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# Transport studies of β-lactam antibiotics and their degradation products across electrified water/oil interface

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**Abstract**

An electrochemical method for quantifying β-lactam antibiotics (cephalexin and ampicillin) and their hydrolysis products is described. Cyclic voltammetry at the water/nitrobenzene interface in a four-electrode system was used. The zwitterionic compounds were ionized to the necessary electrochemical form by pH adjustment. The pH change, however, resulted also in hydrolysis of the antibiotics. Hydrolysis products were characterized across UV-vis spectrum. The various hydrolysis products as well as the ionized antibiotics were studied in voltammetric transfer from water to nitrobenzene using the method of the interface between two immiscible electrolyte solutions (ITIES). It was concluded that this electrochemical method is suitable for the quantification of β-lactam antibiotics and their hydrolysis products. 

*Keywords:* β-Lactam antibiotics; ITIES; Hydrolysis products; Cephalexin; Cephalosporin; Ampicillin; Penicillin; Electroanalysis; Ion transfer

## 1. Introduction

Voltammetric studies at the interfaces between two immiscible electrolyte solutions (ITIES) have seen a steady increase in interest in the past years [1]. In some cases the studies were aimed even towards materials of biological or pharmacological significance [2–7]. Our contribution is oriented toward exploring the possibilities and advantages for developing an electrochemical method for quantifying β-lactam antibiotics and their degradation products. Recent study on the macrolide erythromycin and its hydrolysis products concluded that ITIES is a suitable method for their quantification as well as for distinguishing them from their hydrolysis product [8].

Cephalexin and ampicillin (Fig. 1a,b) are β-lactam antibiotics which share a common, 2-azetidinone four-member ring, fused with a six- or five-member heterocyclic ring, respectively [9]. Cephalexin is a representative of a large group of antibiotics called cephalosporins; ampicillin is in the penicillin group of which penicillin proper was

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the first antibiotic to be used in therapy. Both these compounds are active materials of a number of commercial antibiotics. Their determination is often needed and a new analytical method in addition to the standardized procedures can be always useful. Moreover, the ITIES has been always a method of a study which allowed better understanding of ion transfer across interfaces and ultimately, across membranes. Thus, study of antibiotics on ITIES has the added benefit to gain possibly further insights into drug penetration across biological membranes.

The liquid interface was in the forefront of the electroanalytical chemistry for a number of years. The sensing of ion activities via an ion selective electrode with a liquid ion exchanger is such an example. In that case an oil-soluble mobile species in the membrane establishes equilibrium with the ion of interest in the studied solution. The oil membrane/aqueous interface becomes a site of potential difference which is related to the activity of the analyte in the studied solution. By complementing the membrane with suitable reference electrodes, the interfacial potential, or more correctly, its changes as a function of ion activity, can be measured.

The above paragraph explains one aspect of electroanalysis, potentiometry. Far more intriguing and inherently more complex, but also more useful, are voltamperometric studies on such interfaces. In voltammetry, potential which is usually changing with time, is applied to the interface and resulting current is measured and interpreted. Although a prevalent method in the studies of metal/solution systems, it is relatively rare when applied to the oil/water solution interfaces. Such interfaces are called ITIES (the interface between two immiscible electrolyte solutions [10]) in the literature, and a number of informative reviews on this subject can be found [11–13].

In this study we have focussed on the behavior of pharmaceutically important compounds, the representatives of the $\beta$-lactams, ampicillin and cephalexin. There are two requisite properties a species must have to be detectable by the ITIES voltammetry. First, it must carry a charge, i.e. it must be ionic or, if normally neutral, it must be ionizable or able to bind in a ligand-like manner to an ion. Second, it must be a relatively large species with lipophilic affinity, so that it will enter (i.e. be soluble) even in its charged state in the oil phase. It must not be too lipophilic, though, because it would be extracted entirely in the oil phase with nothing left to contribute to the equilibrium in water.

The second requirement seems to be met well for a number of antibiotics. The requirement of the resident charge is somewhat tricky with antibiotics. Both the $\beta$-lactams in our study are zwitterions and their charge, even polarity, can be changed by a simple pH adjustment. The two $pK_a$ values of cephalexin are 5.2 and 7.3 and for ampicillin 2.7 and 7.3 [14]. However, the complicating factor is the fact that upon pH adjustment these materials can undergo both acid and alkaline hydrolysis. The ability to hydrolyze is due to the strained $\beta$-lactam ring.

A number of studies have indicated that the stability of $\beta$-lactam compounds in solution is a function of pH. So, while ampicillin is more readily soluble in base, it also rapidly loses activity when stored above pH 7.0 [15]. Cephalexin in alkaline solutions undergoes hydrolysis and shows the presence of 2-hydroxy-3-phenylpyrazine or diketopiperazine as the hydrolysis products of $\alpha$-aminocephalosporins [16]. In fact, more concentrated alkaline solutions of cephalexin turn distinctly yellow after a period of time. Cephalexin shows also the presence of degradation products of hydrolysis in acid media [17] and some cephalosporins have been actually assayed spec-

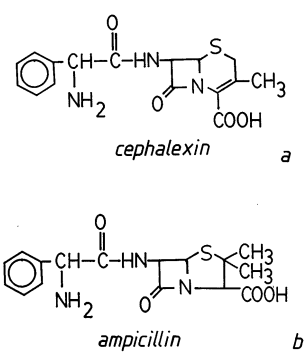


Fig. 1. Chemical structures of cephalexin (a) and ampicillin (b) used in these studies.

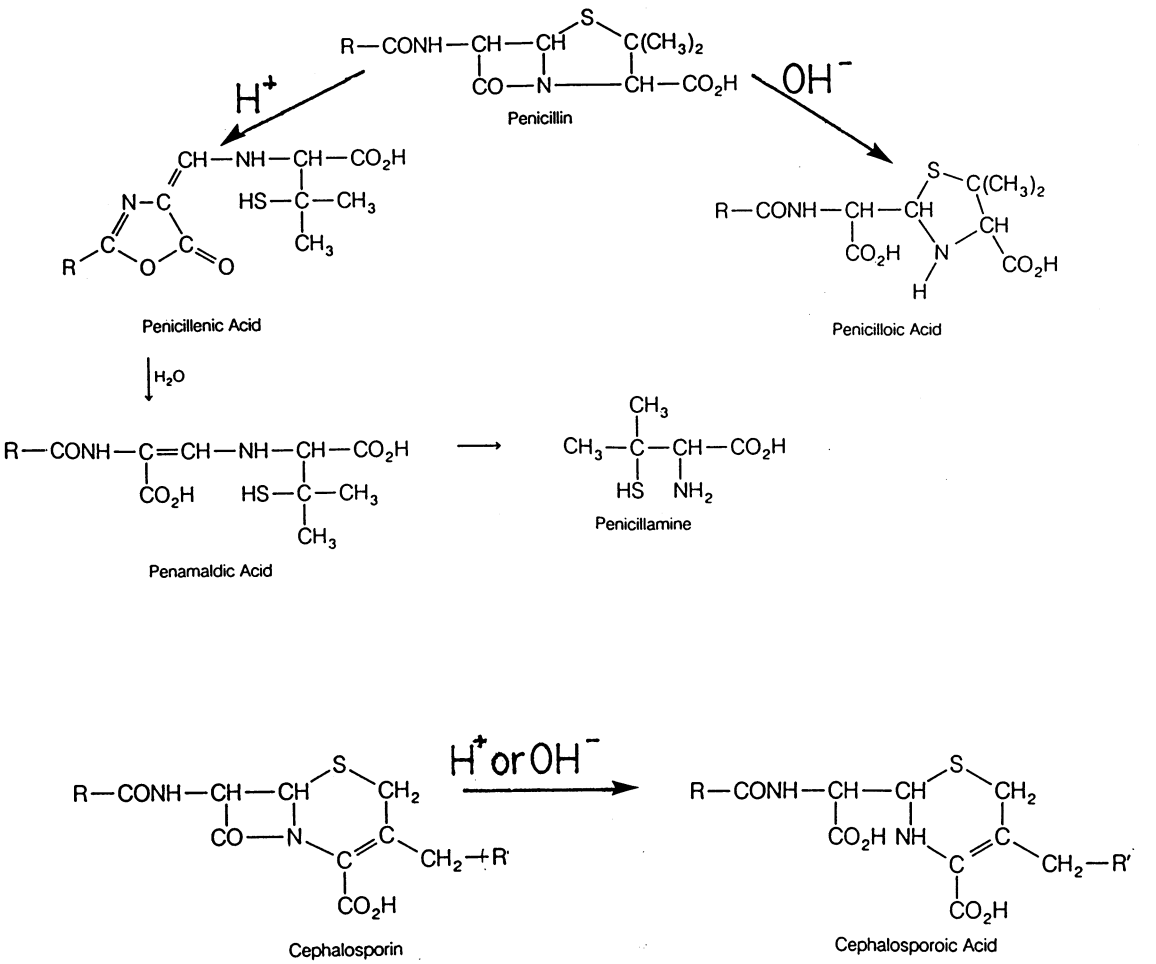


Fig. 2. Degradation pathways for penicillin and cephalosporins, after reference [9].

trophotometrically after preliminary acid hydrolysis [9]. Hence, these antibiotics are not very stable away from the neutral pH, since they undergo, depending on conditions, either acid or basic hydrolysis. Some of the possible degradation pathways are shown in Fig. 2. From a physiological point of view this may be of significant concern. For example these antibiotics do not seem to be successful in treating chronic prostatitis [18]. Since the pH of the prostatic fluid, especially in the infected gland, is 9 or higher, it may well be that the antibiotic degrades before it can exhibit its bacteriostatic or bactericidal ability. Similarly, in analytical chemistry the determination of these antibiotics could be hampered by hydrolysis of the samples. There are also various analytical methods that purposefully hydrolyze the antibiotics, derivatize them and determine them via some sensitive optical method [19]. However, such methods in general assay both the non-degraded and the degraded form of the antibiotic. Several optical methods were reported, which allow to distinguish between the hydrolysis products and the intact antibiotic [20,21].

Because the antibiotics are ionized at particular pH values, they are good candidates for a study in the ITIES approach, with which presence of larger ions can be observed. The essence of the method is ion transfer from one phase (water) into another (organic solvent). In the literature

several such studies on antibiotics were reported [2–7]. We are reporting such work on $\beta$-lactams for the first time.

## 2. Experimental

### 2.1. Chemicals and reagents

Cephalexin hydrate was obtained from Sigma. Ampicillin 99%, tetrabutylammonium tetraphenylborate 99% (TBATPB) and tetrabutylammonium chloride 96% (TBACl) were all from Aldrich, Milwaukee, WI (USA) and LiCl from Fisher Scientific, Pittsburgh, PA (USA). Nitrobenzene (Fisher Certified ACS) was filtered through an activated basic aluminum oxide (J.T. Baker, Phillipsburg, NJ (USA)) column as a standard precaution to eliminate impurities. All measurements were made at 25°C.

### 2.2. Cells, instrumentation and equipment

The experimental cell that was used in this work was similar to the type described earlier [11,22]. The volume of the nonaqueous phase (nitrobenzene) was approximately 7 ml and the aqueous part was filled to about 10 ml. The reference electrodes were silver wires coated with AgCl. The aqueous phase reference electrode was directly immersed in the aqueous solution containing LiCl, identical to the aqueous supporting electrolyte. The nonaqueous phase reference electrode was immersed in an aqueous solution of 0.1 mol $l^{-1}$ TBACl, which in turn was in contact with the nonaqueous phase, containing TBATPB as the supporting electrolyte. The aqueous TBACl solution thus shares the chloride ion with the aqueous Ag/AgCl electrode and the TPB ion with the nonaqueous phase containing TBATPB. Through this sequence of interfaces, of which each has its own determined and fixed potential, an overall constant potential reference potential value is maintained. The purpose of the supporting electrolytes in both phases is to render them conductive. It is important that these electrolytes are preferentially confined in their respective phases. Hence the choice of the very lipophobic LiCl for the water phase and the very lipophilic TBATPB for the nitrobenzene phase. The area of the interfacial contact area between the two phases in the cell was 0.28 $cm^2$.

The electrochemical study was performed with the Solartron 1286 Electrochemical Interface, which was used as a four-electrode potentiostat. Two electrodes are the potential measuring (reference) electrodes described above. The other two electrodes (counter, auxiliary) are current supplying and are platinum spiral wires of about 1 $cm^2$ each. As ions cross the interface and net current flows through the cell, redox processes occur on these electrodes, mostly reduction and oxidation of the solvents. These processes do not contribute to the observed voltammetric behavior due to the function of the potentiostat; the redox products are prevented from reaching the interface by confining the counter electrodes in fritted glass enclosed compartments. The source of the desired voltammetric signal was an EG&G 175 Universal Programmer. The resulting voltammograms were recorded using analog inputs of the Hewlett-Packard 7090A XY recorder.

The spectroscopic measurements were done using a Hitachi V-2000 spectrophotometer to record individual spectra or spectra overlays in the case of hydrolysis studies. A Shimadzu UV-3000 connected to a personal computer was used to record the kinetic data for hydrolysis at a single wavelength. The data were subsequently evaluated using TableCurve by Jandel Scientific Software. Computer simulations of voltammetric curves were done using DigiSim® software by Bioanalytical Systems.

## 3. Results and discussion

$\beta$-Lactam antibiotics exposed to acidic or alkaline media show signs of hydrolysis and the solutions contain the degradation products. Penicillin degradation to penicilloic acid in alkaline media, and penicillamine, penillic acid and penilloaldehyde in acid media has been described in the literature [16]. Certain cephalosporins (cephalexin, cefixime, ceftriaxone, cefotaxime) degrade into their corresponding hydroxamic acids [23]. Acid

degradation proceeds through cleavage of the side chain amide linkage [24] (cf. Fig. 2).

### 3.1. Studies of cephalexin in alkaline media

The UV-vis spectrum of cephalexin in basic medium has a characteristic peak at 472 nm (cf. Fig. 3a). In order to observe the formation of the degradation product of cephalexin, 0.5 ml of 1 mol $l^{-1}$ LiOH were added to 3 ml of cephalexin freshly dissolved in neutral water ($1.61 \times 10^{-3}$ mol $l^{-1}$ in 1 mol $l^{-1}$ aqueous LiCl) and the absorbance signal at 472 nm was registered as a function of time. Absorbance increased rapidly, reaching its maximum value ($A = 0.4$) in 40 min. After this, the signal decreased over a period of time until it leveled off at $A = 0.34$ (Fig. 4, curve a).

The electrochemical response of the supporting electrolyte, consisting of a blank system comprised of 1 mol $l^{-1}$ TBATPB in nitrobenzene and 1 mol $l^{-1}$ LiCl in water, was recorded at 10 mV $s^{-1}$ (Fig. 5), measured versus the 0.1 mol $l^{-1}$ Cl− nonaqueous and aqueous Ag/AgCl reference electrodes. The scan range corresponds to the potential where the charge transport processes across the interface and the resulting current are

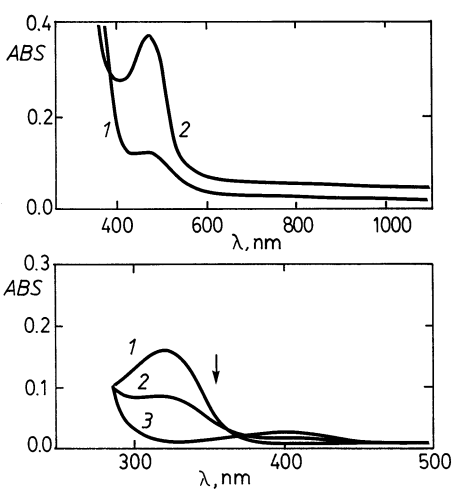


Fig. 3. UV-vis absorption spectra of $\beta$-lactam antibiotics upon change of pH. (a) Cephalexin with LiOH, 1 min between cycles; (b) ampicillin with LiOH 2 min between cycles, 3 ml ampicillin in 1 mol $l^{-1}$ LiCl + 0.5 ml 1 mol $l^{-1}$ LiOH.

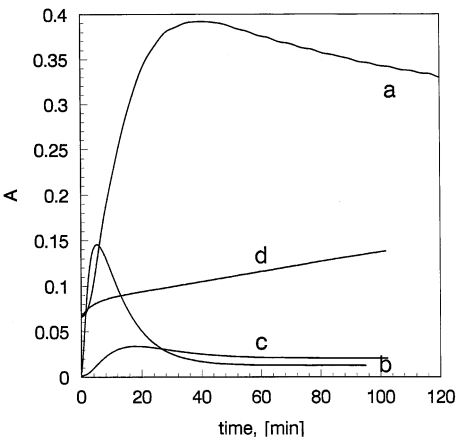


Fig. 4. Photometric absorption of $\beta$-lactam antibiotics upon change of pH. (a, top) Cephalexin with LiOH, 3 ml $1.61 \times 10^{-3}$ mol $l^{-1}$ cephalexin in 1 mol $l^{-1}$ LiCl + 0.5 ml 1 mol $l^{-1}$ LiOH, $\lambda = 472$ nm; (b, bottom) ampicillin with LiOH, 3 ml $1.6 \times 10^{-3}$ ampicillin in 1 mol $l^{-1}$ LiCl + 0.5 ml 1 mol $l^{-1}$ LiOH, $\lambda = 315$ nm; (c) ampicillin with LiOH, 3 ml $1.6 \times 10^{-3}$ ampicillin in 1 mol $l^{-1}$ LiCl + 0.5 ml 1 mol $l^{-1}$ LiOH, $\lambda = 396.5$ nm; (d) ampicillin with HCl, 3 ml $1.6 \times 10^{-3}$ ampicillin in 1 mol $l^{-1}$ LiCl + 0.5 ml 1 mol $l^{-1}$ HCl, $\lambda = 319$ nm.

not influenced by the supporting electrolyte used. In this range it is possible to follow, electrochemically, the interfacial transport of both the antibiotics as well as their degradation products [11].

It is useful to point out the simple, but not immediately obvious fact that the recorded voltammetric response is extremely sensitive to the position of the Luggin capillaries relative to the interface. During the experiment, when an aliquot was added to the cell, it was important to ensure that the height of the interface remained constant, which was accomplished by adjusting the cell volume. If the position of the interface was allowed to move, some change in behavior, due essentially to the change in the amount of the uncompensated solution resistance was observed, which could be erroneously interpreted as an effect of the analyte addition, especially if the expected analyte effect would be only slight.

In preliminary experiments it was verified that addition of 0.01 mol $l^{-1}$ HCl or LiOH to the aqueous supporting electrolyte, without any antibiotic present, does not produce any electrochemical response other than the normal

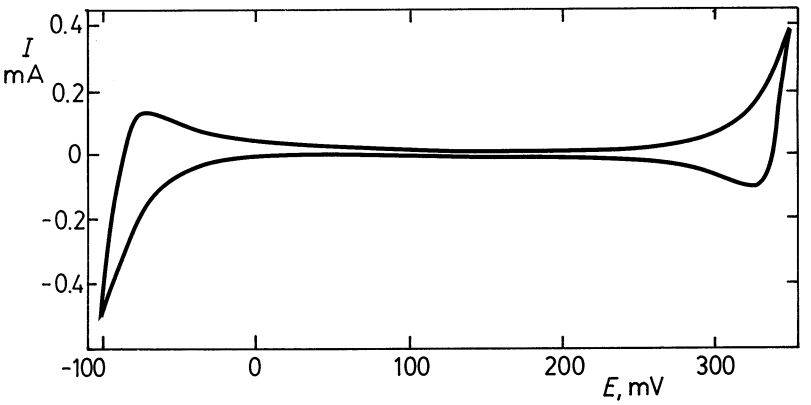


Fig. 5. Voltammogram of the supporting electrolyte. One mol $l^{-1}$ LiCl in water, 0.1 mol $l^{-1}$ tetrabutylammonium tetraphenylborate in nitrobenzene. References: Ag/AgCl in $Cl^-$ aqueous phase, Ag/AgCl in aqueous 0.1 mol $l^{-1}$ TBACl reference interface. Scan rate, 10 mV $s^{-1}$.

signature of the supporting electrolyte cyclic voltammogram (CV). This was done to ascertain whether any response observed was actually due to the presence of the antibiotics and not due to the pH adjustment. HCl or LiOH were chosen to adjust the pH in order to limit the number of foreign ions added to the system, which is made up in neutral solution from LiCl in water.

Fig. 6 shows resulting CV after some cephalexin solution was added to the supporting electrolyte. The stock solution of cephalexin was exposed to the alkaline medium of LiOH (pH 10.6) for 30 min prior to the experiment. Two additions were made, resulting in $7.5 \times 10^{-5}$ mol $l^{-1}$ cephalexin and $1.17 \times 10^{-5}$ LiOH (curve 1) and $1.7 \times 10^{-4}$ cephalexin and $2.6 \times 10^{-5}$ LiOH (curve 2). In comparison to the supporting electrolyte response (Fig. 5), a current increase in the region around 140 mV is being seen.

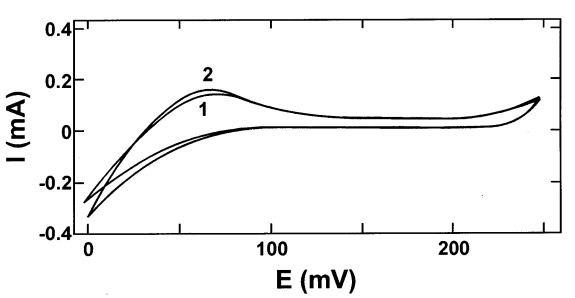


Fig. 6. Voltammogram of addition of cephalexin to the aqueous phase of the ITIES system. Aqueous phase volume 10 ml. Stock solution $2.58 \times 10^{-3}$ mol $l^{-1}$ cephalexin, $4 \times 10^{-4}$ mol $l^{-1}$ LiOH, 1 mol $l^{-1}$ LiCl. Scan rate, 10 mV $s^{-1}$; (1) 300-μl aliquot; (2) additional 400-μl aliquot.

The effect of changing scan rate (Fig. 7) was studied in a potential range between 0 and +220 mV, at rates from 2 to 100 mV $s^{-1}$, and at concentrations of $1.7 \times 10^{-4}$ mol $l^{-1}$ cephalexin and $2.6 \times 10^{-5}$ mol $l^{-1}$ LiOH. There was an obvious increase of peak height with increasing scan rate, but the peak also shifted to more positive values. Such a shift is typically attributed to the effect of residual uncompensated solution resistance. In fact, the ITIES system typically has such residual resistance and our arrangement is

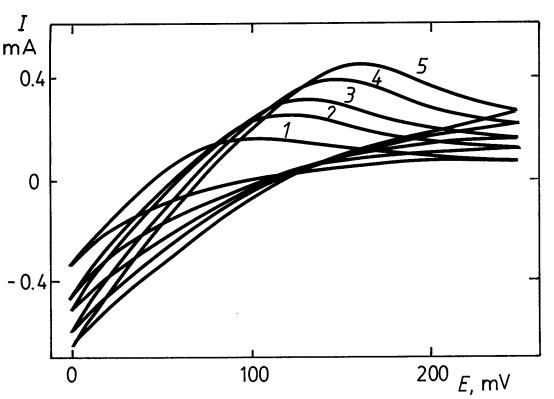


Fig. 7. Scan rate dependence for cephalexin (case of Fig. 6, curve 2): (1) 2 mV $s^{-1}$; (2) 10 mV $s^{-1}$; (3) 20 mV $s^{-1}$; (4) 50 mV $s^{-1}$; (5) 100 mV $s^{-1}$.

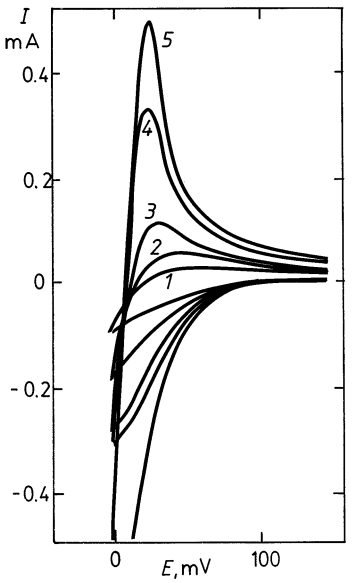


Fig. 8. Time effect after injection of 100 μl of 1 mol $l^{-1}$ LiOH solution to the cell containing, in 10 ml aqueous electrolyte (1 mol $l^{-1}$ LiCl) cephalexin ($1.64 \times 10^{-4}$ mol $l^{-1}$). Scan rate, 10 mV $s^{-1}$. Time after injection: (1) 15 min; (2) 20 min; (3) 25 min; (4) 26 min; (5) 27 min.

not an exception. However, more thorough analysis using voltammetry simulating program DigiSim® revealed that at least part of the shift must be attributed to the sluggishness of the ion transport. The peak shift explanation based solely on solution resistance would require unrealistically high (several MΩ) solution resistance.

In the previous experiment (Figs. 6 and 7) the response of cephalexin was recorded at the stage when the alkaline hydrolysis could reach its completion. The following experiments shows the dynamics of the hydrolysis and voltammetry of its products. Fig. 8 shows voltammetric scan between 0 and + 150 mV, obtained with a cell in which 10 ml of freshly prepared cephalexin of concentration 0.164 mmol $l^{-1}$ was spiked by addition of 100 μl of 1 mol $l^{-1}$ LiOH. It can be observed that the current recorded at the negative extreme has been increasing with the lapsed time. From this it can be concluded that the electroactive species which give a rise to the observed current is the hydrolysis product of cephalexin, not the native cephalexin itself. This figure shows less IR distortion than other voltammograms in this work, presumably due to closer spacing of the reference electrode capillaries, whose position can sometimes vary.

Larger amounts of hydrolysis products were generated by alkalizing a $5.8 \times 10^{-4}$ mol $l^{-1}$ cephalexine solution by LiOH to pH 12.7 as opposed to pH 10.6, used in Fig. 5. The mixture was allowed to react for 30 min, before its response in the voltammetric cell was studied. Fig. 9 shows the electrochemical response after adding subsequent amounts of the stock solution, resulting in concentrations of cephalexin $1.7 \times 10^{-5}$, $3.1 \times 10^{-5}$ and $3.6 \times 10^{-5}$ mol $l^{-1}$, respectively. The lowest concentration corresponds to 5.94 μg $ml^{-1}$ of native cephalexin. An increase in the signal was observed with the increase in the original cephalexin concentration. The three concentrations of added cephalexin allow to obtain a qualitative calibration curve. We chose as the measure of concentration response the current recorded at the left extreme, at + 50 mV. From the three points the current–concentration appears to be more or less linear. However, the intercept is not zero, and it is actually negative. This is likely due to the fact that the measurement was not performed under steady-state conditions. We have observed throughout the work that the open circuit potential drifts with time. Therefore, one scan within a certain potential limit may not

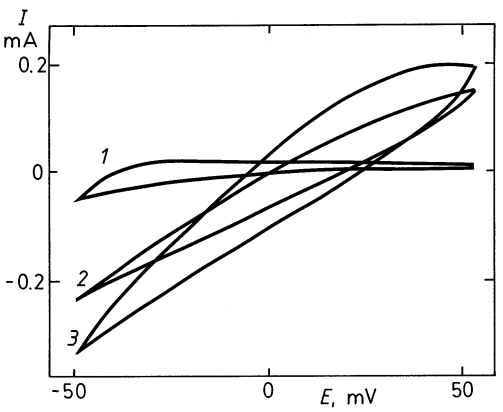


Fig. 9. Voltammetry of concentration dependence of cephalexin previously in contact with LiOH. Scan rate, 10 mV $s^{-1}$. Cephalexin: (1) $1.7 \times 10^{-5}$ mol $l^{-1}$; (2) $3.1 \times 10^{-5}$ mol $l^{-1}$; (3) $3.6 \times 10^{-5}$ mol $l^{-1}$.

be comparable with another scan within the 'same' potential limit, because the base reference potential might have drifted. Whereas this might be detrimental to rigorous analytical application, the same potential shift, if due to concentration effect, may lead to parametric amplification of the recorded current. This advantageous amplification would stem from the fact that the current at the left extreme is proportional exponentially to the shift in the open circuit or reference potential.

### 3.2. Studies of ampicillin in alkaline media

The alkaline hydrolysis of ampicillin with 1 mol $l^{-1}$ LiOH shows two signals attributed to hydrolysis products in the UV-vis range. An intense signal at 25°C appears at 315 nm and a second, smaller in intensity, at 396.5 nm (Fig. 3b). The kinetic responses of both maxima were studied. The reaction was initiated by addition of 0.5 ml of 1 mol $l^{-1}$ LiOH to 3 ml of $1.6 \times 10^{-3}$ mol $l^{-1}$ ampicillin prepared in 1 mol $l^{-1}$ LiCl neutral solution. The signal at 315 nm increased during the first 6 min, reaching a maximum $A = 0.14$, after which the signal decreased exponentially as found through a nonlinear fit, reaching almost zero in 50 min (Fig. 4, curve b). Similar behavior, though not identical in the fit parameters, was observed with the 396.5-nm signal (Fig. 4, curve c). Initially, an increase was observed, reaching a maximum ($A = 0.035$) in about 17 min. From that time on, a decrease to 50% of the original value occurred after 50 min. This response clearly indicates a number of steps and intermediates, as already suggested by the scheme of Fig. 2.

Fig. 10 shows the ITIES voltammetry as the alkaline hydrolysis of ampicillin was unfolding at the scan rate of 10 mV $s^{-1}$. The first cycle of the voltammogram was essentially the same as it would appear if only supporting electrolytes were present. It can be compared to Fig. 5, but a different current sensitivity and a scan range must be noted. Already in the second cycle, after addition of LiOH, it is possible to see some ion transport activity in the range between 0 and +150 mV. The signal increased further in the next cycles, reaching its highest value after 15 min. Upon further cycling (Fig. 11) the signal again begun to decrease. This behavior was similar to the one observed in the spectroscopic signal at 396.5 nm, with similar time (17 min) needed to reach the maximum.

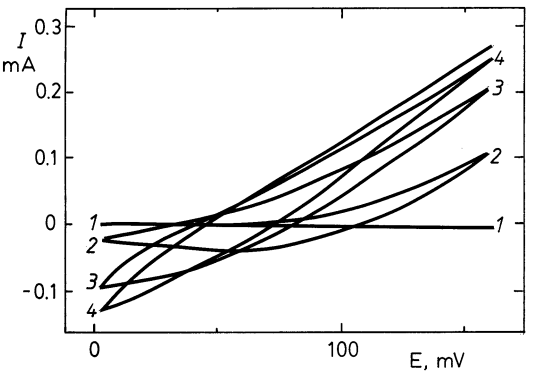


Fig. 10. Voltammogram of ampicillin, time effect/surface concentration dependence. Three ml of $1.6 \times 10^{-3}$ mol $l^{-1}$ ampicillin, 6.5 ml 1 mol $l^{-1}$ LiCl. Scan rate, 10 mV $s^{-1}$. Effect of addition of 0.5 ml 1 mol $l^{-1}$ LiOH to the aqueous phase. (1) first cycle; (2) second cycle; (3) third cycle; (4) fourth cycle.

### 3.3. Studies of ampicillin in acidic media

Ampicillin in acidic medium exhibits just one degradation product with maximum absorption at $\lambda = 319$ nm. In the following experiment, 0.5 ml of 1 mol $l^{-1}$ HCl were added to 3 ml of a

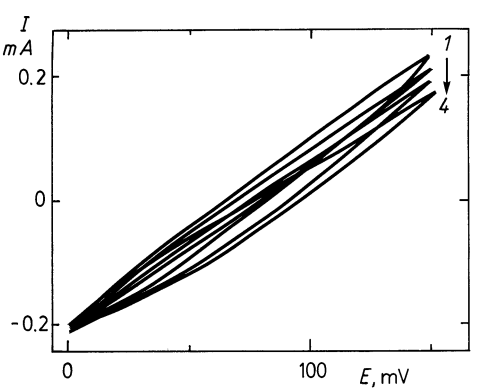


Fig. 11. Same situation as Fig. 10, at later time after LiOH addition. Voltammogram of ampicillin; concentration dependence. Three ml of $1.6 \times 10^{-3}$ mol $l^{-1}$ ampicillin, 6.5 ml 1 mol $l^{-1}$ LiCl. Scan rate, 10 mV $s^{-1}$. 1, first. Effect of addition of 0.5 ml 1 mol $l^{-1}$ LiOH to the aqueous phase. After (1) 15 min; (2) 20 min; (3) 35 min; (4) 55 min.

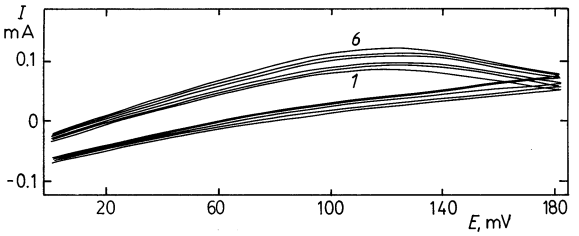


Fig. 12. Voltammetry of ampicillin in acid medium. Three ml of $1.6 \times 10^{-3}$ mol $l^{-1}$ ampicillin, effect of addition of 0.5 ml of 1 mol $l^{-1}$ HCl. Scan rate, 10 mV $s^{-1}$. (1,2) First two cycles; (3) 5 min; (4) 15 min; (5) 30 min; (6) 60 min.

$1.6 \times 10^{-3}$ mol $l^{-1}$ solution of ampicillin and the changes in the absorption signal at $\lambda = 319$ were followed as a function of time. The signal increased in an exponential fashion for 17 min ($A = 0.09$), after which the rate of increase slowed down and became linear, reaching an absorbance value of $A = 0.14$ in 100 min (Fig. 4d).

For the electrochemical work, an acidified solution of $4.8 \times 10^{-4}$ mol $l^{-1}$ ampicillin and $5 \times 10^{-2}$ mol $l^{-1}$ HCl was prepared. Fig. 12 shows a voltammetric peak emerging at about +120 mV. The peak current increased with each cycle, as time progressed. The recorded cycles were the first (number 1) and then 5th, 15th, 30th, and eventually 60th (number 6). The recorded current increase agrees well with the observed spectroscopic evidence of formation of a hydrolysis product (cf. Figs. 3 and 4). The spectroscopic results begin at time $t = 0$ with a zero signal. This only seemingly contradicts the electrochemical data, which already show current for the first cycle. In the electrochemical set-up the measurement does not begin instantaneously. The solution was prepared outside of the cell and the experiment was performed at a time closer to $t = 4$ min. Such results are then in agreement with the spectroscopic data. The difference in appearance of the voltammetric curve in this experiment compared to the previous should be noted. Whereas Sections 3.1 and 3.2 dealt with alkaline medium, this experiment was performed in acidified solution. Consequently, in the first two cases the amphoteric antibiotic and its hydrolysis products become anions, whereas in this case the species which crosses the interface is a cation. Therefore the voltammogram here has a pronounced peak at its right-hand (positive) side, where the water phase is positive and hence the positive species in water crosses into nitrobenzene, whereas the anionic transfer from water to nitrobenzene (Sections 3.1 and 3.2) occurs at the left-hand side of the voltammogram, where the aqueous phase is negative relative to the nitrobenzene phase.

## 4. Conclusions

The experimental electrochemical methods of ITIES have proven to be potentially useful in analytical determinations of antibiotics. The requirement of this method is that the analyzed material is ionized. Therefore, initial pH adjustment in sample preparation may be necessary. With pH adjustments the $\beta$-lactam strained four-member ring undergoes hydrolysis. The degradation products are nevertheless still ionized and the work shows that they can be detected on ITIES. The observed hydrolysis takes place entirely in the aqueous phase, prior to the ion crossing. For the purpose of analytical detection of ion transport across the interface, there is little reason to consider hydrolysis after crossing, in the nonaqueous phase.

The electrochemical knowledge gained with ion transport across immiscible interfaces may be of further use in clinical research. Although it is a known fact that charged particles will travel with difficulty against a potential gradient, the implication of transport of ionized antibiotics across a charged cell membrane is not intuitively obvious. Here one possible situation is considered. Chronic prostatitis is an ailment of uncertain etiology, often refractory to any treatment. Both the bacterial and non-bacterial forms seem to respond on occasion to antibiotics. More often than not, the response to antibiotics is unsatisfactory or temporary, which have lead to speculations that the needed antibiotics do not penetrate into the prostate [18]. Indeed, to facilitate the intake of antibiotics a direct injection rather than oral administration was proposed [25]. The ITIES study can provide two reasons why it may be difficult for the antibiotics to penetrate the prostate. The

pH of an inflamed prostate is high, pH 9 or even more. Under such conditions the antibiotics will be in their anionic form, charged negatively. In that form they will cross only with difficulty any membrane that will have either anchored negative sites (repulsion), or positive sites, if they happen to form a strong complex with the ionized antibiotic. Thus, it can be envisioned how antibiotics from the blood stream may have difficulty entering the prostate capsule. Second, a possible complication is simply chemical. If the alkaline hydrolysis at pH 9 or above is facile enough and the hydrolytic products are biologically inactive, then achieving meaningful concentration of the active antibiotics in the inflamed organ may be impossible.

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