increased to 80 mV, a significant enhancement of the SW peak heights was observed. However, an amplitude greater than 50 mV significantly disturbs the SW peak of ofloxacin. The maximum ratio $I_p/\Delta E_{p/2}$, which appears to be main criterion for selecting particular amplitude for analytical application, at amplitude of 30 mV was observed.

Finally, the following set of experimental conditions has been evaluated as optimal for quantitative determination of ofloxacin, utilizing adsorptive stripping square-wave voltammetry: 0.1 mol/l Britton-Robinson buffer solution with pH of 8.36, frequency of 120 Hz, amplitude of 30 mV and scan increment of 4 mV.

Under these optimal conditions, a linear dependence between peak currents and concentration of ofloxacin exists over a wide concentration range. The calibration plot in 10^{-7} mol/l ofloxacin concentration region was constructed with accumulation time of 90 s (see Fig. 5). The linear line is associated with following equation: $I_p/\mu A = 0.0335 \mu A / \mu mol + 0.505$, with R^2 of 0.998. In the higher concentration region, accumulation time of 10 s was applied and the corresponding equation of the linear regression line reads $I_p/\mu A = 0.0524 \mu A / \mu mol + 0.602$.

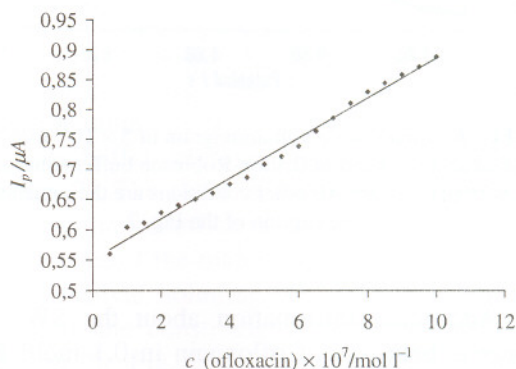


Fig. 5. Calibration plot of ofloxacin recorded in Britton-Robinson buffer solution with pH of 8.36. The experimental were: accumulation time $t_{acc.} = 90$ s, $E_{acc.} = -1.35$ V, $E_{sw} = 30$ mV. Other conditions were the same as in the Fig. 2

Under accumulation time of 90 s, detection limit, estimated as three times to noise, of $4 \cdot 10^{-8}$ mol/l was found. It is the lowest value for the detection limit from all published for ofloxacin. The

relative standard deviation (RSD) of the results varied from 0.2 to 7.1 % (eight measurements at $5 \cdot 10^{-7}$ and $5 \cdot 10^{-6}$ mol/l). The reproducibility of the results ranges from 89.49 to 102.65 %.

CONCLUSION

Ofloxacin is electrochemically active compound that undergoes irreversible reduction at a HMD electrode. The voltammetric response of ofloxacin is sensitive to pH of the supporting electrolyte solutions, as well as the instrumental parameters such as SW frequency, SW amplitude and potential increment. This paper is mainly concerned to elucidate adsorption phenomena of ofloxacin in Britton-Robinson buffer solution with pH of 8.36. We have demonstrated that in this pH medium the

redox reaction of ofloxacin appears as a surface redox process in which the ofloxacin molecules are confined to the mercury electrode surface. These properties enable one to develop a sensitive stripping square-wave voltammetric method for quantitative determination of ofloxacin. An optimization of the experimental parameters for quantitative determination of ofloxacin was also achieved. A detection limit of 4×10^{-8} mol/l of ofloxacin was estimated.

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Резиме

КВАДРАТНО-БРАНОВА ВОЛТАМЕТРИЈА НА OFLOXACIN

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Клучни зборови: ofloxacin; адсорпција; површинска редокс-реакција; квадратно-бранова волтаметрија

Во овој труд, со примена на квадратно-бранова волтаметрија, беа студирани адсорпционите особини на ofloxacin-от на висечка живина капка, во Бритон-Робинсонов пуферски раствор со рН = 8.36. Квадратно-брановиот волтаметриски одговор на ofloxacin-от е функција од рН на основните електролити од времето и потенцијалот на акумулацијата, од концентрацијата на ofloxacin-от и од параметрите на екзитациониот сигнал какви што се фреквенцијата, амплитудата и

потенцијалниот инкремент. Притоа беше испитано и влијанието на концентрацијата на метанол, додаден во основниот електролит, врз волтаметрискиот одговор на ofloxacin. Беше извршена оптимизација на експерименталните услови со цел да се разработи квадратно-бранов волтаметриски метод за квантитативно определување на ofloxacin. При оптималните услови, пресметаната граница на детекција за ofloxacin изнесуваше $4 \cdot 10^{-8}$ mol/l.