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Indirect polarographic and cathodic stripping voltammetric determination of cefaclor as an alkaline degradation product

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Abstract

Cefaclor is not reducible at a mercury electrode, but it can be determined polarographically and by cathodic stripping voltammetry as its initial alkaline degradation product which is obtained in high yield by hydrolysis of cefaclor in Britton–Robinson (B–R) buffer pH 10 at 50°C for 30 min (reduction peak at pH 10, −0.70 V). Differential pulse polarographic calibration graphs are linear up to at least 1×10^{-4} mol l^{-1}. Recoveries of 93% of the cefaclor ($n = 3$) were obtained from urine spiked with 38.6 μg ml^{-1} using this polarographic method with 1 ml urine made up to 10 ml with pH 10 buffer. Using cathodic stripping voltammetry and accumulating at a hanging mercury drop electrode at −0.2 V for 30 s, linear calibration graphs were obtained from 0.35 to 40 μg ml^{-1} cefaclor in B–R buffer pH 10. A relative standard deviation of 4.2% ($\eta = 5$) was obtained, and the limit of detection was calculated to be 2.9 ng ml^{-1}. Direct determination of cefaclor in human urine (1 ml of urine was made up to 10 ml with pH 10 buffer) spiked to 0.39 μg ml^{-1} was made (recovery 98.6%).

Keywords: Cefaclor determination; Human urine analysis; Stripping voltammetry; Alkaline degradation; Polarography

1. Introduction

Numerous analytical procedures have been reported for the determination of cefaclor (1) in the pure drug form, in pharmaceutical preparations, and in biological fluids. The hydroxylamine colorimetric method is in the USP XXII [1]. HPLC methods are given for the determination of cefaclor in human plasma and urine [2–4]. The iodometric determination of cefaclor has been reported [5]. A spectrofluorimetric method is described for its determination in formulations and biological fluids [6]. The present authors have recently reported a cathodic stripping voltammetric method for the indirect determination of cefaclor as a mercury salt (the cefaclor degrades in forming this salt) [7], but no other polarographic or stripping voltammetric method appears to have been reported.

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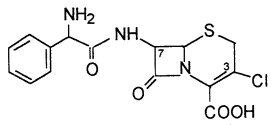
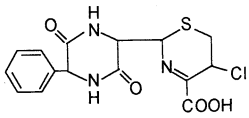
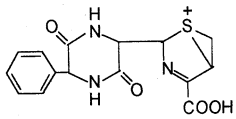


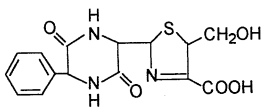
Cefaclor (Compound 1)



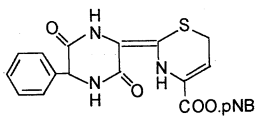
(Compound 2)



(Compound 3)

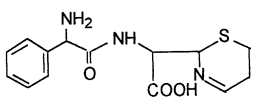


(Compound 4)



(Compound 5)

(pNB = p-nitrobenzyl)



(Compound 6)

Cephalosporins having a substituted 3-methyl group with a substituent which serves as a good leaving group give a polarographic peak at ~ -1.0 V (pH 2–4) owing to the reductive elimination of this group [8]. Clearly, cephalexin (1′), which has an unsubstituted 3-methyl group, does not give this peak, and cefaclor also is not reduced polarographically. Cephalexin, however, has been determined polarographically as a degradation product [9]. The present paper is concerned with the determination of cefaclor after preliminary degradation, and it is therefore pertinent here to compare the information available on the degradation of cefaclor and cephalexin, and to discuss the polarography of degraded cephalexin.

Another characteristic of cephalexin and cefaclor that influences their degradation particularly in neutral solution is that they have an α-aminobenzyl substituent at C-7. Cephalosporins with this substituent degrade in neutral solution by intramolecular aminolysis, which opens the β-lactam ring [10–12]. The initial diketopiperazine derivative that is formed in the case of cephalexin is compound 2′. The diketopiperazine moeity in this compound is not polarographically active, but the compound is reducible owing to the presence of the imine group in the thiazine ring ($E_p = -0.80$ V; pH 7) [8,13,14]. A product of more extreme degradation in neutral solution at 80°C ($E_p = -1.26$ V, pH 7) was used to determine cephalexin polarographically [9]. This compound has not been identified, but the polarographic peak was eliminated on adding sulphite (indicating that the reducible group is a carbonyl moiety) [9,14], and Martin [15] showed that it was a degradation product of the thiolactone (compound 8′). (Dinner [16] isolated compounds 4′ and 8′ from cephalexin solutions degraded at pH 3.3 for 90 min at 75°C). Degradation in alkaline solution and then at pH 5 gives compound 5′ [17]. In the presence of formaldehyde Barbhaiya et al. [18] identified the product as compound 6′. In this latter case, the methyl carbon comes from the formaldehyde molecule. Compound 6′ is formed much more readily than compound 5′ [19]. A method of determining cephalexin polarographically based on hydrolysis to compound 6′ was found by us to be insufficiently precise [9].

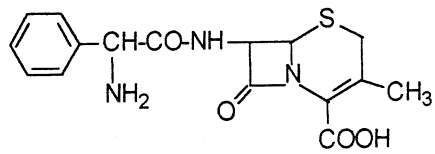
Degradation of ampicillin — the penicillin analogue of cephalexin — gives penicillamine and compound 5′ (incorrectly reported as compound 6′) directly in neutral solution at room temperature [20]. Hydrolysis of cephalosporins in sodium hydroxide solution has been shown spectrophotometrically [21–23] and with the use of a sulphide ion selective electrode [24] to give reproducible yields of sulphide and ammonia. The reproducible yield of sulphide, for example, was from ~ 15 to 65% depending on the cephalosporin. Penicillins give reproducible yields of ammonia [23], but no sulphide is produced as penicillamine is formed instead. An automated UV spectrophotometric method was developed for the determination of traces of cephalosporins in penicillin samples based on this formation of sulphide [22].

Li and Chen [25] have recently applied cathodic stripping voltammetry to the determination of cephalexin after degradation in 0.1 M sodium hydroxide solution at 100°C for 20 min. ($E_p = -0.80$ V, 0.1 M OH^{-1}) (detection limit 5×10^{-10}

M). They concluded that the product is 7′, which would be reducible by virtue of the imine group. (If this molecule were cleaved hydrolytically at the C=N bond it would give compound 4′ and the precursor to compound 8′).

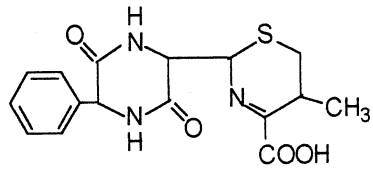
Cefaclor is an orally administered semi-synthetic cephalosporin with a potent antibacterial effect. It belongs to the group of β-lactam antibiotics widely used to treat infants [26]. The drug is excreted unchanged in urine (60–85%) within 24 h after dosages of 0.25–1 g daily. Its antibacterial activity is dependent on the presence of the β-lactam functionality that is hydrolyzed under aqueous conditions [27]. This hydrolytic instability leads to chemical degradation and the formation principally of a piperazine-2,5-dione derivative via intramolecular nucleophilic attack on the β-lactam moiety in neutral solution by the primary amine in the side-chain [28]. In 1996, Vilanova et al. [28] detected the initial hydrolysis product but it was too unstable to isolate: this initial product is the imine derivative which retains the chlorine atom (compound 2). This degrades readily, by means of a 1,3-trans-ring attack at C-3 of the S atom, to form an episulfonium ion (compound 3): the chlorine atom is eliminated in this process. The episulfonium ion rearranges to give a five-membered ring compound which they isolated and characterised (compound 4). This degradation mechanism does not appear to be followed in solutions of higher pH (e.g. pH 10.5), but the chlorine atom is, nevertheless, eliminated [28]. In 1977, Indelicato et al. [29] had expected the chlorine atom to remain when the *p*-nitrobenzyl derivative was degraded in DMSO, but found that it was eliminated. They suggested a structure for the degradation product, viz. the enamine in which the chlorine is replaced by hydrogen (compound 5).

The present paper is concerned with the polarographic and stripping voltammetric determination of cefaclor after alkaline degradation.



1'

Cephalexin



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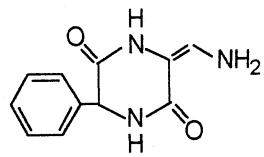

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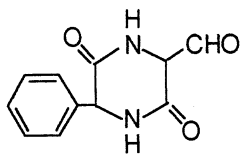

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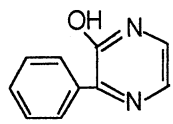

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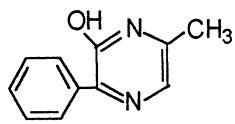

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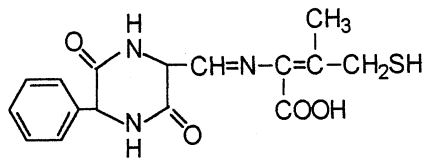

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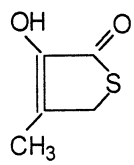

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2. Experimental

2.1. Apparatus

For voltammetric measurements, an EG&G PARC 264A Polarographic Analyzer/Stripping Voltammeter was used with a Houston Instruments Model 2000 x–y recorder. An EG&G PARC 303A stand was used in the DME (for differential pulse polarography) and HMDE (for stripping voltammetry) modes. The three electrode system was completed by means of a glassy carbon auxiliary electrode and an Ag/AgCl (3 M KCl) reference electrode. All potentials given are relative to this electrode.

A model GBC 911A Ultraviolet–visible Spectrophotometer (Brazil) was used for recording absorption spectra.

An ultra thermostatic bath (model Pt-100, NOVA TÉCNICA, Brazil) was used to control the temperature during the hydrolysis steps.

2.2. Reagents

A sample of cefaclor was kindly provided by Eli Lilly (Brazil) and was used as received. All other chemicals were of Suprapur grade. Stock solutions (1×10^{-3} mol l^{-1}) of cefaclor were prepared daily by dissolution of the solid substance in demineralized water from a Milli-Q system (Millipore, Bedford, MA). The supporting electrolyte used was Britton–Robinson (B–R) buffer solution prepared by mixing a solution 0.04 mol l^{-1} in orthophosphoric acid, 0.04 mol l^{-1} in acetic acid and 0.04 mol l^{-1} in boric acid with appropriate volumes of 0.2 mol l^{-1} sodium hydroxide solution.

2.3. Procedures

The general procedure adopted for obtaining polarographic curves was as follows. A 10 ml aliquot of an appropriate buffer solution was placed in the voltammetric cell and the solution was purged with nitrogen for 10 min. Then, the required aliquot of the stock solution of the cefaclor, or of the hydrolysed solution, was added by means of a micropipette and the voltammetric curve was recorded after 2 min purging. (The predeaeration of the buffer allowed a shorter purging time to be used after adding the hydrolysis solution, and thus minimising any further hydrolysis effect).

For obtaining cathodic stripping voltammograms, the accumulation potential was applied to the working electrode while the solution was stirred continuously. After accumulation, a negative-going linear potential scan was initiated and the resulting voltammograms were recorded at a scan rate of 100 mVs^{-1}.

Hydrolysis reactions were carried out by placing 15 ml of cefaclor solution at an appropriate concentration in a B–R buffer in the thermostatic bath. After heating for the required time, the solution was cooled in an ice bath and an aliquot was removed by micropipette and diluted with 10 ml of B–R buffer solution in a thermostatted cell at 25°C. In carrying out analyses of urine samples spiked with cefaclor, the hydrolysis reaction was carried out by heating the solution directly in the cell.

3. Results and discussion

3.1. Differential pulse polarography

Differential pulse polarography was used to follow the degradation of cefaclor in B–R buffer in the pH range 2–11. Polarography was carried out at the same pH as the hydrolysis. At pH 6 cefaclor gave two small peaks at ~ -0.5 and -1.0 V after the prepared solution had been kept for 2 h at room temperature (see curve 2 of Fig. 1). However, a large peak appeared at -0.5 V (peak A) after 30 min of heating at 50°C (curve 3, Fig. 1), showing that this initial hydrolysis product of cefaclor is reduced electrochemically. The size of peak A and that at -1.0 (peak B) are affected by the pH and the temperature at which degradation is carried out, and by the heating time. The effect of increasing the pH of the solution being degraded up to pH 11 on the peak current corresponding to the first reduction step (peak A) was investigated using a heating time of 60 min at 25 and 50°C. The results are shown in Fig. 2. When heating at 50°C, the maximum peak

height occurred for degradation at pH 10. At 25°C, no appreciable formation of the compound responsible for this peak occurs until pH 11 is reached. The second reduction peak at −1.1 V (pH 10) (peak B) also increases on heating but it is always smaller than the first peak.

The effect of the degradation temperature on the height of the dp polarographic peaks at −0.7 V (peak A) and −1.1 V (peak B) for 1×10^{-3} mol l^{-1} cefaclor in B–R buffer pH 10, after heating for 30 min, is shown in Fig. 3. The height of the first peak increased when the temperature was increased from 30 to 50°C, but decreased above this temperature. An increase in temperature above 50°C produced a marked increase in the height of peak B, which may indicate that the compound responsible is produced by further degradation of the compound responsible for peak A.

The effect of the length of time of heating on the heights of the peaks obtained after hydrolysis of cefaclor in B–R buffer at pH 10 and 50°C is shown in Fig. 4. The height of peak A increased for heating times up to 60 min. After that time, peak A became smaller, whereas the height of peak B continued to increase. These results suggest that the compound responsible for peak A is an intermediate which is relatively stable at $T <$ 60°C, heating times lower than 60 min, and pH < 11. Under more extreme conditions, it degraded further.

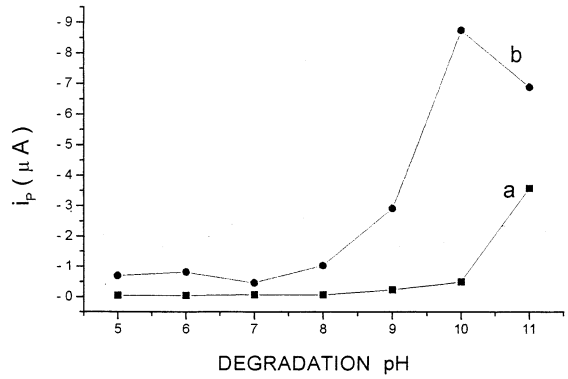

Fig. 2. Effect of pH on the first reduction peak current of 1×10^{-3} mol l^{-1} of cefaclor after 60 min in B–R buffer at 25°C (Curve a) and 50°C (Curve b). The solutions were polarographed at the pH at which hydrolysis had been carried out.

For a 1×10^{-3} mol l^{-1} cefaclor solution, the potential of peak A was found to vary rectilinearly with pH in the range 6–11, following the equation: E_p (mV) = −670 + 137 pH, $r = 0.998$ ($\eta = 7$). The width of peak A obtained over the whole pH range was constant with $w_{1/2} = 48$ mV,

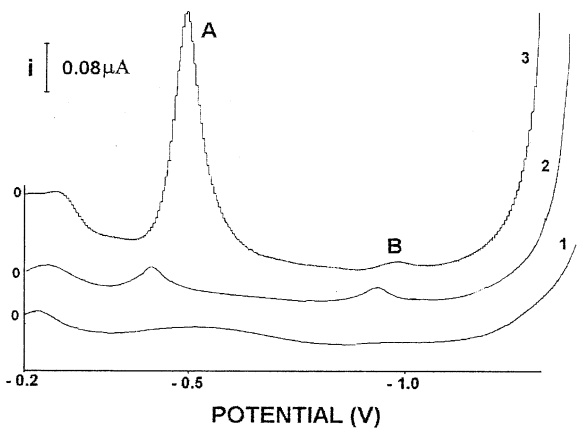

Fig. 1. Differential pulse polarograms of 1×10^{-3} mol l^{-1} cefaclor in B–R buffer pH 6.0: (Curve 1) Supporting electrolyte (B–R buffer pH 6.0); (Curve 2) solution of 1×10^{-3} mol l^{-1} cefaclor after 2 h hydrolysis at pH 6.0 at room temperature and (Curve 3) solution of 1×10^{-3} mol l^{-1} cefaclor after 2 h hydrolysis at pH 6.0 at 50°C.

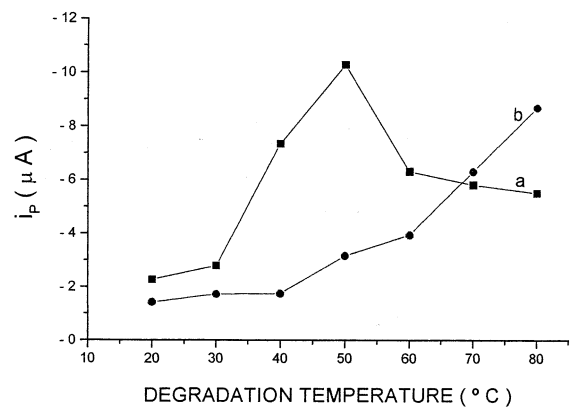

Fig. 3. Effect of degradation temperature on peak currents (1×10^{-3} mol l^{-1} cefaclor) after 60 min of heating in B–R buffer pH 10. (Curve a) peak at −0.70 V and (Curve b) peak at −1.1 V.

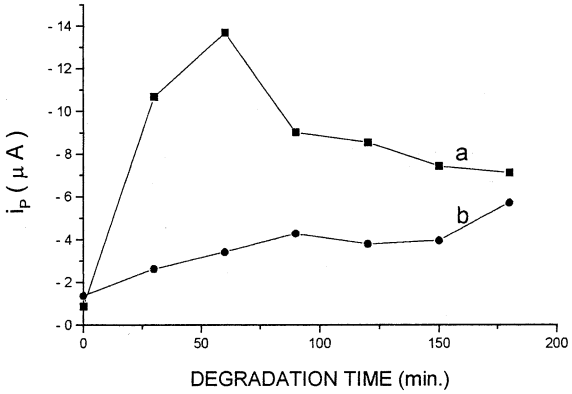

Fig. 4. Effect of degradation time on peak currents (1×10^{-3} mol l^{-1} cefaclor) in B–R buffer pH 10 at 50°C. (Curve a) peak at -0.70 V and (Curve b) peak at -1.1 V.

suggesting that the reduction process is close to that predicted for a reversible process involving two electrons [30]. The pH dependence with a slope of 137 mV per pH unit suggests that one (1.16) proton is consumed in the electrode reaction.

Cefaclor can be determined by means of its initial degradation product, which produces a well-defined polarographic peak at -0.70 V on being produced and polarographed in B–R buffer pH 10. Using a 60 min heating time at $T = 50$°C, calibration graphs were obtained for cefaclor in the concentration range 3.86–38.6 μg ml^{-1} (equation: i_p (nA) $= 38.3 + 26.9\ C$ ($C =$ μg ml^{-1}), $r = 0.996$ ($\eta = 7$). At 3.86 μg ml^{-1}, the relative standard deviation was 3.5% ($\eta = 3$).

The direct determination of cefaclor in human urine can be made using the degradative heating step prior to the polarographic measurements. The degradation step probably also denatures the proteins that can interfere in voltammetry. Urine aliquots of 1 ml spiked to a concentration of 38.6 μg ml^{-1} in cefaclor, diluted in 9 ml of B–R buffer pH 10 and heated for 60 min at 50°C, after cooling to 25°C gave a well-defined peak at $E_p = -0.70$ V. Quantification of the drug content of the urine was accomplished by nine standard additions of cefaclor 3.86 μg ml^{-1}, as shown Fig. 5. A recovery of 93.5% ($\eta = 3$) was obtained. The limit of determination obtained using the proposed method was 0.386 μg ml^{-1} ($\eta = 3$) and detection limit obtained using the proposed method was calculated to be 0.154 μg ml^{-1} ($\eta = 3$). The limit of determination was the smallest concentration that gave an good signal. The detection limit was calculated as three times the standard deviation of a single current measurement divided by the slope of the regression line.

3.2. Cathodic stripping voltammetry

If a determinant adsorbs on mercury, it is logical to complement the polarographic method with a stripping voltammetric method in order to increase sensitivity and to lower the detection limit. The compound responsible for the peak at -0.70 V was found to adsorb on a hanging mercury drop electrode, and so a cathodic stripping voltammetric method was developed. Optimum hydrolysis and measurement conditions were found to be the same as for polarography. Typical linear scan cathodic stripping voltammograms of a 1×10^{-6} mol l^{-1} solution of the hydrolysed product of cefaclor produced in B–R buffer pH 10, with 30 min of heating at 50°C after 0 and 180 s accumulation at -0.20 V are shown in Fig. 6.

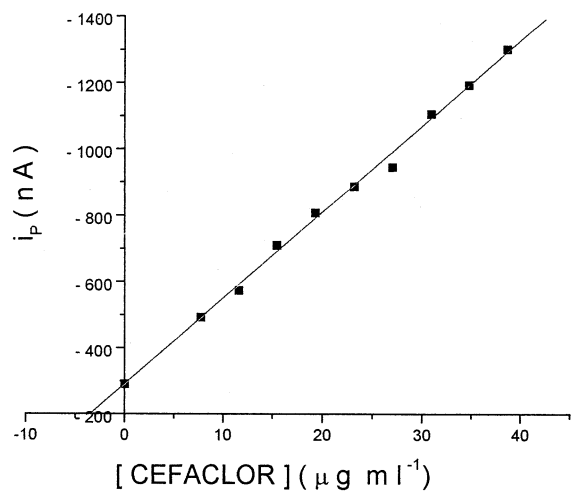

Fig. 5. Analytical plot obtained by the standard addition method for the determination of cefaclor in human urine as its hydrolysis product. Hydrolysis reaction conditions: temperature 50°C, hydrolysis time 60 min, in B–R buffer pH 10.

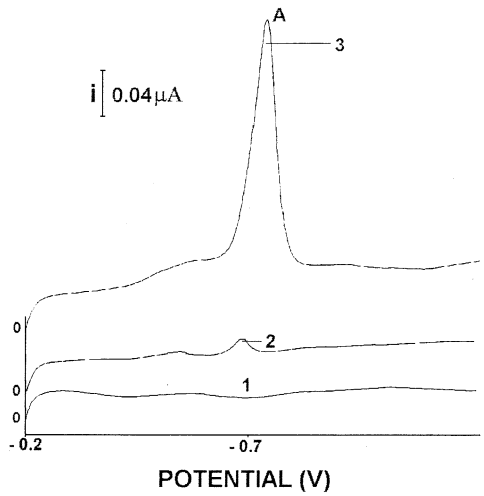

Fig. 6. Linear sweep cathodic stripping voltammograms of 1×10^{-6} mol l^{-1} cefaclor in B–R buffer pH 10. Accumulation potential -0.20 V, scan rate 100 mV s^{-1}. (Curve 2) Accumulation time 0 s; (Curve 3) Accumulation time 180 s and (Curve 1) Supporting electrolyte ($t_{acc} = 180$ s).

A single well-defined peak at -0.67 V (A), corresponding to the reduction of the alkaline degradation product, can be seen. Peak A obtained without accumulation is much smaller, indicating the adsorptive nature of the process. Peak B which was observed polarographically was not observed in the stripping experiments. Comparison of the voltammetric response of cefaclor in these conditions over a period of time have shown that the product generated is stable for at least 2 h. The effect of pH on the differential pulse adsorptive stripping voltammograms of cefaclor was investigated from pH 5 to 11. Maximum signal was observed at pH 10, which was therefore chosen for the adsorptive stripping voltammetric determination.

The influence of accumulation potential on the stripping current at potentials from $+0.20$ to -0.20 V is shown in Fig. 7. An accumulation potential of -0.20 V was chosen as satisfactory. Drop size 0.4 mm^2, stirring speed 1500 rev min^{-1}, rest time 15 s and scan rate 100 mV s^{-1} were chosen as giving the optimum experimental conditions.

The effect of accumulation time for 1×10^{-6} mol l^{-1} cefaclor previously hydrolysed was investigated from 0 to 240 s, as shown in Fig. 8. A rectilinear relationship is observed in all accumulation time range, following the equation: i_p (nA) $= 55.9 + 1.95\ t$, $r = 0.992$ ($n = 8$). Above these times, the peak current is constant suggesting electrode surface saturation.

The peak height was linearly dependent on the cefaclor concentration. Linear calibration graphs were obtained from voltammograms recorded at accumulation times of 30 s from 0.386 to 3.86 μg ml^{-1} cefaclor, following the equation: i_p (nA) $= -70.1 + 275 \times C$ (C = concentration of cefaclor in μg ml^{-1}). A determination limit of 0.0386 μg ml^{-1} was obtained using 60 s accumulation. The reproducibility of the method was determined by successive measurements of seven solutions of 1×10^{-6} mol l^{-1} cefaclor in B–R pH 10 submitted to previous heating for 30 min at 50°C. Relative standard deviations of 4.2% were obtained with a preconcentration time of 30 s.

The determination of cefaclor in urine was possible without prior extraction. A urine sample (1 ml, spiked with 0.386 μg of cefaclor) in 9 ml of B–R buffer pH 10, gave the voltammogram shown in Fig. 9 (curve 2). The respective curves obtained after standard additions of 1×10^{-7} mol l^{-1} of cefaclor solution are shown in Fig. 9

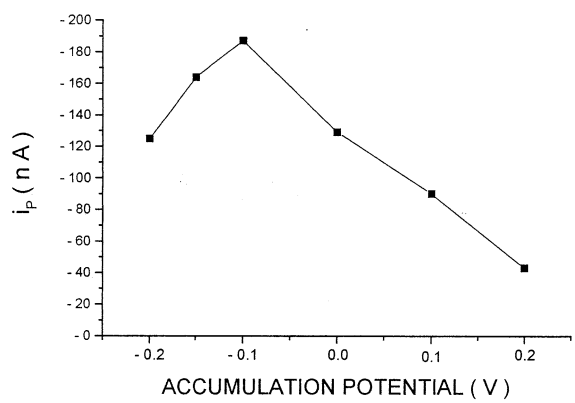

Fig. 7. Effect of accumulation potential on peak current for 2×10^{-6} mol l^{-1} cefaclor in B–R buffer pH 10 hydrolysed for 60 min at 50°C; $t_{acc} = 30$ s and $v = 100$ mV s^{-1}.

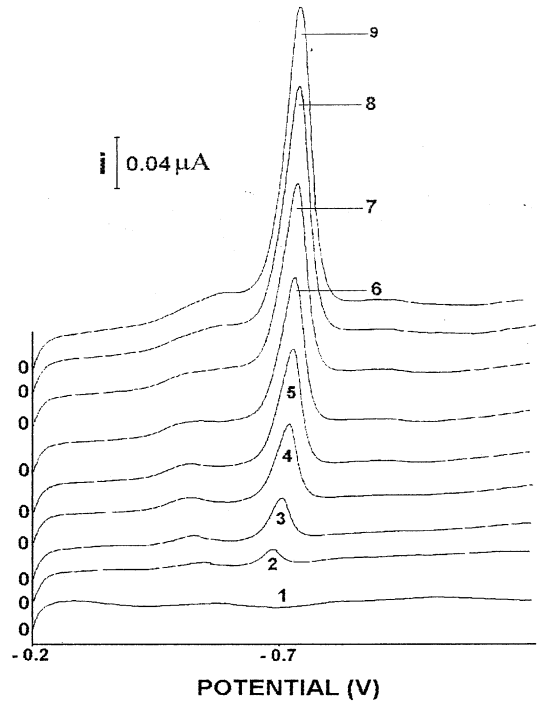

Fig. 8. Linear sweep cathodic stripping voltammograms of 1×10^{-6} mol l^{-1} cefaclor hydrolysed for 60 min at 50°C; $E_{acc} = -0.20$ V. (Curve 1) Supporting electrolyte; Accumulation time: curve 2, 0 s; curve 3), 10 s; curve 4, 30 s; curve 5, 60 s; curve 6, 90 s; curve 7, 120 s; curve 8, 180s; and curve 9, 240 s.

(curves 3–9). A mean recovery of 98.6% was obtained and a limit of detection of 2.94 ng ml^{-1} was calculated for the determination of cefaclor in the (1 + 9 v/v) diluted urine. The limit of detection was calculated as above.

3.3. Nature of the degradation product

The degradation product upon which the above polarographic and cathodic stripping voltammetric methods for the determination of cefaclor are based cannot be identified conclusively from the results obtained so far. The alkaline conditions used here are relatively mild in terms of hydroxide concentration and temperature. It is possible that they are sufficiently mild for the mechanism of Vilanova et al. [27] to be operative, in which case the degradation product might be one of compounds 2–4. If the alkalinity is too high for intramolecular aminolysis to occur and for the chlorine atom to be eliminated by another (hydrolytic) mechanism, then the product might be compound 6. Compounds 2, 3 and 6 (and probably 4) might be expected to be reducible by means of the C=N bond. Martin [15] has shown that under slightly more aggressive alkaline degradation conditions (0.025 M OH^{-1} at room temperature) many cephalosporins (whether with the C-7 α-aminobenzyl substituent or not) give a peak at −0.39V (pH 2), and that as this peak decreases in size another peak appears at −0.50 V (pH 2). Further work is now in progress to decide under which conditions these various peaks are obtained and to identify the compounds responsible for producing them.

UV spectroscopy of the solutions of cefaclor before and after degradation in this study seem to indicate that a diketopiperazine compound is be

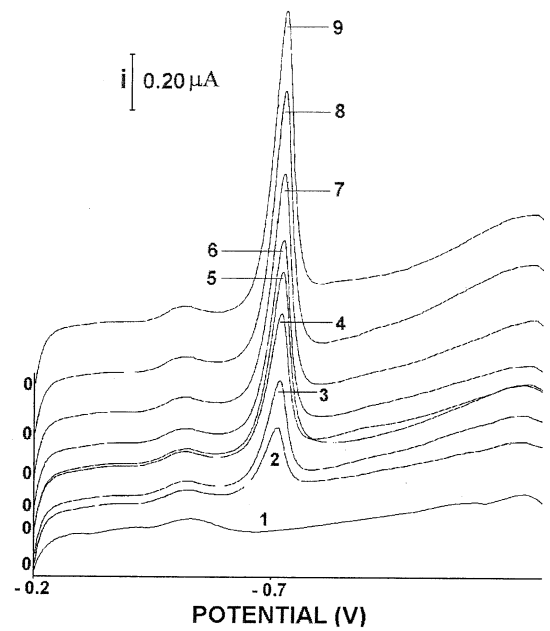

Fig. 9. Cathodic stripping voltammograms of human urine 1 ml containing 0.386 μg of cefaclor in 9 ml of B–R buffer pH 10 and submitted to 60 min of heating at 50°C (Curve 2). $t_{acc} = 30$ s; $E_{acc} = -0.20$ V. Supporting electrolyte (Curve 1); Successive additions of 1×10^{-7} mol l^{-1} cefaclor (Curves 3–9) in the final solution in the cell.

ing formed. Before degradation, two absorption maxima are observed at 214 nm (band A) and 264 nm (band B) which can be attributed to –C=C–COO– and O=C–N–C=C– groups [25] respectively. After degradation band B is absent, presumably as a result of cleavage of the –C–N– bond in the four membered β-lactam ring. A new absorption band (C), apparently due to the diketopiperazine group, appears at 340 nm, as had been observed previously for cephalexin [25].

A further polarographic study was made to try to decide whether the compound responsible for the analytical peak used here was a diketopiperazine compound. Polarographic studies were made of cefaclor solutions in B–R buffer pH 10 using acetic anhydride as a protecting group for the amine moiety. Differential pulse polarograms obtained for cefaclor that had been hydrolysed for 30 min at $T = 50°C$ and then submitted to reaction with acetic anhydride, showed the same polarographic behaviour as that observed without the protection. Polarograms obtained for cefaclor which had been protected previously with acetic anhydride in B–R buffer pH 10, and then subjected to hydrolysis and polarography did not give a reduction peak. The derivatisation of the α-aminobenzyl group of cephaclor would prevent the intramolecular degradation reaction and the formation of any diketopiperazine derivative. This seems to indicate clearly that the peak is due to compound 2, but further work is required to confirm this.

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