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Determination of cefonicid in human urine by adsorptive square-wave stripping voltammetry

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Abstract

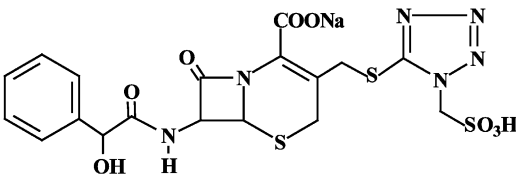
The adsorption behavior of cefonicid on the hanging mercury drop electrode (HMDE) has been examined using cyclic voltammetry and square-wave voltammetry techniques in Britton–Robinson (B–R) buffers in the pH range of 2.0–11.0. The effect of different parameters on the accumulation behavior of the adsorbed species has been evaluated. Sensitive measurements can be achieved after controlled adsorption on the surface of HMDE followed by square-wave voltammetry. Under optimal conditions, a detection limit of 4.0×10^{-8} M and a linear calibration graph in the range 1.0×10^{-7}–1.0×10^{-6} M were obtained. Direct simple determination of cefonicid in urine was established with no manipulation of urine sample other than dilution and subsequent adsorptive stripping voltammetric determination. The detection limit of the method was 1.0 μg ml^{-1} of cefonicid in urine.

Keywords: Cefonicid; Square-wave; Adsorptive stripping voltammetry; Urine analysis

1. Introduction

The β-lactam antibiotics are used most frequently in human medicine on account of their broad antibacterial activity spectrum and low toxicity. Their activity is exerted through the inhibition of some enzyme reactions vital to bacteria, which hinders the formation of the bacterial wall.

Cefonicid, 7-[(*R*)-Mandelamido]-3-(1-sulfomethyl-1*H*-tetrazol-5-ylthiomethy)-3-cephem-carbooxylic acid (**I**),



(I)

is a second generation cephalosporin, which is highly protein bound and has a prolonged serum

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elimination half-life after intravenous and intramuscular administration [1].

Up to now the most commonly-employed techniques for the determination of the drug in biological fluids have been based on HPLC [2,3] and microbiological assay [4]. Such techniques for the measurement of biological concentration are necessary in clinical environment to ensure that adequate drug levels can be maintained while avoiding toxic concentrations for such drugs. The main problems encountered using such methods are either the need for derivatisation or the need for time-consuming extraction procedures. Since the these techniques have a slightly expensive instrumentation and running costs, the use of simpler, faster and less expensive, but still sensitive, electrochemical techniques can be an interesting alternative, mainly those based on adsorptive stripping techniques.

The cefonicid molecule has electroactive groups, but nothing appears to have been published concerning its electrochemical behavior or its voltammetric measurement in particular. Adsorptive stripping voltammetry has been demonstrated as a sensitive analytical method for several electroactive cephalosporins that exhibit adsorption capabilities on the electrode surface [5–10]. The high sensitivity of adsorptive stripping methods makes it possible to work with very diluted samples with a corresponding decrease in possible interferences in analysis.

The purpose of the present work was to explore and exploit the adsorption of cefonicid on to hanging mercury drop electrode as an effective preconcentration step before square-wave cathodic stripping voltammetry. In this way, a methodology for direct and simple determination of drug in urine at the low level found in urine after therapeutic doses of cefonicid can be established.

2. Experimental

2.1. Reagents

A stock solution 1.0×10^{-3} M of cefonicid sodium was prepared daily in de-mineralized water from the pure compound supplied by Smith Kline Beechman (Brentford, England). The supporting electrolyte used was Britton–Robinson (B–R) universal buffer solution, which was prepared by mixing solutions orthophosphoric acid, acetic acid and boric acid, 0.04 mol l^{-1} each, adjusted with appropriate volumes of 0.2 sodium hydroxide solution. All reagents used were of analytical-reagent grade. De-mineralized water from a Milli-Q system (Millipore, Bedford, MA) was used throughout the work.

2.2. Apparatus

Voltammetric measurements were obtained with an 394 EG&G PARC electrochemical trace analyzer. An EG&G PARC 303A stand was used in HMDE mode (drop size: large, area of the drop: 0.026 cm^2). The three-electrode system was completed by means of a platinum auxiliary electrode and an Ag/AgCl (3 M KCl) reference electrode. All potentials given are relative to this electrode. A magnetic stirrer (PAR 305) and stirring bar provided the convective transport during preconcentration.

2.3. Procedure

A 10 ml volume of an appropriate buffer solution was added to the voltammetric cell and the solution was purged with nitrogen for 10 min (and for 60 s before each adsorptive cycle). The accumulation potential was applied to the working electrode for a selected time while the solution was stirred continuously. The stirring was then stopped and after 5 s, a negative-going potential scan was initiated and the resulting voltammogram was recorded. Then, the required aliquot of the stock solution of cefonicid was added by means of micropipette and the adsorptive stripping cycle was repeated using a new mercury drop. All data were obtained at ambient temperature.

2.3.1. Analysis of cefonicid in urine

Urine samples, 1 ml each, spiked with 10–100 μl aliquots of 1000 μg ml^{-1} cefonicid working solution were made up to 10 ml with de-ionized water to obtain urinary concentrations of 10–100 μg cefonicid per ml of urine. A 1 ml volume of the

resulting cefonicid–urine solution was transferred into a voltammetric cell containing 9.0 ml B–R solution pH 4.0. After removal of oxygen for 10 min the square-wave voltammograms were recorded following the optimized conditions. Quantification was performed by means of the calibration curve method.

3. Results and discussion

3.1. Effect of pH

Cefonicid was reduced readily at mercury electrode in B–R buffer in pH range 2.0–11.0. Square-wave voltammograms gave rise to two reduction peaks in acidic solutions. Fig. 1 shows representative square-wave voltammograms for 5.0×10^{-7} M cefonicid in B–R buffer pH 4.0 ($t_{acc.} = 90$ s; $E_{acc} = -0.35$V). The voltammetric response is markedly dependent on pH. The current of the first peak is maximum in pH interval 2.0–4.0, decreases continuously above pH 4.0 and is not seen at pH 8.0. The second peak was observed only in pH range 1.0–5.0. In neutral or alkaline solutions (pH 7.0–11.0) a small reduction peak was observed at more negative potentials. The peak potential of the first reduction process is shifted towards more negative values with increasing pH up to pH 4.0 by 25 mV per pH unit. A break appears at this pH value and the slope becomes equal to 55 mV per pH unit at higher pH value. The slope change at pH 4.0, which coincide with the maximum value found for the peak intensity probably corresponds to pK_a value of the carboxylate function [11]. This behavior indicates clearly that protons involved in the electrode reaction [12].

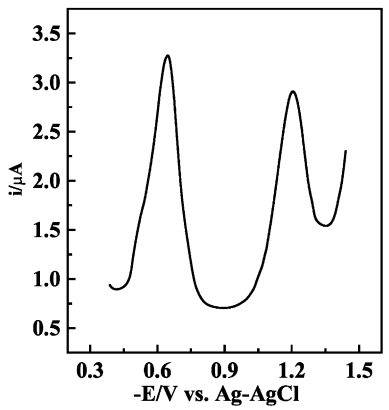

Fig. 1. Stripping square-wave voltammogram of 5.0×10^{-7} M cefonicid solution in the B–R buffer pH 4.0 ($f = 120$ Hz, $\Delta E = 10$ mV and $a = 100$ mV) following accumulation, $t_{acc} = 90$ s at $E_{acc} = -0.35$ V and equilibration time 5 s.

Probably the protonated carboxylate form present at pH 4.0 is adsorbed extensively at the electrode surface and the increase of the adsorption phenomena in acidic medium is likely to be related to maximum response reached within pH interval 2.0–4.0.

On the basis of the mechanism proposed for other cephalosporins [13,15], it seems reasonable to consider that the less cathodic peak (first one) is due to a two-electron reduction of the C=N double bond of the tetrazolyl substituent of the side chain at position 7. The more cathodic peak (second one) appears at potential close to the reduction potential of 7-aminocephalosporanic acid, indicating that the peak is probably due to the reduction of the double bond in the dihydrothiazine ring of the cephem nucleus. The reduction of the double bond in the dihydrothiazine ring involves cleavage of the molecule and consequently, detachment of the thioester linkage at position 3.

From the analytical point of view, i.e. with respect to the peak height, peak shape, repeatability and facile evaluation, the less cathodic peak at pH 4.0 emerged as optimum. Thus, we focused our study mainly on this reduction peak because of its analytical interest.

3.2. Cyclic voltammetry

Fig. 2 shows the first two scans of the cyclic voltammograms, run at B–R pH 4.0 for cefonicid concentration of 1.0×10^{-5} M at HMDE following an accumulation time of 90 s with stirring at -0.35 V and scanning in the negative direction at scan rate of 100 mV s^{-1}. Cefonicid produces large cathodic adsorptive peak at -0.60 V and another small one at -1.15 V, respectively. These peaks decreased in subsequent scans with the same drop. This behavior gives an indication of an adsorption reaction. The log–log plot of peak current versus

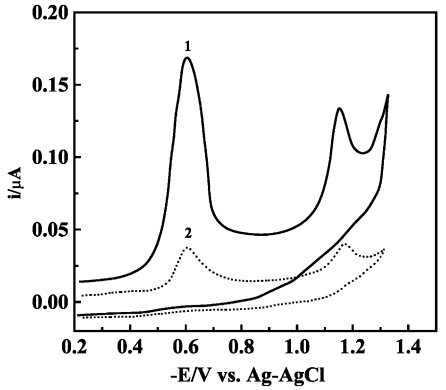

Fig. 2. Repetitive cyclic voltammograms for 1.0×10^{-5} M cefonicid in the B–R buffer pH 4.0 with scan rate $v = 100$ mV s^{-1} following accumulation, $t_{acc} = 90$ s at accumulation potential $E_{acc} = -0.35$ V.

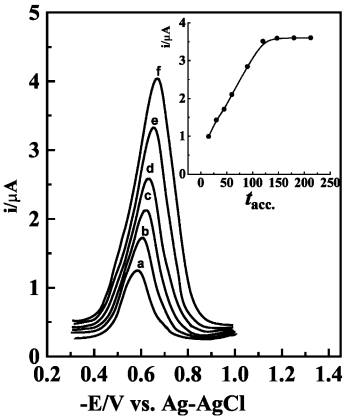

Fig. 3. Effect of accumulation time on the stripping peak current for 5.0×10^{-7} M cefonicid in the B–R buffer pH 4.0 following accumulation at $E_{acc} = -0.35$V. Inset is the current/time plot.

scan rate gives a slope of 0.85, clearly indicative of adsorption-controlled process. The fact that such slope is less than the theoretically expected slope (1.0) can be attributed to the fact that the response is the result of the reduction not only of the antibiotic molecules that were already adsorbed on the electrode surface prior to the sweep, but also of those that reach the electrode by diffusion.

On the reverse scan, no anodic peak was observed at scan rate of 10–300 mV s^{-1}, a characteristic behavior of irreversible process. The reduction peak is displaced to more negative potentials when the scan rate increases, confirming the irreversible nature of the reduction reaction.

3.3. Effect of accumulation potential and time

The effect of accumulation potential on SWV peak height was examined at a constant time of accumulation of 90 s in stirred solution. Some dependence of peak height on potential of accumulation, thought not very pronounced, was observed. The other dependences were therefore measured at a potential of accumulation of −0.35 V.

The effect of accumulation time for 5.0×10^{-7} M cefonicid was investigated from 0 to 240 s. As shown in Fig. 3, a rectilinear relationship is observed in an accumulation time range from 15 to 120 s, following the equation: $i_p = 0.666 + 0.024t_{acc}$. Above 120 s, saturation coverage of the electrode occurs and curvature of the graph is observed.

The optimum instrumental conditions were then chosen from a study of the variation of the peak current (i_p) of 5.0×10^{-7} M cefonicid in B–R pH 4.0 with frequency f, scan increment ΔE and pulse height a. The highest i_p values were found with high frequencies of the order 120 Hz. Higher currents were given by increasing the pulse height or the scan increment without loss of resolution, i.e. the peak half–width of cefonicid remained almost constant. The optimal SW parameters are established at $f = 120$ Hz, $\Delta E = 10$ mV and $a = 100$ mV and hence these values were used for further measurements.

3.4. Analytical application

When using optimal conditions (90 s accumulation time; −0.35 V accumulation potential; B–R buffer pH 4.0). The response is linear in concentration range 1.0×10^{-7}–1.0×10^{-6} M following the equation: i_p (μA) = (0.433 ± 0.0427) + (4.101 ± 0.068)C (μM) with correlation coefficient of 0.998. Above a concentration of 1.0×10^{-6} M, saturation of the electrode occurs which leads to the constancy of the calibration graph, the detection limit, calculated following the expression $a + 3S_{yx}$,

[16] where a = intercept and S_{yx} = error standard deviation, was 4.0×10^{-8} M. The precision was good; the relative standard deviation of ten determinations at 5.0×10^{-7} M level was 2.3%. It was found that the voltammetric response of cefonicid at the pH value chosen was practically stable to at least 1 h, with a maximum decrease of less than 1.0% being quite satisfactory for analytical purpose.

3.5. Analysis of cefonicid in urine

Practical application of adsorptive stripping analysis would suffer from interferences due to the presence of surface-active compounds present in urine. The adsorption of such compounds on the electrode surface would inhibit the accumulation of the analyte and exhibit depression of the stripping signal. Appling the proper dilution of urine would conveniently diminish the effect of competitive adsorption of urine components. Fig. 4 illustrates the adsorptive square-wave voltammetric response to different concentrations of cefonicid in diluted urine samples using the optimal conditions. The response was linearly related to the cefonicid concentration within the range 10–100 μg per 1.0 ml of urine according to the regression equation: i_p (μA) = (4.818 ± 0.036) + (0.012 ± 0.005)C (μg ml^{-1}), $r = 0.996$. The peak current was measured in all instances against the base line of the blank urine sample. A detection limit of 1.0 μg ml^{-1} was obtained. The precision and recovery were calculated from five measurements of five urine samples spiked with cefonicid at concentration from 20 to 100 μgml^{-1}. The mean recovery was 97.5% with a mean relative error of 3.4%.

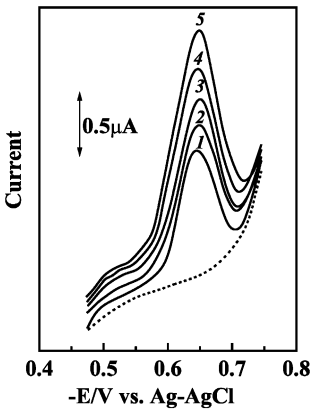

Fig. 4. Adsorptive square-wave stripping voltammograms obtained for the determination of cefonicid in urine. Dotted lines represent the blank; (1, 2, 3, 4, and 5) urine spiked with increasing concentration of cefonicid; (1) 20 (2) 40 (3) 60 (4) 80 and (5) 100 μg ml^{-1}.

4. Conclusion

Cefonicid in biological fluids is normally determined by chromatographic methods [2,3]. For analysis of cefonicid in urine, the drug was extracted from diluted urine with methanol and then analysed by HPLC with UV detection at 265 nm. The urine concentration of cefonicid have been detected down to 0.03 μg ml^{-}1with linear range 1–50 μg ml^{-1} of urine. Although the proposed method is less sensitive than the HPLC, the limit of detection obtained was low enough to reach the urine levels of cefonicid after normal therapeutic doses [4]. This, together with facts that no manipulation of urine sample is needed other than dilution and short analysis time greatly approves the proposed to be well suitable for urine level monitoring in clinical practice and pharmacological studies

References

[1] D.H. Pitikin, J. Dubb, P. Actor, F. Alexander, S. Ehrlich, R. Familiar, R. Stote, Clin. Pharmcol. Ther. 30 (1981) 587.
[2] E. Brendel, M. Zschunke, I. Meineke, J. Chromatogr. Biomed. Appl. 359 (1985) 339.
[3] R. Phelps, E. Zurlinden, J.E. Conte, E. Lin, J. Chromatogr. Biomed. Appl. 111 (1986) 375.
[4] R.P. Sterlin, D.J. Connor, J.C. Norman, D.A. Coolley, Antimicrob. Agents Chemother. 790 (1983) 23.
[5] D. Peled, C. Yarnitzky, W.F. Smyth, Analyst 112 (1987) 959.
[6] B. Ogorevc, A. Krasina, V. Hudnik, S. Gomiscek, Mikrochim. Acta I (1991) 131.
[7] S. Altinoz, D. Ozer, A. Temizer, N. Yukse, Analyst 1575 (1994) 119.
[8] V.S. Ferreirra, M.V.B. Zanoni, M. Furlan, A.G. Fogg, Anal. Chim. Acta 105 (1997) 351.
[9] L.N.C. Rodrigues, M.V.B. Zanoni, A.G. Fogg, J. Pharm. Biomed. Anal. 497 (1999) 21.

[10] N. Abo El-Maali, Talanta 957 (2000) 51.
[11] M. Zhou, R.E. Notari, J. Pharm. Sci. 534 (1995) 84.
[12] P. Zuman, in: P. Zuman, L. Meites (Eds.), Progress in Polarography, vol. 3, Wiley, New York, 1992, p. 73.
[13] E.C. Rickard, G.G. Cooke, J. Pharm Sci. 379 (1977) 66.
[14] L. Camacho, J.L. Avila, A.M. Heras, F. Garcia-Blanco, Analyst 1507 (1984) 109.
[15] B. Ogorevc, S. Gomiscek, J. Pharm. Biomed. Anal. 9 (1991) 225.
[16] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry; Ellis Horwood Series, PTR Prentice Hall, New York, 1993, p. 119.