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A Study of Stripping Voltammetric Behaviour of Cefadroxil Antibiotic in the Presence of Cu (II) and its Determination in Pharmaceutical Formulation

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Abstract

Square-wave voltammetry was used to explore the adsorption property of cefadroxil complex with copper ions on the hanging mercury drop electrode (HMDE). By employing the adsorptive stripping voltammetric approach, a sensitive electroanalytical method for the quantitative analysis of cefadroxil antibiotic was achieved. A well-developed voltammetric peak was obtained in pH 10 Britton-Robinson buffer (B-R buffer) at -650 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, scan rate, pulse amplitude, SW frequency, working electrode area and convection rate has resulted in the recognition of optimal experimental conditions for cefadroxil analysis. The studied electroanalytical signal showed a linear response for cefadroxil in the concentration range 6×10^{-7} - 2×10^{-6} mol L^{-1} (r = 0.999). A limit of detection of 2×10^{-9} mol L^{-1} with relative standard deviation of 2.783 RSD% and mean recovery of 99% was obtained. Possible interferences by several substances usually present in pharmaceutical formulation were also evaluated. The analytical quantification of cefadroxil in commercially available pharmaceutical formulation was performed and compared with data from HPLC technique.

Keywords: adsorptive stripping voltammetry, square wave voltammetry, cefadroxil antibiotic, drug-metal ions complex.

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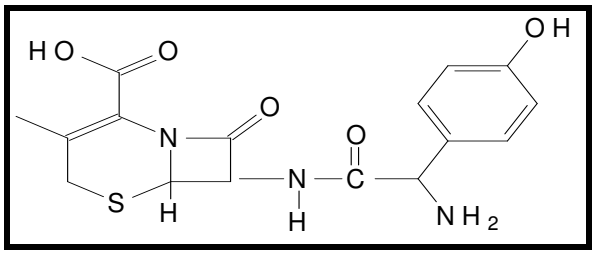
Introduction

Square Wave Adsorptive Stripping Voltammetry (SW-AdSV) has been well characterized as an extremely sensitive source for electroanalytical measurements since its establishment about 25 years ago. Such electrochemical approach with improved sensitivity and selectivity has promoted the development of numerous analytical applications of ultra-trace determinations of a variety of organic or inorganic substances. 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 interference of dissolved oxygen in the analysed samples [1-3]. 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 [4,5], organic analytes [6], pharmaceutical drugs and biomedical compounds [7,8].



Scheme 1. Chemical structure for cefadroxil.

Cefadroxil is a broad-spectrum antibiotic, considered as a first-generation in the cephalosporin family of drugs. This antibacterial drug is used primarily in the treatment of milt to moderate susceptible infections. In fact, it is used to treat bacterial infections of the skin, ear, soft tissues, strep throat and the urinary tract [9-11]. 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 [12-14], high performance liquid chromatography (HPLC) [15,16], and electrochemical methods such as polarography and differential pulse voltammetry [17,18]. However, no literature data were found on the square wave voltammetry in general, or on the adsorptive stripping determination of this drug in particular. Consequently, the development of SW-AdSV method for the analysis of cefadroxil after complex formation with copper ions, and its application to determination in pharmaceutical formulation, is described.

Experimental

Apparatus

All square wave adsorptive stripping voltammetric measurements were carried out with 797 VA computrace (Metrohm, Herisau, Switzerland) in connection with Dell computer and controlled by (VA computrace 2.0) control software.

Stripping voltammograms were printed via a hp deskjet 5150 printer. A conventional three electrodes 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 a Metrohm 632 pH meter. Biohit adjustable micropipette (AU), and Brand adjustable micropipette (Germany), were used to measure microliter volumes of the standard solutions.

Reagents

All chemicals used were of analytical reagent grade and were used without further purification. Cefadroxil drug (Sigma-Germany) stock solution of 1×10^{-2} mol L^{-1} was prepared by dissolving the appropriate amount of cefadroxil in distilled water in a 25 mL volumetric flask and this stock solution was stored in the dark. Similarly, Cu (II) stock solution of 1×10^{-3} mol L^{-1} was prepared by dissolving the appropriate amount of copper nitrate salt in distilled water in a 50 mL volumetric flask. Britton-Robinson supporting buffer (pH ≈ 2, 0.04M 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_2PO_4(winlab, UK) and 0.1 M H_3PO_4] was prepared by dissolving 12 g of NaH_2PO_4 and 6.78 g of H_3PO_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. While, 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. Finally, ammonia buffer was prepared by dissolving 4.5 g of ammonium chloride in 20 mL distilled water and then adding 35 mL of concentration ammonia and diluting to 1 L with distilled water.

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 cefadroxil complex were added. The test solutions were purged with nitrogen for 5 minutes initially, while the solution was stirred. The accumulation potential of +0.1 V vs. Ag/AgCl was applied to a new mercury drop while the solution was stirred for 90 seconds. Following the preconcentration period, the stripping was stopped and after 20 seconds had elapsed, cathodic scans were carried out over the range 0.0 to –1.0 V. All measurements were made at room temperature.

Results and discussion

The electroanalytical properties of cefadroxil complex

Preliminary stripping voltammetric experiments showed that cefadroxil pharmaceutical molecule did not yield any cathodic reduction response at mercury electrode as can be seen from Fig. 1, which exhibited a flat background line (line A) for 5×10^{-7} mol L^{-1} cefadroxil in pH 10 Britton-Robinson buffer.

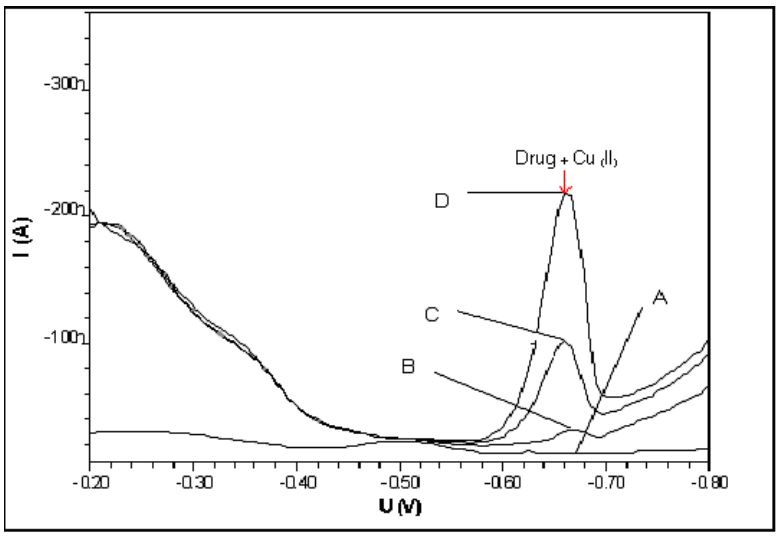
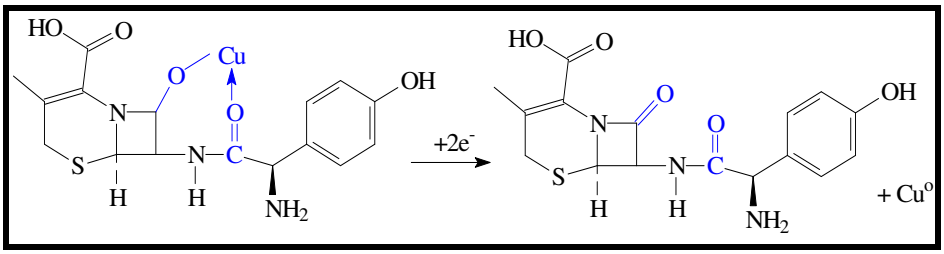


Figure 1. Electrochemical behaviour of cefadroxil complex with copper ions: A = B-R buffer pH 10 + $5x10^{-7}$ M cefadroxil, B = A + $5x10^{-6}$ M Cu(II), C = A + B + $1x10^{-6}$ M cefadroxil and D = A + B + C + $5x10^{-6}$ M Cu^{+2}.

However, the addition of 5×10^{-6} mol L^{-1} Cu (II) to the previous cefadroxil test solution provided a well-defined (line B) cathodic peak at -650 mV (versus Ag/AgCl reference electrode). Furthermore, the free copper ions still existing in the test solution also produce an extra voltammetric peak at E_p=-210 mV. In fact, the copper ions exhibited a good affinity towards cefadroxil molecules forming a very stable cefadroxil-Cu (II) complex, which is strongly adsorbed onto the HMDE surface. This obtained well-developed stripping voltammetric peak was found to respond sharply to the addition of either cefadroxil or Cu (II) concentrations (lines C and D), which probably reflects the formation and adsorption of the suggested complex. The observed AdSV peak is most probably due to the cathodic reduction of Cu (II) in the adsorbed complex with cefadroxil and the electrochemical mechanism for the reduction process of cefadroxil-Cu (II) complex is illustrated in scheme 2.

Clearly, this proposed electrochemical reduction mechanism suggested an irreversible reductive process for copper ions in the adsorbed complex, an assumption which was confirmed by cyclic voltammetric measurement of 1×10^{-4} mol L^{-1} cefadroxil drug and 1×10^{-3} mol L^{-1} Cu (II) in pH 10 B-R buffer at 50 mV s^{-1} scan rate. As can be noticed from Fig. 2, which exhibits the cyclic voltammogram of cefadroxil-Cu (II) complex, 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 cefadroxil complex with Cu (II) were carried out, a well developed AdSV peak

was observed at the first cathodic scan; however, succeeding cathodic scans exhibit a gradual decrease 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 cefadroxil molecules.



Scheme 2. Mechanism of the studied electrochemical reduction process for cefadroxil-Cu(II) complex.

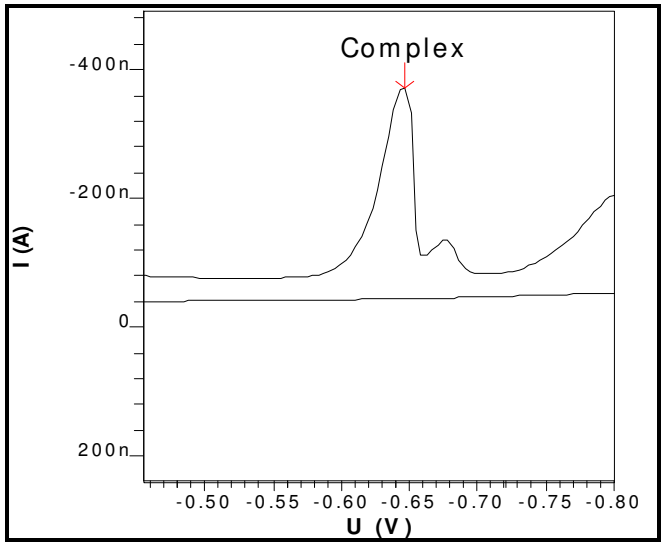


Figure 2: Cyclic voltammograms for 1×10^{-4} mol L^{-1} cefadroxil complex with Cu (II) in pH 10 B-R buffer at 50 mV s^{-1} scan rate. Accumulation time 60 s at E_{acc}: 0.0 V.

Optimisation of experimental parameters

Effect of supporting buffer constituents and pH

Since the adsorptive phenomena of cefadroxil complex on the HMDE were utilized as a suitable collection step prior to their electrochemical determination, it was rational to characterize various variables and experimental conditions 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, ammonia 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 work.

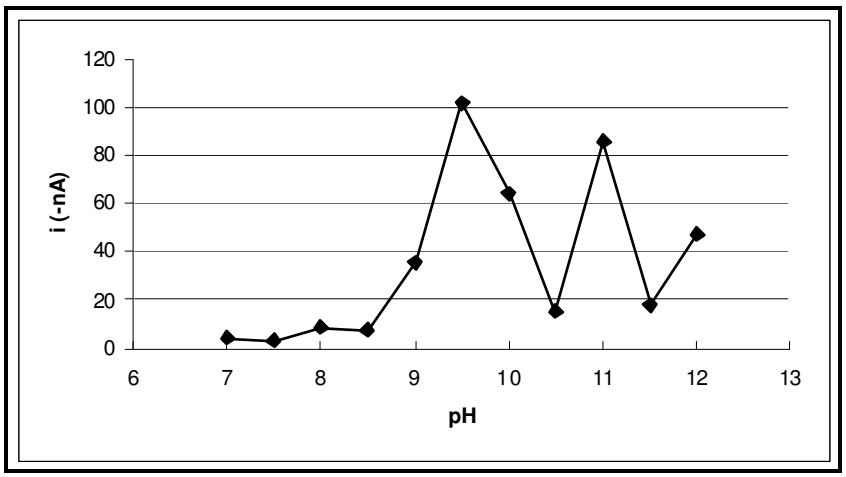


Figure 3. Effect of pH on SW-AdSV peak current of 7.5×10^{-7} mol L^{-1} cefadroxil drug and 5×10^{-6} mol L^{-1} Cu (II) in B-R buffer after an accumulation period of 90 s at E_{acc}: + 0.1 V and 900 mV s^{-1} scan rate.

Generally, the AdSV signal was mainly pH dependent since the monitored voltammetric signal was only observed at alkaline media. When the stripping voltammetric peak current was measured as a function of pH over the range 7-12, the peak current increased gradually at first and enhanced sharply beyond pH 8.5, then it reached its maximum value at pH 9.5, 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 shift 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 are not consumed.

Effect of accumulation factors

The interfacial accumulation of cefadroxil 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 Cu (II) complex. Therefore, the effect of accumulation time on the efficiency of the collection of 7.5×10^{-7} mol L^{-1} cefadroxil drug in the presence of 5×10^{-6} mol L^{-1} copper 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_p-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 0-90 s and thereafter the peak intensity nearly levelled probably due to the saturation of the HMDE. Hence, 90 s accumulation time was selected for all future experiments. Furthermore, variation of the accumulation potential over the range from +0.4 V to -0.8 V at 90 s accumulation time, revealed that a preconcentration potential of +0.1 V was the ideal choice for optimal sensitivity.

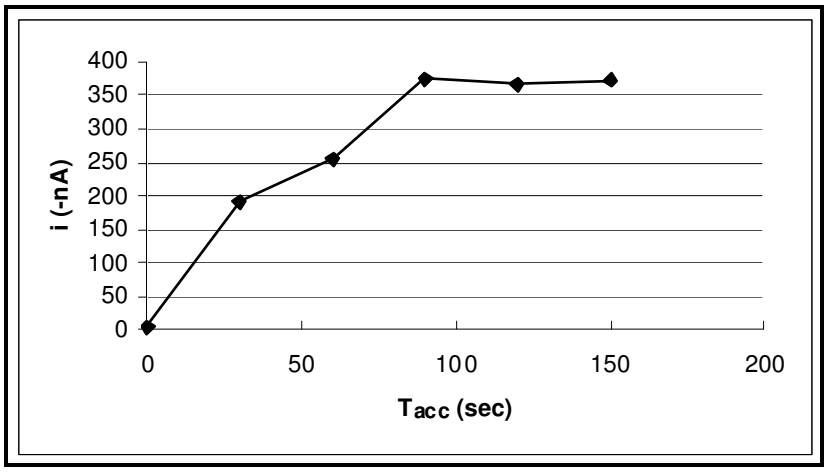


Figure 4. Effect of accumulation time on the stripping voltammetric peak current of 7.5×10^{-7} mol L^{-1} cefadroxil drug and 5×10^{-6} mol L^{-1} Cu (II) in pH 9.5 B-R buffer. Accumulation potential: + 0.1 V and scan rate: 900 mV s^{-1} .

Effect of metal ion concentration

The dependence of the WS-AdSV voltammetric current of 7.5×10^{-7} mol L^{-1} cefadroxil in a B-R buffer of pH 9.5 on the concentration of copper ions was also investigated. As shown in Fig. 5 the monitored voltammetric signal was approximately linear over the range from 1×10^{-7} mol L^{-1} to 5×10^{-6} mol L^{-1} Cu (II), then decreased sharply and this behaviour may be attributed to competitive coverage of the analyte interest (cefadroxil) and free copper ions on the working electrode surface. Hence, the metal ion concentration of choice will be 5×10^{-7} mol L^{-1} cefadroxil Cu (II).

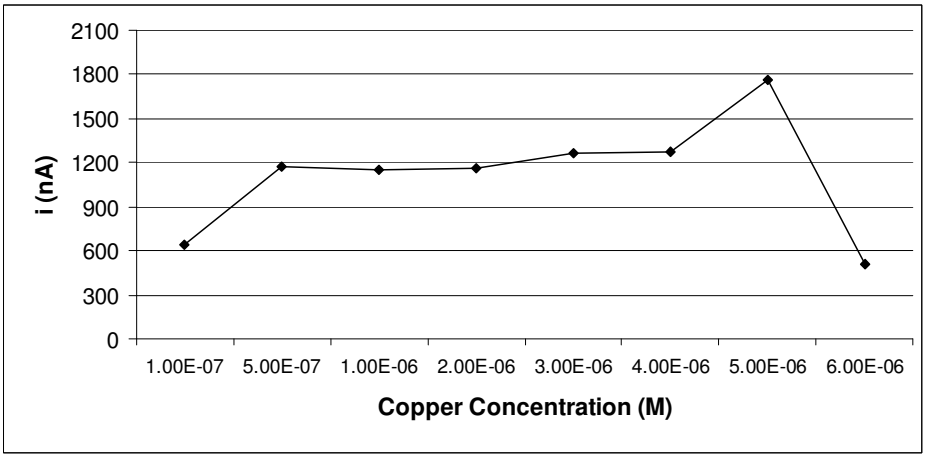


Figure 5. Effect of copper ion concentration on the stripping voltammetric peak current of 7.5×10^{-7} mol L^{-1} cefadroxil drug in pH 9.5 B-R buffer. Accumulation potential: + 0.1 V and scan rate: 900 mV s^{-1} .

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 100-900 mV s^{-1} scan rate. However, when scan rates faster than 900 mV s^{-1} were employed, the peak current decreased slightly. Consequently, scan rate value of 900 mV s^{-1} would be adequate 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 and it was concluded that in order to assure maximum peak current, 50 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 10-100 Hz resulted in a substantial enhancement of the voltammetric peak current, particularly at range 10-70 Hz. Accordingly, for future work 70 Hz SW frequency value was adopted.

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 cefadroxil 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 rang 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 optimised experimental conditions for SW-AdSV measurements of cefadroxil drug were accumulating its complex for 90 s at +0.1 V preconcentration potential with stirring rate of 3000 rpm. These voltammetric measurements were carried out in Britton-Robinson buffer at pH 9.5 in the presence of 5×10^{-6} mol L^{-1} Cu (II). The applied potential was scanned at 900 mV s^{-1} with 70 Hz SW frequency rate and 50 mV pulse amplitude.

Analytical performance
Calibration graph and detection limit
Once the optimal chemical conditions and instrumental parameters for the SW-AdSV determination of cefadroxil were established, several analytical characteristics of the proposed were evaluated. Under the optimised conditions, a linear correlation between SW-AdSV peak intensity and the drug concentration was obtained over the range 6×10^{-7} - 2×10^{-6} mol L^{-1}, (see Fig. 6). The calibration equation was calculated by least-squares method and it has the form:

$$I_p\ (\text{nA}) = 4.1\times10^{9}\ C\ (\text{mol L}^{-1}) - 1847 \qquad r = 0.999, \quad n = 8$$

where I_p is the stripping voltammetric peak current in nanoamperes, C is cefadroxil concentration and r is the correlation coefficient.

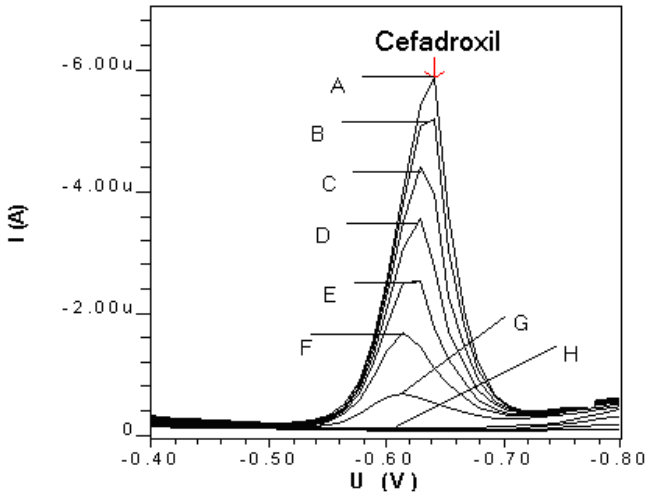


Figure 6. AdSV voltammogram for cefadroxil in the presence of 5×10^{-6} mol L^{-1} Cu(II) in B-R buffer pH=9.5, T_{acc} = 90 sec, E_{acc}= + 0.10 V and scan rate = 900 mV/s. Drug concentrations: (A= 2×10^{-6} M, B = 1.8×10^{-6} M, C = 1.6×10^{-6} M, D = 1.4×10^{-6} M, E = 1.2×10^{-6} M, F = 1×10^{-6} M, G = 8×10^{-7} M, H = 6×10^{-7} M).

The effective preconcentration step during the adsorption process of the analyzed drug allows a very low detectability. The detection limit estimated based on the signal-to-noise ratio (S/N=3) was 2×10^{-9} mol L^{-1}. This obtained sensitivity was significantly preferable than those reported for other analytical techniques currently used for determination of cefadroxil drug, such as flow injection analysis methods with 1.1 $\times10^{-6}$ M [13] detection limit, chromatography method (HPLC) with 2.75 $\times10^{-6}$ M [15] detection limit and polarography method with 8 $\times10^{-6}$ M [17].

Precision, accuracy and stability

The reproducibility of the developed procedure was evaluated from eight repeated measurements of 7.5×10^{-7} mol L^{-1} cefadroxil drug in the presence of 5×10^{-6} mol L^{-1} copper ion. The precision of the method in terms of the relative standard deviation (RSD%) was 2.7%. The accuracy of the electrochemical method was checked by calculating the recovery of known amount (7.5×10^{-7} mol L^{-1}) cefadroxil drug spiked in buffer solution and analysed by the optimised procedure. The results of four measurements obtained by the standard addition method have a recovery mean of 99% with standard deviation of ± 0.82%. When the SW-AdSV signal of 7.5×10^{-7} mol L^{-1} cefadroxil drug solution in the presence of 5×10^{-6} mol L^{-1} Cu(II) was monitored every ten minutes, it was found to be nearly stable for a period of 1.5 hours at least.

Interferences

The competitive co-adsorption interference was evaluated in the presence of various substances which occur in the pharmaceutical formulation as tablet ingredients or additives. For these investigations, the interfering species (lactose, sucrose, cellulose, starch and magnesium stearate) were added at different

concentrations (twice, 5-fold and 50-fold) higher than the concentration of cefadroxil (7.5×10^{-7} mol L^{-1} cefadroxil). The addition of magnesium stearate even at high concentration level, caused no significant effect on the SW-AdSV response of cefadroxil. In contrast, the addition of 50-fold of sucrose higher than the quantity of the assayed drug caused the AdSV peak current to decrease by about 80% of its original signal. While the addition of 5-fold and 50-fold of cellulose caused the AdSV peak current to decrease by about 13% and 31%, respectively. Furthermore, the addition of 2-fold, 5-fold and 50-fold of lactose, yielded negative interferences by 8%, 18% and 76%, respectively. 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. The presence of starch at 5-fold and 50-fold excess of cefadroxil caused nearly 42% and 75%, respectively, enhancement in the monitored AdSV peak current.

Analytical applications

To assess the reliability of the proposed electrochemical procedure described above, it was applied for resolving the determination of cefadroxil in a pharmaceutical preparation. The drug content of commercially available droxil capsulate (United Pharmaceutical, Jordon: contain 500 mg of cefadroxil) was determined via the optimized SW-AdSV procedure directly after the required dissolving and filtration steps and dilution to the required concentration level. The electrochemical measurements were done by the standard addition method in order to minimize matrix effects. Four aliquots of this sample were analyzed by the proposed SW-AdSV method and HPLC technique. Table 1 shows the analytical results obtained by the two methods and, as can be seen, good agreement was found between them, and this was statistically proved according to the F-test approach. The means of both analytical methods did not differ significantly, since the calculated F-test value (5.8) was less than the tabulated value (9.12).

Table 1. Comparative determination of cefadroxil complex in commercial drug by the proposed SW-AdSV method and the reference chromatographic method.

	SW-AdSV	**HPLC**
	Found (mg)	**Found** (mg)
Labeled Content droxil capsulate 500 mg	495	490
	495	492
	497.5	494
	492.5	510
	487.5	
	Mean= 493.5 mg (98.7%) S.D.= ±0.8 mg	**Mean= 496.5 mg (99.3%)** S.D.= ±1.8 mg

Acknowledgement

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References

1. J. Wang, *Analytical Electrochemistry*, VCH Publishers Inc., New York, 1994.
2. J. Osteryoung, J.J. O`Dea, in *Electroanalytical Chemistry*, Vol. 14 (A.J. Bard, ed.), Marcel Dekker, New York, 1986.
3. A. Economou, P.R. Filden, *Anal. Chim. Acta* 273 (1993) 27- 34.
4. P.M. Zaitsev, R.M.F. Salikhdzhanova, N.K. Zaitsev, *Indust. Lab Diagno. Mater.* 65 (1999) 1-15.
5. A.Z. Abu Zuhri, W. Voelter, Fresenius, *J. Anal. Chem.* 360 (1998) 1-9.
6. K.H. Brainina, N.A. Malakhova, N.Y. Stojko, *Fresenius J. Anal. Chem.* 368 (2000) 307-325.
7. A.H. Alghamdi, *J. Saudi Chem. Soc.* 6 (2002) 185-198.
8. J.C. Vire, J.M. Kauffmann, G.J. Patriarche, *J. Pharma. Biomed. Anal.* 7 (1998) 1323-1335.
9. S. Budavari (ed.), The Merck Index, MercCo., Inc., Whitehouse Station, N J, 1996.
10. J.E.F. Keynolds, *Matrindale: the Extra Pharmacopoeia*, Royal Pharm. Soc., London, 1996.
11. L.K. Morton, *Medicine, the comprehensive guide*, Bloomsbury, London, 1991.
12. A.M. El-Walily, A.A. Gazy, S.F. Belal, E.F. Khamis, *J. Pharm. Biomed. Anal.* 20 (1999) 643-653.
13. F.H. Metwally, A.A. Al-Warthan, S.A. Al-Tamimi, *Farmaco* 56 (2001) 601-607.
14. C. Thangpoon, B. Liawruangrath, S. Liawruangrath, R. Alan Wheatley, A. Townshend, *Anal. Chim. Acta* 553 (2005) 123-133.
15. J.A. McAteer, M.F. Hiltke, B.M. Silber, R.D. Faulkner, *Clinic. Chem.* 33 (1987) 1788-1790.
16. A. El-Gindy, A.F. El Walily, M.F. Bediar, *J. Pharm. Biomed. Anal.* 23 (2000) 341-352.
17. S.A. Ozkan, N. Erk, B. Uslu, N. Yilmaz, I. Biryol, *J. Pharm. Biomed. Anal.* 23 (2000) 263-273.
18. A. Ivaska, F. Nordstrom, *Anal. Chim. Acta* 146 (1983) 87-95.
19. G. Stubauer, D. Obendorf, *Analyst* 121 (1996) 351-356.