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Determination of Norfloxacin by square-wave adsorptive voltammetry on a glassy carbon electrode

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Abstract

The adsorptive and electrochemical behavior of norfloxacin on a glassy carbon electrode were investigated by cyclic and square-wave voltammetry. Cyclic voltammetric studies indicated that the process was irreversible and fundamentally controlled by adsorption. To obtain a good sensitivity, the solution conditions and instrumental parameters were studied using square-wave voltammetry. In acetate buffer of pH 5.0, norfloxacin gave a sensitive adsorptive oxidative peak at 0.920 V (versus Ag–AgCl). Applicability to measurement of norfloxacin at submicromolar levels in urine samples was illustrated. The peak current was linear with the norfloxacin concentration in the range 5–50 μg ml^{-1} urine. The detection limit was 1.1 μg ml^{-1} urine. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Norfloxacin {1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolonecarboxylic acid} is considered to be the first commercially available member of the modern fluoroquinolones [1,2]. The chemical structure is shown in Scheme 1. This group of drugs is bactericidal over a wide range of therapeutically achievable concentrations and act via selective inhibition of bacterial DNA synthesis [1]. Norfloxacin has a broad range of action against both gram negative and gram positive bacteria. It is widely used in the treatment of respiratory tract and urinary tract infections [3]. Single oral dose of 400 mg gave peak serum level of 1.58 μg ml^{-1} and ~30% of the administrated dose was recovered in urine as unmetabolized norfloxacin [4].

Many methods have been developed for the determination of norfloxacin. Methods based on the spectrophotometric determination of norfloxacin in pharmaceutical formulations have been reported [5–7]. A high-performance chromatographic procedure for the determination of the drug has been used [8]. Only one official method

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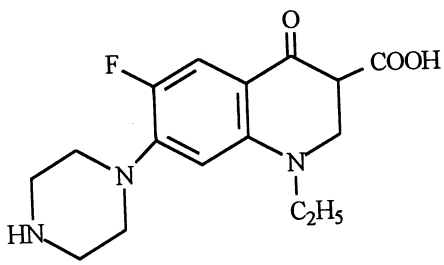

Scheme 1.

based on the non-aqueous titration of norfloxacin dissolved in glacial acetic acid with perchloric acid is considered [9].

During the past few years several papers on the polarographic behavior of norfloxacin and some related fluoroquinolones have appeared in the literature, including polarographic investigation of the Redox mechanism and the application of adsorptive stripping voltammetry at the hanging mercury drop electrode (HMDE) for the determination of norfloxacin [10–13]. However, the electroanalytical method for this drug with a non-mercury electrode has not yet been reported up to now.

This work is concerned with a study of the voltammetric behavior using the particularly rapid and sensitive technique of square wave voltammetry on a glassy carbon electrode. Norfloxacin is adsorbed onto the glassy carbon electrode in acetate buffer and this phenomena was put to analytical advantage in the design of an adsorptive stripping method for the determination of norfloxacin at low ppb levels, i.e. 10^{-7}–10^{-8} M concentrations. Its applicability to the determination of norfloxacin levels in urine samples was evaluated.

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed with a 394 Electrochemical Trace Analyzer (EG&G/PAR). The three-electrode system consisted of a glassy carbon electrode (G0197, Model 303A, 7 mm^2 surface area), Ag–AgCl saturated KCl reference electrode and a platinum wire auxiliary electrode. All measurements were carried out at room temperature.

2.2. Reagents

Norfloxacin and Noroxin® tablets were obtained from the Egyptian International Pharmaceutical Industries (EIPICO). The stock solution of norfloxacin (1×10^{-3} M) was prepared with 0.01 M hydrochloric acid. Diluted solutions were then made with de-ionized water. Acetate buffer (0.05 M, pH 5.0) was prepared with sodium acetate and acetic acid. All the chemicals used were of AnalaR–reagent grade and all solutions were prepared with deionized water. The human urine samples were collected daily from at least five healthy individuals and a pool of these was used.

2.3. Procedure

The GCE was polished, at the start of the work, with aqueous slurry of 0.5 μm alumina powder (K0015) on a damp silk cloth until a mirror-like finish was obtained. The accumulation of norfloxacin at the working electrode was carried for a selected time while the solution was stirred at 1600 rpm. The stirring was then stopped, and after a 2-s rest period, a cyclic or a square-wave voltammetric stripping, initiated in the anodic direction, was performed either in the same electrolyte solution or in the blank electrolyte after medium exchange. For electrode regeneration, the working electrode was transferred to a blank electrolyte solution and series of cyclic scans was continued until a voltammogram corresponding to the residual current was obtained. The electrode was then ready for use in a next measurement cycle.

2.3.1. Tablets analysis

Five series of single tablet of Noroxin® (400 mg) was sonicated in 5 ml of 0.01 M HCl and a series of dilution with de-ionised water was then performed to give a final concentration of 1×10^{-4} M norfloxacin. An aliquot of the clear supernatant liquor was then transferred to a voltammetric cell containing 10 ml of acetate buffer (pH 5.0) to yield a final concentration of

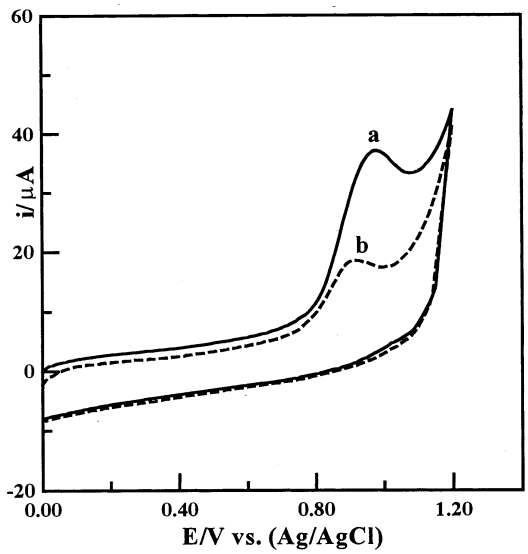

Fig. 1. (a) Cyclic voltammograms for 5.0×10^{-5} M norfloxacin in 0.05 M HOAc–NaOAc buffer at pH 5.0 and scan rate 100 mV s^{-1} following 180 s stirring at 0.0 V. (b) An analogous voltammogram without the accumulation.

1×10^{-6} M norfloxacin. The square-wave voltammograms was then recorded after 30 s pre-concentration time at open circuit condition. The content of the drug in tablet was determined referring to the regression equation.

2.3.2. Urine analysis

Urine samples, 1 ml each, were spiked with varying amounts (ranged from 5 to 50 μg) of norfloxacin. The analysis were carried out by adding 0.1 ml of norfloxacin-urine solution to the cell containing 9.9 ml of acetate buffer (pH 5.0). The solution was stirred at 1600 rpm at open circuit condition, and the glassy carbon electrode was immersed for 300 s (pre-concentration step). The electrode was then washed with water, dried, and placed in the measurement cell containing 10 ml of acetate buffer (pH 5.0) and the square-wave voltammograms was recorded following the optimized conditions. Quantification was performed by means of calibration curve method.

3. Results and discussion

3.1. Cyclic voltammetry

The interfacial accumulation of norfloxacin on a glassy carbon electrode is indicated from the cyclic voltammograms of 1×10^{-6} M norfloxacin in 0.05 M acetate buffer (pH 5.0) recorded before (a) and after (b) 180 s stirring at 0.0 V as shown in Fig. 1. The short pre-concentration time results in a large anodic peak at 0.950 V. A substantial decrease of the anodic peak is observed in subsequent scans. Such behavior indicates rapid desorption of drug from the electrode surface or fouling of the GCE electrode by oxidation products. Repeated voltammetric scans on the same electrode surface without prior accumulation in unstirred solution were used to evaluate the adsorption of the oxidation products. The ratio of peak current (i_p) of second scan to that of initial scan was ~ 0.92, suggesting that no fouling of the electrode surface by the adsorbed oxidation products occurred under the condition used. The fact that no peaks were observed in the anodic branch scans suggests that the process is irreversible. The effect of scan rate (v) on stripping peak current (i_p) was examined under the above conditions with a plot of log i_p versus log v (i_p), giving a straight line which may be expressed by the equation: $i_p = 1.12 \log v - 0.72$ ($r = 0.999$). The slope (1.12) is close to the theoretically expected (1.0) for an ideal reaction of surface species [14]. The peak potential shifts to more negative values on increasing the scan rate, which confirms the irreversibility of the reduction process.

The plot of log i_p versus E_p at different scan rates gives a linear relation that fits the equation: $\log E_p = 0.140 \log v + 0.688$ ($r = 0.997$). Using the value of the slope and supposing that the electrode process includes one electron, an $\alpha = 0.38$ of the charge transfer was obtained [15].

3.2. Square-wave voltammetry

The solution conditions affect the enhancement of the peak associated the preconcentration step. Various electrolytes such as Britton-Robinson buffer, borate buffer, ammonia/ammonium chlo-

ride, potassium chloride and potassium nitrate were examined and exhibited varying degrees of accumulation. Best results were obtained with 0.05 M acetate buffer (pH 5.0); this electrolyte was used throughout this study. The square wave-mode yielded improved response compared to the linear scan one, and was used in all subsequent work.

Fig. 2 shows the dependence of the adsorptive peak current on the preconcentration time at two concentration levels, i.e. (a) 1.0×10^{-7} M; and (b) 5.0×10^{-7} M. The peak current increases with increasing pre-concentration time, indicating enhancement of norfloxacin concentration at the electrode surface. As the accumulation time increases the peak current tend to level off, showing that the adsorptive equilibrium is reached. The higher is the concentration, the shorter is time to reach the equilibrium. For 1.0×10^{-7} M norfloxacin solution following 200 s, an approximately 6-fold enhancement of the peak current is observed over that attained without accumulation. Thus, considerable increase in sensitivity can be achieved by application of adsorptive stripping voltammetry to determination of norfloxacin.

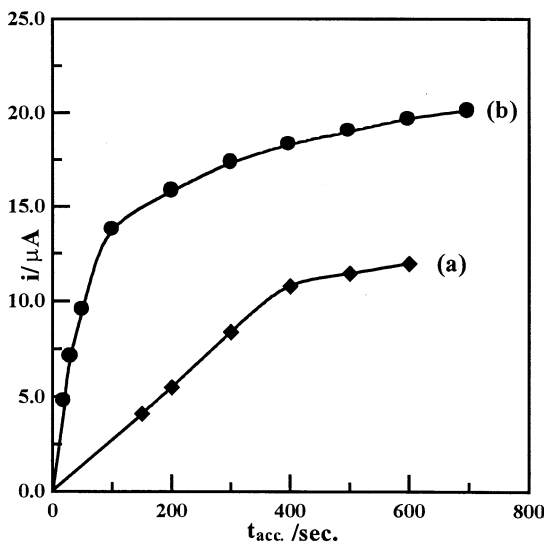

Fig. 2. Effect of the accumulation time on the peak current for: (a) 1.0×10^{-7}; and (b) 5.0×10^{-7} M norfloxacin. Square waveform with $f = 60$ Hz, $\Delta s = 10$ and 50 mV pulse amplitude. Other conditions were as in Fig. 1(a).

The effect of the accumulation potential on peak intensity was also evaluated for 5.0×10^{-6} M norfloxacin solution following 60 s pre-concentration time over the range 0.0 to +0.6 V. No great effect of accumulation potential on peak intensity was observed. Therefore, in most cases an accumulation potential of 0.0 V was chosen for analytical process.

The optimum instrumental conditions were then chosen from a study of the variation of the peak current of 5.0×10^{-6} M norfloxacin in acetate buffer with frequency, scan speed and pulse height. Higher peak currents were observed with high frequency of the order of 120 Hz (mV s^{-1}) or by increasing the pulse height from 25 to 100 mV. The peak increases linearly with the scan increment up to 10 mV. The best peak definition was found with 60 Hz frequency (mV s^{-1}), 10 mV scan increment and 50 mV pulse height.

The SW peak potential is linearly dependent on the SW frequency with a slope $\Delta E_p/\Delta \log f = 2.3 RT/2\alpha n$ for the irreversible Redox reaction process [16]. The relationship between the oxidation peak potential of norfloxacin and the logarithm of the SW frequency gives a straight line with slope of 86 mV/decade of square frequency. An $\alpha = 0.42$ of the charge transfer was obtained for one electron electrode process. This value is in quite agreement with that obtained from cyclic voltammetry.

3.3. Quantitative aspects

Fig. 3 shows the dependence of the square-wave peak current on norfloxacin concentration following (a) 100 and (b) 300 s pre-concentration. It can be seen that the linearity range depends on the accumulation time used. Concentration range from 1.0×10^{-7} M up to 1.0×10^{-6} M can therefore be determined by this adsorptive stripping technique using accumulation time of 100 s. For longer accumulation time, e.g. 300 s, surface saturation for this accumulation time is reached at lower concentration of $\sim 1.25 \times 10^{-7}$ M. Thus, the ultimate choice of preconcentration time depends on the concentration range studied. The detection limit was 2.5×10^{-9} M, with preconcentration time of 300 s. The precision expressed

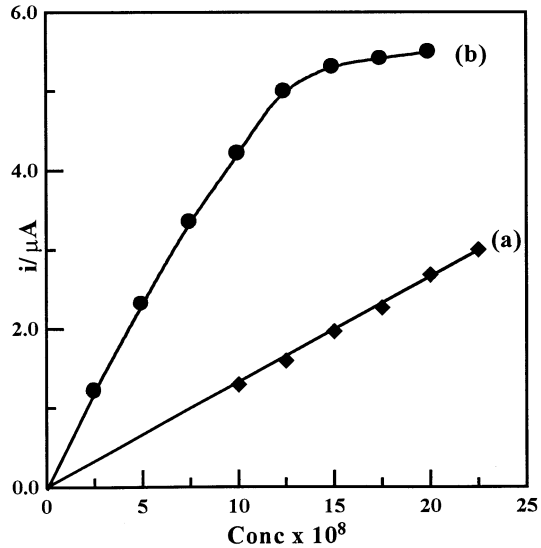

Fig. 3. Dependence of the peak current on the norfloxacin concentration using: (a) 100; and (b) 300 s preconcentration. Other conditions were as in Fig. 2.

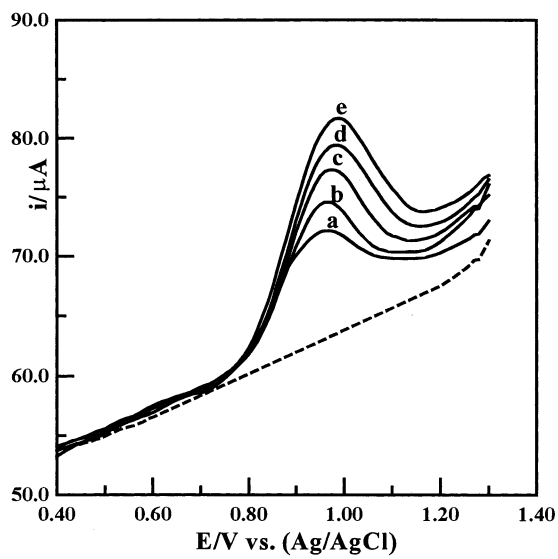

Fig. 4. Adsorptive square wave voltammograms obtained for the determination of norfloxacin in urine samples after medium exchange: dashed lines represent the blank (a–e) urine spiked with increasing concentration of norfloxacin: (a) 8, (b) 16, (c) 24, (d) 32, and (e) 40 μg ml^{-1}, respectively.

by relative standard deviation was 4.87% ($n = 7$) for a concentration of norfloxacin of 1.0×10^{-6} M at electrode surface cleaned before each measurement. The cleaning step was achieved by means of cycling potential scan for 5 cycles between 0.0 and 1.3 versus Ag/AgCl at scan rate of 100 mV s^{-1} in acetate buffer.

3.3.1. Norfloxacin assay in Noroxin® tablets

Five samples from different dissolved Noroxin® (400 mg) tablets were analysed using the proposed stripping voltammetric method. A mean value of 394 mg with relative standard deviation of 0.69% was obtained. This compares reasonably well with other reported methods. The proposed method is more sensitive than spectrophotometric methods [5–7]. Moreover, it has merits such as simplicity and time efficiency. Statistical analysis of the results obtained by the proposed and the official method using the *t*-test and variance ratio *F*-test shows no significant difference between the two methods with regard to accuracy and precision.

3.3.2. Norfloxacin assay in urine

Direct determination of norfloxacin in urine was not possible due to the huge interfering oxidation peaks of blank urine, and so a medium exchange had to be performed. However, after medium exchange experiment a large peak at + 0.280 V was recorded. This peak did not increase with increasing accumulation time and was well differentiated from that of norfloxacin. Thus, the peak did not interfere with the determination of norfloxacin but only comparably high concentration could be determined. Fig. 4 illustrates the square-wave wave voltammetric response to different concentrations of norfloxacin in urine samples. The response was linearly related to the norfloxacin concentration within the range 5–50 μg per 1.0 ml of urine (1.5×10^{-6}–1.5×10^{-5} M) according to the regression equation: i_p (μA) = 0.135 C (μg ml^{-1}) + 2.781, $r = 0.995$. A detection limit of 1.1 μg ml^{-1} (3.0×10^{-7} M) was obtained. The precision expressed by relative standard deviation was 4.98% ($n = 7$) at 25 μg ml^{-1}.

4. Conclusion

The adsorptive stripping voltammetry on a

glassy carbon electrode can be used to determine norfloxacin at trace levels because of its low detection limit. Norfloxacin can be effectively accumulated from aqueous solutions or urine samples onto the surface of a glassy carbon electrode. Moreover, the proposed method is fast and purging of the norfloxacin solutions with nitrogen is not required. The electrochemical renewal of the electrode surface in acetate buffer is efficient and ensures the reproducibility of individual measurements. The detection limit found for norfloxacin on a glassy carbon electrode for urine samples after medium exchange is low enough to reach the levels expected in urine after therapeutic doses.

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