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Electrochemical study of cefetamet–Na and its polarographic determination

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Abstract

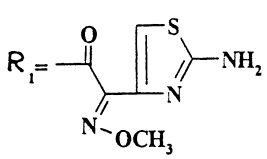
Polarographic behavior of cephalosporin cefetamet–Na (Cef–Na) in aqueous solutions of pH ranging from 1.7 to 12.5 was investigated by applying direct current (dc) polarography, differential pulse polarography (dpp), alternating current (ac) polarography, cyclic voltammetry and electrolysis at constant potential. The characteristics of the corresponding electrode reaction are presented and discussed. The electrode reaction was found to be affected by strong adsorption, strongly and slightly pronounced in acidic and alkaline media, respectively. The methoxyimino group electroreduction was carried out and the mechanistic scheme was suggested. In addition, a sensitive dpp method was proposed for analytical determination of a very low concentrations of Cef–Na. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Cefetamet–Na electrode reduction; Mechanistic scheme; Dpp determination

1. Introduction

Cephalosporins are semisynthetic antibiotics of the β-lactam family and are thus closely related in structure to penicillins. Because of their antibacterial activity, β-lactamase resistance and pharmacokinetic properties, the cephalosporin antibiotics have gained importance in pharmaceutical research over the last two decades and are now widely used in clinical practice for the treatment of severe infections [1].

The basic structure of cephalosporins shows the cephem nucleus (Fig. 1(a)), and side chains at positions 3 and 7 that determine their properties and bioactivity [2]. The compound depicted in Fig. 1(b) with $R_3 = R_2 = H$, and



represents cefetamet–Na (Cef–Na), or chemically [6R-[(6α,7β(z))]] - 7 - [[(2 - amino - 4 - thiazolyl)(methoxyimino)acetyl]amino] - 3 - methyl - 8 - oxo - 5 - thia - 1 - azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid–Na salt.

Most cephalosporins are electroactive and give a faradaic response at an electrode, mercury or solid, immersed in their solution.

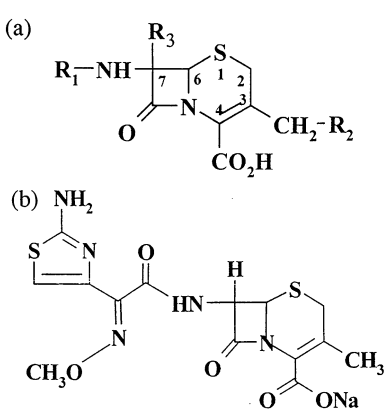


Fig. 1. (a) The basic cephem nucleus structure. (b) Molecular structure of Cef–Na.

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The majority have been reported to be electroreducible and the common electrode reaction has been shown to be two-electron reduction of the Δ3 double bond of the cephem nucleus. Since the C=C bond is very difficult to reduce, its polarographic signal occurs at relatively high negative potentials (at −1.0 V versus Ag/AgCl or SCE electrode), in acidic media pH 2–4, and is dependent upon the presence and nature of the substituent at position 3 (R_2 in Fig. 1(a)). Ochiai et al. [3,4] were the first to present evidence for electroreduction of the cephem double bond, proving the cleavage of the substituent at position 3. They found that all derivatives with a substituent R_2 different from H gave 3-methylenecepham derivatives as a result of cathodic reduction. These findings suggest the inability of the cephem ring to undergo cathodic reduction if $R_2 = H$. The authors proposed a reduction mechanism involving a double one-electron transfer step via a very unstable intermediate radical anion, and splitting off of the R_2 group to obtain an intermediate anion with final solvent protonation of its C4 carbon to form the 3-methylencepham derivative. Hall et al. [5,6] suggested a reduction mechanism of cephalothin that differed in part from that shown by Ochiai et al. The authors also found that this reduction was totally irreversible and influenced by adsorption phenomena.

Many cephalosporins contain additional or possibly other reducible groups, incorporated into side chains at positions 3 and 7, and usually give rise to a less cathodic reduction signal compared with that of the Δ3 double-bond reduction. In the first related paper by Sengun et al. [7] the differential pulse polarography (dpp) behavior of ceftriaxone, cefuroxime, cefotaxime and ceftriaxime was described. All examined cephalosporins contained the methoxyimino group of the side chain at position 7, and the first three also contained reducible groups at position 3 of the cephem nucleus.

The first three cephalosporins gave rise to two peaks, the more negative one being ascribed to the known reductive cleavage of substituent at position 3, and the ceftizoxime, with no side chain at position 3, exhibited only a single peak. The polarographic peaks observed at ca. −0.4 to −0.5 V for all examined cephalosporins were probably due to the methoxyimino group at position 7. Ogorevc et al. [8,9] studied the reduction of ceftriaxone, cefuroxime and cefotaxime, which all contain methoxyimino group at position 7, and suggested a reduction mechanism. A mechanism was proposed that involved a double two-electron reduction of the methoxyimino group, via a hydroxylamine intermediate, to the corresponding amine and methanol.

Bernacca et al. [10] studied the polarographic behavior of the cefuroxime and suggested a reduction mechanism, but only in acidic media. Even the cefuroxime contains both the 3–4 double bond of the cephem nucleus and the methoxyimino group, so the authors focused on the mechanism of reduction of the methoxyimino group. The results of potential controlled electrolysis carried out at limiting current potentials involved four electrons in the whole electroreduction. The products of electrode reaction were found to be methanol and aminic derivative of the cephalosporin. Investigations were limited to acidic media; no investigations were performed in alkaline and neutral solutions.

The present article is dealing with polarographic and voltammetric investigation of electroreduction of Cef (Fig. 1(b)), which is a microbiologically active part of an orally absorbed prodrug ester–Cef pivoxil. Cef is classified as a third-generation cephalosporin according to its antimicrobial activity.

No literature data were found on the electrochemical behavior of Cef, either in acidic or in neutral and slightly alkaline media. Therefore, the aim of this work was to elucidate the electrochemical behavior of Cef, the mechanism of reduction of the methoxyimino group, by using direct current (dc) polarography, differential pulse polarography (dpp) and alternating current (ac) polarography, and also cyclic voltammetry and electrolysis at constant potential. The studies were carried out in the entire pH range, from acidic to alkaline medium.

Based on the extensive electrochemical study of Cef, the optimum conditions were established for its polarographic determination.

2. Experimental

2.1. Apparatus

Polarographic measurements (dc and dp polarograms) were performed with a PAR 174 polarographic analyzer (Princeton Applied Research Corp.). Three-electrode operation was employed: a dropping mercury electrode was used as the working electrode, while a platinum spiral-shaped wire and a saturated calomel electrode served as auxiliary and reference electrodes, respectively. The dc polarographic measurements were performed under the following conditions: the drop time of 2 s was kept constant by shearing off the drop with an automatic hammer, scan rate was 5 mV s^{-1}, and the mercury column height was 80 cm. A modulation amplitude of 50 or 100 mV, and scan rate of 2 mV s^{-1} were used when dpp was performed.

A PAR model 173 potentiostat with PAR model 176 current to voltage converter was used for coulometry. The coulometric cell consisted of a 40 mm (diameter) × 45 mm Pyrex weighing bottle fitted with a rubber stopper containing the appropriate cell connections. The working electrode was a 12.7 cm^2 stirred mercury pool. A platinum wire counter electrode was

separated from the working electrode by a medium porosity sintered-glass disc. The saturated calomel electrode was used as a reference electrode.

Cyclic voltammograms were recorded with a PAR Model 164A polarographic analyzer, coupled with a PAR 303A static mercury drop electrode (SMDE) (drop size: medium; area of the drop 0.017 cm^2). The polarographic cell (PAR model K 0060) was fitted with an Ag/AgCl saturated KCl reference electrode, and platinum wire was connected to the 303 SMDE. The capillary of the mercury electrode had a bore diameter of 0.016 cm and a length of 12.7 cm.

Ac polarographic measurements were performed with a PAR M-372 ac analyzer and PAR-M-124 lock-in amplifier with PAR 303 static or dropping ($t = 2$ s) mercury electrode. The ac polarographic measurements were performed with a scan rate of 5 mV s^{-1}. The frequency of the ac voltage was 40 Hz and the amplitude was 5 mV. The phase angle was 90°.

A Radiometer PHM 62 pH-meter with appropriate standard buffer solutions was used.

2.2. Reagents

All investigations were carried out with Cef–Na (standard) produced by Hoffmann La Roche (Basel, Switzerland). Boric, orthophosphoric and acetic acids were p.a. (E. Merck). NaOH, KNO_3 and KCl were p.a. All other reagents and chemicals used in this study were of analytical grade.

2.3. Solutions

Stock solutions of Cef–Na were prepared daily, dissolving a suitable amount of Cef in bidistilled water. Britton Robinson (BR) [11] universal buffer was prepared from stock buffer solutions of 0.04 M boric, orthophosphoric and acetic acids with the appropriate volumes of 0.2 M NaOH. The ionic strength was maintained constant by adding KNO_3 or KCl.

2.4. Procedure

BR buffer (10.50 ml) and KNO_3 (2 M, 1.5 ml) (for dc polarography and dpp) or 7 ml of BR buffer, 1 ml KNO_3 (for ac polarography and cyclic voltammetry) were transferred into a polarographic cell and purged with nitrogen. After addition of 3 ml Cef 1×10^{-3} M standard drug solution, the final concentration of Cef was 2×10^{-4} M and the total ionic strength 0.2 M; the whole solution was purged with nitrogen for another 60 s before the corresponding polarogram or voltammogram was recorded.

In the coulometric determination of the number of electrons exchanged, 19 ml of the supporting electrolyte was measured into the coulometric vessel and purged with nitrogen, with simultaneous initiation of pre-electrolysis at the selected constant potential. After about 120 min, when the residual current value decreased to below 10% of the initial current and no longer changed, the circuit parameters were adjusted for automatic residual current compensation. Then, 1 ml of a molar Cef–Na solution prebubbled with nitrogen was added and the reduction was carried out at a constant potential with constant stirring and bubbling with nitrogen. Completion of the reduction was indicated by a decrease in the electrolytic current to the residual value (after 180–200 min) and the charge passed was found by electronic integration.

3. Results

3.1. Direct current polarographic behavior of Cef

The polarographic reduction of Cef–Na at the drooping mercury electrode was investigated in the pH range 1.7–12.5, as shown in Fig. 2. As seen from Fig. 2, only one wave is present in acidic medium, while in neutral and alkaline media it splits into two waves: the first one is probably an adsorption prewave, and the second one is the main wave. At pH greater than 10.5 only the first wave is obtained, and the second one probably merges with the hydrogen discharge.

The shape and the position of the waves are strongly pH dependent, indicating that protonation of the reactive part of the molecule is involved in the overall electrode reaction mechanism. An S-shaped curve for half-wave potential versus pH was obtained (Fig. 3(a)). The $E_{1/2}$ of the main wave shifts toward more negative potential, and four regions of linearity are evident: in the range pH (1.7–3.1) the slope is 56 mV pH^{-1}; in the pH range (3.1–5.5) it is 90 mV pH^{-1}, while at $5.5 < pH < 7.5$ this slope increases to 250 mV pH^{-1}, and at $pH > 7.5$ the slope is again 56 mV pH^{-1}.

The very complex dependence of the limiting current of both waves, as well as the height of each wave, are presented in Fig. 3(b).

Effects of temperature and mercury column height show that the limiting current of the main wave is diffusion controlled. The limiting current was proportional to the square root of the corrected mercury column height, and it had a temperature coefficient [12] of 1.52% per degree (in acidic medium) to 1.81% per degree (in alkaline medium).

Fig. 4 shows plots of $\log i/i_d - i$ versus E at different pH values in the pH region from 2.0 to 11.0. The plots obtained are nonlinear, except at pH 2.0, 4.0 and 11.0 where only one wave is present, so the plots are linear. Polarographic waves are irreversible, and the slope of this semilogarithmic plot is $\alpha nF/RT$, where α denotes the experimental value of the cathodic transfer coeffi-

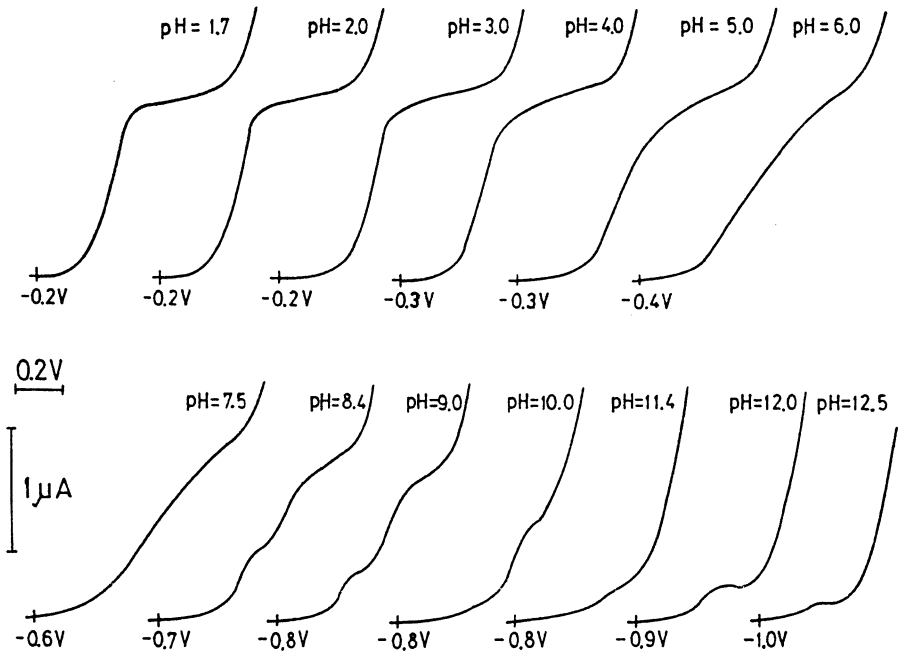


Fig. 2. Dc curves of Cef–Na at different pH values ($c_{Cef} = 2 \times 10^{-4}$ M, BR buffer with constant ionic strength of 0.2 M KNO_3).

cient. The value of αn changes from 0.60 at pH 2.0 and from 4.0 to 0.97 when the pH reaches 11.0.

3.2. Controlled potential coulometry

Analytical data were performed using a cell with separated anode and cathode compartments. Potential controlled electrolysis was carried out at the limiting current potentials at three different pH values. In the acidic media at pH 2.0 ($E = -0.700$ V), and in the alkaline medium at pH 8.4 ($E = -1.400$ V) it was found that four electrons are involved in the whole electroreduction at each pH investigated (Fig. 5(a)). On the contrary, when the coulometry was performed at pH 8.4, but at the limiting current potential of the first polarographic wave ($E = -1.135$ V), as well as at pH 12.0 ($E = -1.300$ V), it was found that no electrons are exchanged under these conditions. When the logarithm of the current was investigated as a function of the electrolysis time, under the circumstances when four electrons are involved in the reduction process, a plot with two linear regions was obtained, indicating a two-step process (Fig. 5(b)).

3.3. Cyclic voltammetry

Cef–Na shows well-defined CV reduction peaks. Fig. 6 shows the change in the position and shape of the cyclovoltammetric peaks obtained at three selected pH values (a, pH 2.0; b, 12.0; and c, 8.4, respectively). In the study of the reversibility of the system no anodic peak was observed at polarization rates of 5–1000 mV s^{-1}. From the measured effect of the polarization rate on the height and position of the cathodic peak at pH

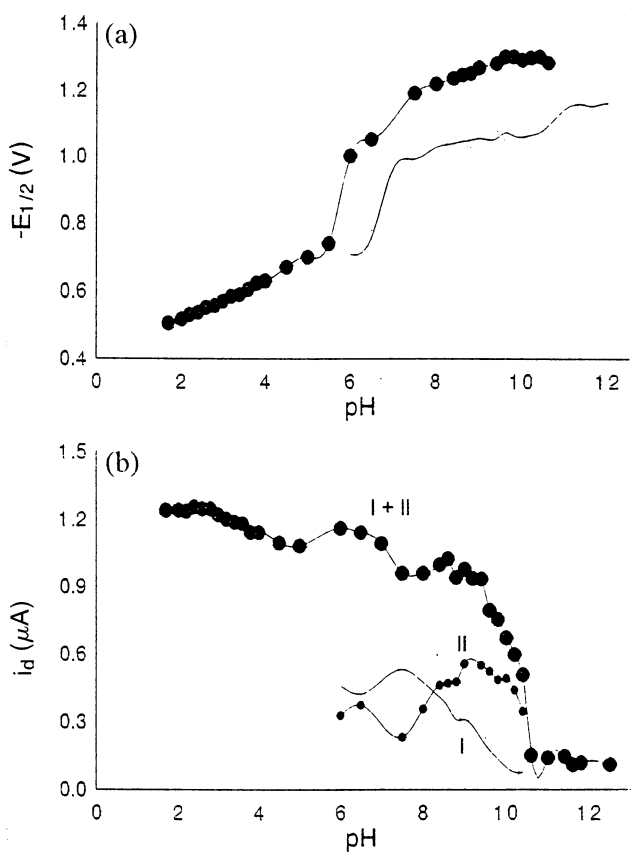


Fig. 3. (a) $E_{1/2}$–pH dependence. (b) i_d–pH dependence. ●, The main wave; ○, the prewave (c_{Cef} 2×10^{-4} M BR buffer).

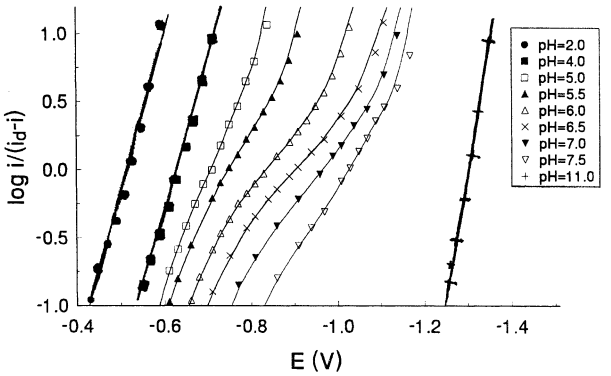


Fig. 4. Plots of $i/i_d - i$ vs. E at different pH values (c_{Cef} 2×10^{-4} M BR buffer).

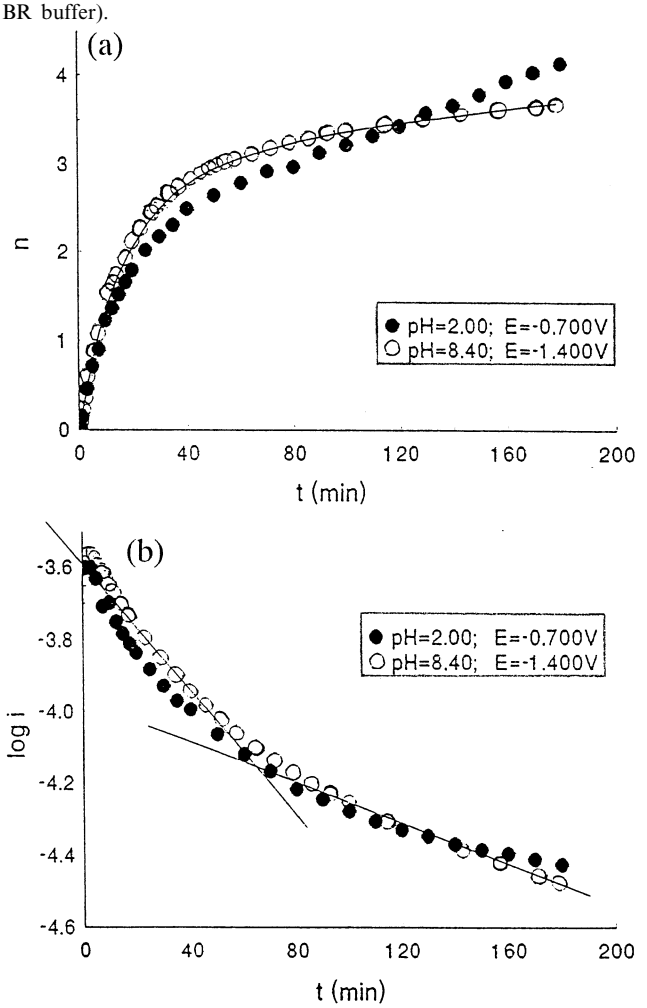


Fig. 5. (a) Dependence of the number of electrons involved in the electrode process vs. time of electrolysis. (b) Logarithm of the current as a function of the electrolysis time ($c_{Cef} = 5 \times 10^{-5}$ M, $I = 0.2$ M). ●, pH 2.0; $E = -0.7$V. ○, pH 8.4; $E = -1.40$ V.

2.0 and for the second peak at pH 8.4 it follows that the peak height increases with the square root of the polarization rate. However, this dependence shows two regions of linearity with different slopes depending on the polarization rate applied. The plot of $i_p/v^{1/2} = f(v)$ presented in Fig. 7 of the cathodic peak at pH 2.0 and for the second peak at pH 8.40, again shows two linear parts. Extrapolating them to the zero polarization rate ($v = 0$), the obtained values for $i_p/v^{1/2}$ were 1:2. On the other hand the first peak at pH 8.4, and the cyclovoltammetric peak obtained at pH 12.0 show unusual behavior of peak current with the square root of the

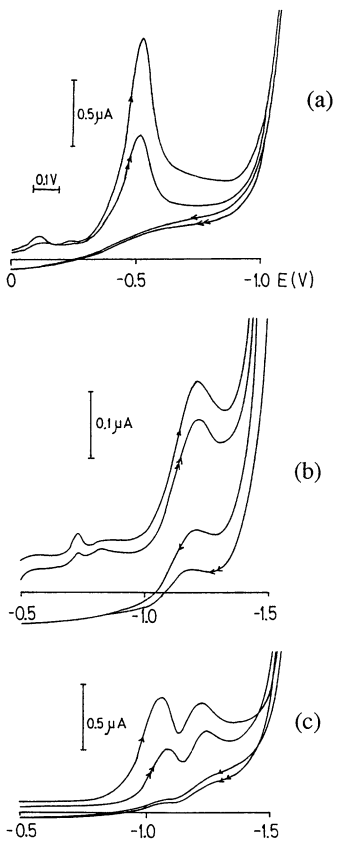


Fig. 6. Cyclovoltammetric curves of 1×10^{-4} M Cef–Na in BR buffer: (a) pH 2.0; (b) pH 12.0; (c) pH 8.4.

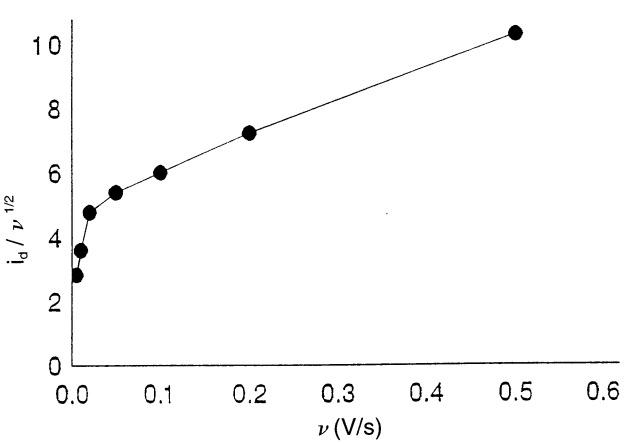


Fig. 7. $i_p/v^{1/2}$ as a function of scan rate v; 1×10^{-4} M Cef–Na in BR buffer pH 2.0.

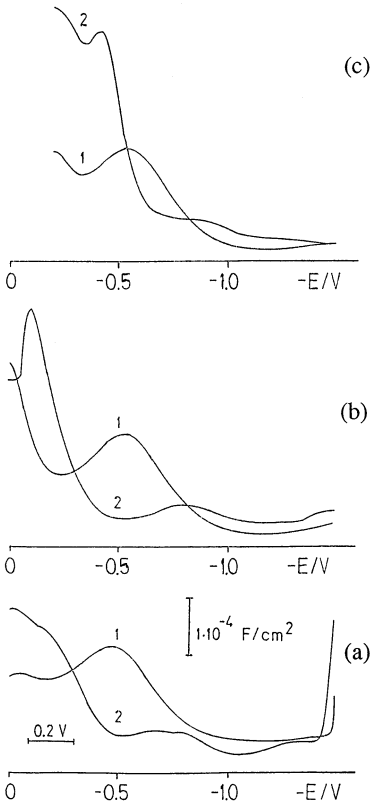


Fig. 8. Ac curves of 1×10^{-4} M Cef–Na in BR buffer: (a) pH 2.0; (b) pH 8.4; (c) pH 12.0 (curve 1: supporting electrolyte; curve 2: 1×10^{-4} M Cef–Na in supporting electrolyte).

polarization rate: for the rates smaller that 100 mV s^{-1} it slightly increases, and then it becomes independent of the polarization rate.

3.4. Alternating current polarography

Adsorption of the studied cephalosporin on the mercury electrode was also confirmed by ac polarography. The C–E curves of 1×10^{-4} M Cef where predominantly cationic, zwitterionic and anionic forms exist in the solution [13] are presented in Fig. 8(a,b and c), respectively. The shape of C–E curves obtained at selected pH values at which these ionic forms exist indicates a decrease in the double-layer capacitance with respect to the capacitance of the supporting electrolyte, as a result of Cef adsorption at the electrode surface. As seen, the adsorption of the zwitterionic form is very similar to those obtained at pH 2, but with moderately pronounced decreased electrode capacitance. A considerably weaker adsorption of Cef anionic form is noticed and this finding is in a good agreement with our previous results related to the adsorption of other cephalosporins [14].

4. Discussion

Investigating the nature of the Cef reduction process at different pH values clearly shows that two waves are present only when Cef is present as a zwitterion and anion. Under those conditions a wave occurring at more positive potentials can be described as a prewave, and the second one as a main cathodic wave. The presence of the prewave causes a decrease in the main-wave limiting current. When Cef–Na is dominantly present as a cation, only the main wave exists.

4.1. The nature of the main-wave electrode process

Polarographic investigation of the reduction of Cef–Na showed that in the entire pH range investigated the main polarographic wave current and the $E_{1/2}$ value are strongly influenced by the change in pH. An S-shaped curve obtained for half-wave potential versus pH indicated the participation of hydrogen ions in the overall electrode mechanism. Data obtained by the usual means of polarographic criteria (height of Hg column and temperature coefficient) showed that in all cases the limiting current of the main wave was diffusion controlled. From logarithmic analysis of the dc polarographic curves, and from the observed dependence of $E_{1/2}$ on the concentration of the Cef–Na, it follows that the process is irreversible. Nonlinear plots obtained for the logarithmic analysis of the zwitterionic form of Cef–Na present in the solution, as well as the results obtained by ac polarography indicate adsorption of the Cef or the occurrence of the adsorbed film of the reduction product on the electrode surface. The irreversible character of the process was also confirmed by cyclic voltammetry on the hanging mercury drop. Measured effects of the polarization rate on the height and the position of the cathodic peak (corresponding to the main wave) showed that the peak height increases with the square root of the polarization rate. However, this dependence, as well as the $i_p/v^{1/2} = f(v)$ plot, shows two linear parts, which indicates that a two-step reduction process, with different rates, is occurring. The peak currents obtained for $v = 0$, the values of which are in the ratio 1:2, leads us to the assumption that the overall electrode process involved twice as many electrons as the first step. The number of electrons involved in the electrode mechanism, obtained by control potential electrolysis, showed that four-electron reduction is occurring in the entire pH region investigated. Logarithmic analysis $\log i = f(t)$, shown in Fig. 9, confirmed that the process is occurring in two steps. Since these two slopes of $\log i = f(t)$ dependence are in the ratio of 1:2, it can be concluded that there are two electrons involved in each step, while the overall Cef–Na reduction process consumed four electrons.

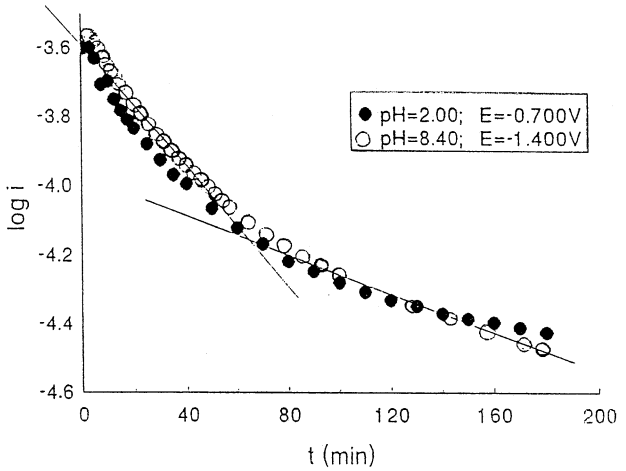


Fig. 9. Dependence of the log i vs. time of electrolysis, t, at pH 2.0 and 8.4 ($c_{Cef} = 5 \times 10^{-5}$ M, $I = 0.2$ M).

4.2. The nature of the prewave electrode process

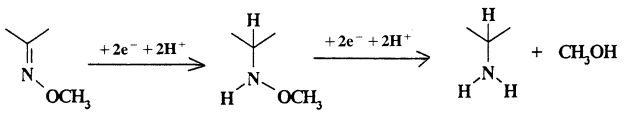
Investigations of the influence of pH, temperature, mercury column height and concentration on the $E_{1/2}$ and limiting current of the prewave showed that the prewave is diffusion controlled and irreversible, as is the main wave. The only difference is that this prewave is occurring only at a relatively high Cef–Na concentration (5×10^{-5} M) when the electrode surface is, most probably, completely covered with the adsorbed film of the reduction product. The prewave is caused by reduction at a more positive potential than the main wave, indicating that less energy is needed for its reduction than for the reduction of the main wave.

4.3. Mechanism of Cef reduction

According to these results it can be concluded that the whole process is followed by the adsorption of the Cef–Na reduction process which formed the adsorbed film on the mercury electrode surface. This adsorbed film is not reducible, but causes the energetic barrier for the reduction of Cef–Na in the solution.

It should be noted that due to the pK values [13] of Cef ($pK_1 = 3.11$, and $pK_2 = 10.56$), the reactant can be in the form of either cation, zwitterion or anion. All three forms are electroreducible and differ in their diffusion coefficients and their adsorptivities. The decrease of adsorption phenomena on increasing the pH value is likely to be related to the minor adsorptivity [14] of the zwitterion and the negatively charged form of Cef–Na.

According to the results presented, our investigation has lead us to the assumption of a four-electron reaction mechanism, involving a reduction of the methoxyimino group via a hydroxylamine intermediate to the corresponding amine and methanol. This reaction probably involves a two-step mechanism according to the following scheme.



This is in agreement with the results of some other authors [8,10] for the acidic medium. We assumed that the overall mechanism is the same for the entire pH range, only the rate of the reactions varying with pH.

4.4. Analytical application

On the basis of the electrochemical investigation of Cef, an analytical method for Cef determination was suggested, and the necessary analytical parameters were established. These parameters are summarized in Table 1 and the representative dp curves obtained at pH 8.4 are shown in Fig. 10. These two pH values (2.0 and 8.4) were selected for analytical investigation for two reasons: in these media Cef–Na exists in one of three ionic species as a predominant one (cation and zwitterion, respectively), according to the values of its protonation constants previously determined. Besides, under these conditions the highest and best developed waves (peaks) were obtained. For analytical application only the main wave was used. It was found that the polarographic response of Cef–Na at the pH values chosen was practically stable to at least 1 h, with a maximum decrease of less than 1% being quite satisfactory for analytical purposes. The dependence of the peak characteristics (peak height, half-width and peak potential) on the pulse amplitude was also investigated, since the dpp mode was applied as the preferable one for analytical purposes. One of the important parameters when using dpp is also the magnitude of the pulse amplitude applied. It was found that the most favorable signals

Table 1
Statistical parameters for the polarographic determination of Cef–Na

pH	Technique	Regression equation	Linear concentration range (M)	Limit of determin. (M)
2.0	dpp	$y = 0.041x - 2.01 \times 10^{-8}$, $r = 0.9980$	1×10^{-6}–1×10^{-5}	8×10^{-7}
	dc	$y = 0.0063x + 1.034 \times 10^{-7}$, $r = 0.9946$	1×10^{-5}–2×10^{-4}	1×10^{-6}
8.4	dpp	$y = 0.444x + 7.01 \times 10^{-9}$, $r = 0.9952$	1×10^{-6}–1×10^{-5}	8×10^{-7}
	dc	$y = 0.003x + 1.41 \times 10^{-8}$, $r = 0.9969$	1×10^{-5}–2×10^{-4}	1×10^{-6}

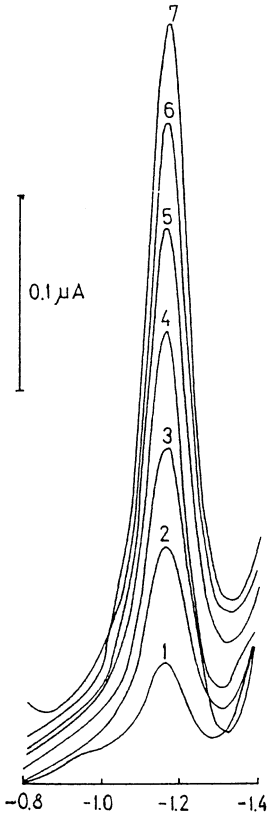


Fig. 10. Dpp curves of Cef–Na from 1×10^{-6} (curve 1) to 7×10^{-6} M (BR buffer, pH 8.400, $I = 0.2$ M, modulation amplitude 50 mV s^{-1}).

are gained by applying a pulse height of 50 and 100 mV (in the direction of potential scan). This must be connected to the irreversibility of the corresponding electrode processes.

As a criteria of linearity, the correlation coefficient of the corresponding calibration curve at a minimum condition of $r = 0.990$ has been taken. The limit of determination was established as a concentration related to the lowest wave or peak, which was significantly distinguishable from the base line of the supporting electrolyte under given conditions.

Two polarographic techniques were applied, dc polarography and dpp, in an acid medium (pH 2.0, − 0.63 V) and in slightly alkaline medium (pH 8.4, − 1.15 V). Linearity was achieved from 1×10^{-5} to 2×10^{-4} M in dc with a detection limit of 1×10^{-6} M, and from 1×10^{-5} to 1×10^{-6} M with a detection limit of 8×10^{-7} M in dpp.

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