A study of adsorptive stripping voltammetric behavior of ofloxacine antibiotic in the presence of Fe(III) and its determination in tablets and biological fluids

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Keywords
Adsorptive stripping voltammetry; Square-wave voltammetry; HMDE; Ofloxacine antibiotic; Drug-metal ion complex

Abstract
Square-wave voltammetry was used to explore the adsorption property of ofloxacine complex with iron ion on the hanging mercury drop electrode (HMDE). By employing the adsorptive stripping voltammetric approach, a sensitive electroanalytical method for the quantitative analysis of ofloxacine antibiotic was achieved. A well-developed voltammetric peak was obtained in pH 7.5 Britton–Robinson buffer (B–R buffer) at \(-1400 \text{ mV}\). The cyclic voltammetric studies indicated that the reduction process was irreversible and primarily controlled by adsorption. An investigation of the variation of adsorptive voltammetric peak current with supporting electrolyte, pH, accumulation time, accumulation potential, ion concentration, scan rate, pulse amplitude, SW frequency, working electrode area and convection rate has resulted in the recognition of optimal experimental conditions for ofloxacine analysis. The studied electroanalytical signal showed a linear response for ofloxacine in the concentration range \(5 \times 10^{-7} - 1.7 \times 10^{-6} \text{ mol l}^{-1}\) \((r = 0.999)\). A limit of detection of \(1.1 \times 10^{-8} \text{ mol l}^{-1}\) (3.98 ppb) with relative standard deviation of 1.21 RSD% and mean recovery of 99.6% were obtained. Possible interferences by several substances usually present in pharmaceutical formulation were also evaluated. The analytical quantification of ofloxacine in commercially available pharmaceutical formulation was performed and compared with data from HPLC technique.

1. Introduction

Square Wave Adsorptive Stripping Voltammetry (SW-AdSV) has been well characterized as an extremely sensitive source for electroanalytical measurements since its establishment half a century ago. Such electrochemical approach with improved sensitivity and selectivity have promoted the development of numerous analytical applications of ultra-trace determinations of a variety of organic or inorganic substances, alike.
SW-AdSV method involves a stripping step carried out by using a square wave time-potential waveform imposed on the working electrode. The principle advantages of SW-AdSV over other AdSV techniques (namely differential pulse and linear sweep) are its enhanced powers of detection, speed of analysis and freedom from sensitivity of dissolved oxygen in the analysed samples (Wang, 1994; Osteryoung and O’Dea, 1986; Economou and Filden, 1993). There have been many reviews devoted to emphasize and illustrate the wide spectrum and scope of AdSV applications and potentialities in the analysis of metal ions (Zaitsev et al., 1999; Abu Zuhri and Voelter, 1998) organic analytes (Brainina et al., 2000) and pharmaceutical drugs and biomedical compounds (Alghamdi, 2002; Vire et al., 1998).

Ofloxacine belongs to a group of broad spectrum antibiotics called the quinolones. It works by entering the bacterial cell and inhibiting a chemical called DNA-gyrase which is involved in the production of genetic material (DNA). This antibiotic drug prevents the bacteria from reproducing and their growth is stopped. Ofloxacine is effective against several types of bacteria that tend to be resistant to other commonly used antibiotics. It is used to treat a wide range of infections, including infections of the chest, urinary tract, pneumonia, bronchitis, venereal disease (VD), and prostate, skin. It is also used as a single dose treatment for gonorrhea. Ofloxacine is the racemic mixture of a chiral compound. Its chemical IUPAC name (+/-)-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 (Hayakawa et al., 1983; Truste Cerner Multum Inc. Version, 2006; Bethesda, 2006). The structural formula of this pharmaceutical compound is exhibited in Scheme 1. This antibiotic drug has been analysed in pharmaceutical formulations and biological samples by various analytical methods such as spectrophotometry (Hopkata and Kowalcuk, 2000; Garcia et al., 2005; Lisiane and Schapoval, 2002; Kapetanovic et al., 1996; Patel et al., 2007), high performance liquid chromatography (HPLC) Lisiane and Schapoval, 2002; Kraas and Hирrle, 1986; Arai and Hiroko, 1989; Espinosa-Mansilla et al., 2005; Tadashi et al., 1991 and electrochemical method such as polarography linear sweep voltammetry and cyclic voltammetry (Rizk et al., 1998; Gerong and Jinghao, 1995; Nan and Xiaoli, 2007; Azcurra et al., 2001; Chun-Hai et al., 2007). However, no literature data was found on the square wave voltammetry in general or the adsorptive stripping determination of this drug–Fe(III) ions, and its application to determination in pharmaceutical formulation, is described.

Scheme 1  Chemical structure of ofloxacine drug.

2. Experimental

2.1. Apparatus

All adsorptive stripping measurements were carried out with 797 VA computrace (Metrohm, Switzerland) in connection with Dell computer and controlled by (VA computrace 2.0) control software. Stripping voltammograms were obtained via a hp deskjet 5150 printer. A conventional three electrode system was used in the hanging mercury drop electrode (HMDE) mode. Chromatographic determination of this antibiotic drug was obtained by HPLC instrumental model LC-20AT Shimadzu in connection with Dell computer. HPLC chromatograms were printed via a hp LaserJet 1020 printer. pH values were measured with Metrohm 632 pH meter (Swiss made). Biohit adjustable micropipette (AU), and Brand adjustable micropipette (Germany), were used to measure microliter volumes of the standard solutions.

2.2. Reagents

All chemicals used were of analytical reagent grade and were used without further purification. Ofloxacine drug stock solution of $1 \times 10^{-2}$ mol l$^{-1}$ was prepared by dissolving the appropriate amount of ofloxacine in ethanol in 25 ml volumetric flask and this stock solution was stored in the dark. Similarly, Fe(III) stock solution of $1 \times 10^{-3}$ mol l$^{-1}$ was prepared by dissolving the appropriate amount of iron nitrate salt in dilute nitric acid in 50 ml volumetric flask. Britton–Robinson supporting buffer (pH 2, 0.04 M in each constituent) was prepared by dissolving 2.47 g of boric acid (winlab, UK) in 500 ml distilled water containing 2.3 ml of glacial acetic acid (BDH, UK) and then adding 2.7 ml of ortho-phosphoric acid (Riedal-deHaen, Germany) and diluting to 1 l with distilled water. In addition, phosphate supporting buffer [0.1 M NaH$_2$PO$_4$ (winlab, UK) and 0.1 M H$_3$PO$_4$] was prepared by dissolving 12 g of NaH$_2$PO$_4$ and 6.78 g of H$_3$PO$_4$ in 1000 ml distilled water. Acetate supporting buffer (0.02 M in each constituent) was prepared by dissolving 1.68 g of sodium acetate (winlab, UK) in 500 ml distilled water containing 1.12 ml of acetic acid and diluting to 1 l with distilled water. Finally, carbonate supporting buffer (0.1 M in each constituent) was prepared by dissolving 10.6 g of sodium carbonate (BDH, UK) and 8.4 g of sodium hydrogen carbonate (winlab, UK) in 1 l distilled water.

2.3. Procedure

The general procedure adopted for obtaining square wave adsorptive stripping voltammograms was as follows: A 10 ml aliquot of B–R supporting buffer (unless otherwise stated) at desired pH was pipetted in a clean and dry voltammetric cell and the required standard solutions of ofloxacine complex were added. The test solutions were purged with nitrogen for 5 min initially, while the solution was stirred. The accumulation potential of 0.0 V vs. Ag/AgCl was applied to a new mercury drop while the solution was stirred for 100 s. Following the preconcentration period, the stripping was stopped and after 20 s had elapsed, cathodic scans were carried out over the range 0.0 to $-1.7$ V. All measurements were made at room temperature.
3. Results and discussion

3.1. The electroanalytical properties of ofloxacine complex

Preliminary stripping voltammetric experiments show that ofloxacine pharmaceutical molecule \((5 \times 10^{-7} \text{ mol l}^{-1})\) gave a well-defined (line B) cathodic peak at \(-1400 \text{ mV (vs. Ag/AgCl reference electrode)}\) in pH 7 Britton–Robinson buffer as can be seen from Fig. 1. Then, the addition of \(5 \times 10^{-6} \text{ mol l}^{-1} \text{ Fe(III)}\) to the previous ofloxacine test solution increased a well-defined (line C) cathodic peak. Also, the addition of \(1 \times 10^{-6} \text{ mol l}^{-1}\) ofloxacine drug to the solution increased the cathodic current (line D), all additions were made at potential \(-1400 \text{ mV}\). In fact, the iron(III) ions exhibited a good affinity towards ofloxacine molecules forming a very stable ofloxacine–Fe(III) complex which is strongly adsorbed onto the HMDE surface. This obtained well-developed stripping voltammetric peak was found to response sharply to the addition of either ofloxacine or Fe(III) concentrations (lines B, C and D), which probably reflect the formation and adsorption of the suggested complex. The observed AdSV peak is most probably due to the cathodic reduction of Fe(III) in the adsorbed complex with ofloxacine and the electrochemical mechanism of this reduction process for ofloxacine–Fe(III) complex is illustrated in Scheme 2.

Clearly, this proposed electrochemical reduction mechanism suggested an irreversible reductive process for iron ion in the adsorbed complex, an assumption which was confirmed by cyclic voltammetric measurement of \(1 \times 10^{-4} \text{ mol l}^{-1}\) ofloxacine drug and \(1 \times 10^{-3} \text{ mol l}^{-1}\) Fe(III) in pH 7.5 B–R buffer at 50 mV s\(^{-1}\) scan rate. As can be noticed from Fig. 2, which exhibits the cyclic...

![Figure 1](image1.png)

**Figure 1**  Electrochemical behavior of ofloxacine complex with iron(III) ions: (A = buffer, B = buffer + \(5 \times 10^{-7} \text{ M}\) ofloxacine, C = A + B + \(5 \times 10^{-6} \text{ M} \text{ Fe}^{+3}\), D = A + B + C + \(1 \times 10^{-6} \text{ M}\) ofloxacine).

![Figure 2](image2.png)

**Figure 2**  Cyclic voltammogram for \(1 \times 10^{-4} \text{ mol l}^{-1}\) ofloxacine with \(\text{Fe}^{3+}\) (\(1 \times 10^{-3} \text{ mol l}^{-1}\)) in pH 7.5 B–R buffer at 50 mV s\(^{-1}\) scan rate. Accumulation time 60 s at \(E_{\text{acc}}: 0.0 \text{ V}\).

![Scheme 2](image3.png)

**Scheme 2**  Suggested mechanism of the studied electrochemical reduction process for ofloxacine–iron(III) complex.
voltamogram of ofloxacin–iron(III), the absence of the anodic peak at the reverse scan confirmed the irreversible nature of the evaluated reduction process. Furthermore, when repetitive cyclic voltammetric measurements for ofloxacin complex with Fe(III) were carried out, a well-developed AdSV peak was observed at all cathodic scans, however, succeeding cathodic scans exhibit a gradual increase in the voltammetric peak intensity, that seemed to indicate the adsorptive characteristic of this complex at the surface of the employed working electrode. Anyhow, the interfacial accumulation of this drug–metal ion complex onto the HMDE surface can be used as an effective accumulation step in order to enhance the electroanalytical determination of ofloxacin molecules.

3.2. Optimization of experimental parameters

3.2.1. Effect of supporting buffer constituents and pH

Since the adsorptive phenomena of ofloxacin complex on the HMDE was utilized as a suitable collection step prior to its electrochemical determination, it was rational to characterize various variables and experimental conditions that affecting the engaged adsorption process. In fact, the sensitivity of the adsorptive stripping procedure for a particular analyte is usually significantly influenced by the composition of the supporting buffer and pH value. Consequently, several supporting buffers such as Britton–Robinson, phosphate, acetate and carbonate buffers at different pH values were evaluated after 60 s accumulation time at 0.0 V accumulation potential. Among these supporting electrolytes the best electroanalytical signal in terms of SW-AdSV peak current intensity and shape was obtained with B–R buffer, which was selected as optimal for further works.

Generally, the AdSV signal was mainly pH dependent since the monitored voltammetric signal was only observed at low alkaline media. When the stripping voltammetric peak current was measured as a function of pH over the range 5–9, the peak current increased gradually at first and enhanced sharply beyond pH 7 then it reached its maximum value at pH 7.5, which was adopted as optimum pH value for subsequent investigations. The influence of pH factor on the SW-AdSV signal is illustrated in Fig. 3. In addition, it was observed that the voltammetric peak potential of this complex did not shifted when pH was varied over the studied pH range, which indicates that $E_p$ was pH independent as expected for an electrochemical reaction in which hydrogen ions did not consumed.

3.2.2. Effect of accumulation factors

The interfacial accumulation of ofloxacin complex onto the HMDE surface depends on some operational factors, which worth additional investigations in order to ensure high sensitive determinations of this drug via its Fe(III) complex. Therefore, the effect of accumulation time on the efficiency of the collection of $5 \times 10^{-7}$ mol l$^{-1}$ ofloxacin drug in the presence of $5 \times 10^{-6}$ mol l$^{-1}$ iron(III) ions onto the working electrode was evaluated by rising the accumulation time over the range 0–150 s. The resulting peak current-accumulation time ($i$–$T_{acc}$) profile is exhibited in Fig. 4 and as can be seen from this plot, a steadily enhancement in the peak current was observed over the range 0–30 s and thereafter the peak intensity nearly decreased probably due to the saturation of the HMDE. Hence, 30 s accumulation time was selected for all future experiments. Furthermore, variation of the accumulation potential over the range from +0.4 to –1.2 V (see Fig. 5) at 30 s accumulation time, revealed that a preconcentration potential of –1.0 V was the ideal choice for optimal sensitivity.

3.2.3. Effect of metal ion concentration

The dependence of the WS-AdSV voltammetric current of $5 \times 10^{-7}$ mol l$^{-1}$ ofloxacin in a B–R buffer of pH 7.5 on the concentration of iron(III) ions was also investigated. As shown in Fig. 6 the monitored voltammetric signal was approximately

![Figure 3](image3.png)

**Figure 3** Effect of pH on SW-AdSV peak current of $5 \times 10^{-7}$ mol l$^{-1}$ ofloxacin drug and $5 \times 10^{-6}$ mol l$^{-1}$ Fe(III) in B–R buffer after an accumulation period of 60 s at $E_{acc} = 0.0$ V.

![Figure 4](image4.png)

**Figure 4** Effect of accumulation time on the stripping voltammetric peak current of $5 \times 10^{-7}$ mol l$^{-1}$ ofloxacin drug and $5 \times 10^{-6}$ mol l$^{-1}$ Fe(III) in pH 7.5 B–R buffer. Accumulation potential: 0.0 V.

![Figure 5](image5.png)

**Figure 5** Effect of accumulation potential on the stripping voltammetric peak current of $5 \times 10^{-7}$ mol l$^{-1}$ ofloxacin drug and $5 \times 10^{-6}$ mol l$^{-1}$ Fe(III) in pH 7.5 B–R buffer. Accumulation time: 30 s.
linear over the range from $5 \times 10^{-7}$ mol l$^{-1}$ to $1 \times 10^{-5}$ mol l$^{-1}$ Fe(III). For getting up good shape of cathodic peak, the metal ion concentration of choice will be $5 \times 10^{-6}$ mol l$^{-1}$ ofloxacine–Fe$^{3+}$.

3.2.4. Effect of potential sweep parameters

The observed stripping voltammetric signal can be further maximized by adjusting the way the applied potential was scanned. The relationship between the measured peak intensity and scan rate was found to be directly proportional over 100–700 mV s$^{-1}$ (from studied range 100–1000 mV s$^{-1}$). However, when scan rates faster than 700 mV s$^{-1}$ were employed, the peak current decreased slightly. The influence of scan rate on the observed voltammetric signal is illustrated in Fig. 7, which indicates that scan rate value of 700 mV s$^{-1}$ would be adequate optimum for succeeding investigations. In addition, the impact of varying the excitation wave pulse amplitude on the voltammetric current intensity was also evaluated. The effect of this operating variable was studied over the range 10–100 mV (see Fig. 8) and it was concluded that in order to assure maximum peak current, 90 mV pulse amplitude is the ideal choice for this operational parameter. Moreover, varying the value of square wave frequency also plays an important role for the measured signal of SW-AdSV approach. Varying this parameter over the range 10–140 Hz resulted in a substantial enhancement of the voltammetric peak current particularly at range 10–120 Hz as can be seen from Fig. 9, then the peak of current become constant. Accordingly, for future work 120 Hz SW frequency value was adopted.

3.2.5. Effect of other instrumental variables

The influence of other operating parameters such as the size of the adsorption area (HMDE) and convection rate on the efficiency of the adsorption accumulation of ofloxacin complex was additionally checked. As expected, a linear enhancement for the electrochemical peak intensity was observed when the surface area of HMDE was increased over the range 0.15–0.6 mm$^2$ drop size area. Besides, the SW-AdSV peak current can be maximized further by increasing the stirring rate of the rotating rod over the range 0–3000 rpm. Hence, for optimal sensitivity, 0.6 mm$^2$ drop size and 3000 rpm stirring speed were selected. In conclusion, for electroanalytical purposes, the optimized experimental conditions for SW-AdSV measurements of ofloxacin drug–Fe(III) were accumulating his complex for 30 s at −1.0 V preconcentration potential with stirring rate of
3000 rpm. These voltammetric measurements were carried out in Britton–Robinson buffer at pH 7.5 in the presence of 5 × 10⁻⁶ mol l⁻¹ Fe(III). The applied potential was scanned at 700 mV s⁻¹ with 120 Hz SW frequency rate and 90 mV pulse amplitude.

3.3. Analytical performance

3.3.1. Calibration graph and detection limit

Once the optimal chemical conditions and instrumental parameters for the SW-AdSV determination of ofloxacine were established, several analytical characteristics of the proposed procedure were evaluated. Under the optimized conditions, a linear correlation between SW-AdSV peak intensity and the drug concentration was obtained over the range 5 × 10⁻⁷ to 1.7 × 10⁻⁶ mol l⁻¹, (see Fig. 10). The calibration equation was calculated by least-squares method and it has the form:

\[ I_p (\text{nA}) = 3.2 \times 10^8 C \text{ (mol l}^{-1}\text{)} + 1250 \quad r = 0.999, \quad n = 5. \]

where \( I_p \) is the stripping voltammetric peak current in nanoamperes, \( C \) is ofloxacine concentration and \( r \) is the correlation coefficient.

The effective preconcentration step during the adsorption process of the analyzed drug allow a very low detectability. The detection limit estimated based on the signal-to-noise ratio (S/N = 3) was 1.1 × 10⁻⁸ mol l⁻¹ (3.98 ppb). This obtained sensitivity was significantly preferable than those reported for other analytical techniques currently used for determination of ofloxacine drug such as chromatography method (HPLC) with 14.5 ppb (Espinosa-Mansilla et al., 2005) and 20 ppb (Tadashi et al., 1991) detection limits and polarography method with 108.4 ppb (Rizk et al., 1998) and 1445.5 ppb (Gerong and Jinghao, 1995).

3.3.2. Precision, accuracy and stability

The reproducibility of the developed procedure was evaluated from ten repeated measurements of 5 × 10⁻⁷ mol l⁻¹ ofloxacine drug in the presence of 5 × 10⁻⁶ mol l⁻¹ Fe(III) ion. The precision of the method in terms of the relative standard deviation (RSD%) was 1.21%. The accuracy of the electrochemical method was checked by calculating the recovery of known amount (2 × 10⁻⁷ mol l⁻¹ ofloxacine drug spiked in buffer solution and analysed by the optimized procedure. The results of five measurements obtained by the standard addition method have a recovery mean of 99.6% with standard deviation of ±0.55%. When the SW-AdSV signal of 5 × 10⁻⁷ mol l⁻¹ ofloxacine drug solution in the presence of 5 × 10⁻⁶ mol l⁻¹ Fe³⁺ was monitored every 10 min, it was found to nearly stable for a period of 1.5 h at least.

3.3.3. Interferences

The competitive co-adsorption interference was evaluated in the presence of various substances usually occur in the pharmaceutical formulation as tablet ingredients or additives. For these investigations, the interfering species (lactose, sucrose, cellulose, starch and magnesium sulfate) were added at different concentrations (twice, 5-fold and 50-fold) higher than the concentration of ofloxacine (5 × 10⁻⁷ mol l⁻¹ ofloxacine). The additions of magnesium sulfate, cellulose and starch at high concentration level, caused the AdSV peak current to decrease by about 8%, 17% and 11% respectively. In contrast, the additions of sucrose and lactose at the drug solution no significant effect on the SW-AdSV response of ofloxacine.

This inhibition response is possibly due to the competitive co-adsorption of these interfering substance (particularly at higher concentration levels) on the adsorption sites of HMDE.

3.4. Analytical applications

The reliability of the proposed AdSV method for the determination of ofloxacine was investigated by assaying this drug in some real samples. Following the developed electroanalytical procedure described above, ofloxacine–Fe(III) was analysed in pharmaceutical formulation. The ofloxacine content of commercially available tablets (oflacin/200 mg ofloxacine, manufactured by Ram Pharmaceutical, Jordon) was determination directly by the SW-AdSV method after the required dissolving and filtration steps. Five aliquots of the dissolved sample were diluted to the required concentration level and measured via the standard additions approach. For these studies, results obtained gave a recovery mean 103.5% with standard deviation of ±1.3%. As can be seen from Table 1, these results achieved by the optimized AdSV procedure were in good agreement with those obtained by HPLC technique for the analysis of the same pharmaceutical tablets (oflacin-200 mg). Based on the statistical evaluation (F-test approach) for these results, there is no significant difference between the results obtained by the developed AdSV procedure and that obtained by the reference method. When comparing the variances of the developed AdSV procedure and the chromatographic reference method (HPLC), the calculated \( F \) value is 6.76. Whereas the calculated \( F \)-test value (6.76) was less than the critical value (9.28) at the 95% confidence level. There is no statistical evidence that the variance of the proposed method differ significantly from the variance of the reference method.

In addition, the applicability of the AdSV procedure for the analysis of ofloxacine–Fe(III) in biological samples was also evaluated by estimating its recovery from spiked human urine and serum samples. A simple and fast pretreatment (clean-up) procedure, which is in fact a slight modification of the sample preparation method develop for the determination of some antagonist drugs (Stubauer and Obendorf, 1996) was used. By adding a small amount of 5% ZnSO₄·7H₂O solution, NaOH and methanol to the urine or serum samples and
Centrifuging the mixture, most of the interfering substances (mainly proteins) were simply removed and eliminated by precipitation. As can be extracted from Table 2, this AdSV method (after appropriate dilution) allowed the determination of ofloxacin–Fe(III) in spiked urine and serum samples with mean recoveries 103.7% ± 0.6 and 105.33% ± 0.6, respectively (note: conc. of Fe(III) equal 5 × 10^-6 M in all measurements).

Acknowledgement

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References


Table 1 Comparative determination of ofloxacin–Fe(III) in commercial drug by the proposed SW-AdSV method and the reference chromatographic method.

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<th>% Recovery</th>
<th>Found (mg)</th>
<th>% Recovery</th>
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<td>Standard deviation</td>
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Table 2 Analytical result for ofloxacin–Fe(III) recovery from biological fluids.

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<tr>
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<th>% drug recovery</th>
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<tr>
<td>Standard deviation</td>
<td>± 0.6</td>
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