"Uilleadh eile a dtaobh na néithean iodhbhartar d'iodhaluibh. Atá a fhios againn go bhfuil eolus againn uile. Arduighe an t-eolus [intinn duine] acht do ghní an grádh follamhnughadh"

1 Corintianacha VIII, 1. (CORINTHIANS).

POLAROGRAPHIC STUDIES OF BETA-LACTAM DEGRADATIONS

by

MICHAEL JOSEPH MARTIN, B.Sc., G.R.S.C.

A DOCTORAL THESIS

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Supervisor: DR. A.G. FOGG, B.Sc., Ph.D., A.R.T.C.S.,
C. Chem., F.R.S.C.
Reader in Analytical Chemistry,
Department of Chemistry.

November, 1982

DEDICATION

i gcuimhne mó mhaimín, Máire Úna, meadú ar a glóire sna flaithis.

to my mother, Mary Agnes, in blessed memory.

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CONTENTS

ABSTRACT

Alkaline degradation studies were followed polarographically for eleven cephalosporins. Correlations are apparent in that most compounds give waves at -0.39 V (pH 2) and -0.50 V (pH 2); these are the initial and secondary polarographically active alkaline degradation products respectively. Possible structures for these degradation products are suggested which contain a reducible carbon-nitrogen double bond in the 6-membered thiazine ring analogous to 1,4-benzodiazepines. These compounds are considered to arise via expulsion of a leaving group at the C-3 position to form the reducible imine compound, as in the formation of diketopiperazine derivatives from α-aminobenzylcephalosporins.

Reproducible yields of hydrogen sulphide, ranging from 18.5% (cephalothin) to 65.6% (cephradine) have been recorded from the alkaline degradation at 100°C for all the cephalosporins investigated. The yields have been determined using a normal pulse polarographic method based on the anodic oxidation of mercury in the presence of sulphide to mercury(II) ions. The analytically useful range of this method was from 0.3×10^{-4} M to 6.0×10^{-4} M. The high values obtained for cephalonium, cephoxazole and cefazolin when compared with the spectrophotometrically and potentiometrically determined values, are explained by interference from thiol type compounds also produced in the alkaline hydrolysis of these compounds.

The role of formaldehyde and acetaldehyde in the production of a polarographically active pyrazine compound from α-aminobenzyl-β-lactams has been investigated. Addition of the aldehydes markedly increases the maximum production of these analytically useful pyrazine derivatives, typically by a factor of 1.5 to 2.5, in both strong acid (5 M HCl, 12.5% H_2SO_4) and dilute acid (pH 5) solutions of α-aminobenzyl-β-lactams. Polarographic data has confirmed the mechanism of Barbhiaya *et al.*[58] in

which formaldehyde is incorporated into the pyrazine molecule as the 6-methyl derivative. Acetaldehyde has a lesser effect on pyrazine production due to the steric hindrance imparted by the formation of the 6-ethylpyrazine derivative. Synthesis and hydrolysis of known degradation products of α-aminobenzyl-β-lactams has enabled mechanisms to be suggested for the formation of these pyrazine derivatives. The initial alkaline degradation products, the cephalosporoic and penicilloic acids, undergo degradation to pyrazines via their penaldic and penicilloaldehyde derivatives. The addition of an aldehyde to these degrading solutions ensures that oxidation of the cyclised penicilloaldehyde is not required.

The neutral degradation of three α-aminobenzylcephalosporins, cephaloglycin, cefaclor and cephalexin have been compared. Major differences are in the change in reduction potential of the primary diketopiperazine degradation products and the absence of the polarographically active carbonyl compound found in degraded cephalexin solutions which reduces at -1.26 V (pH 7.4). A thiophenone, present in cephalexin solutions degraded in the pH range 3.3 to 0.1 M sodium hydroxide, has been shown to be a key intermediate in the formation of the polarographically active carbonyl compound (-1.26 V, pH 7.4) and in the formation of hydrogen sulphide. This compound is also an intermediate in the degradation of 7-ADCA, and the diketopiperazine derivative of cephalexin. Isolation and degradation of the thiophenone and the diketopiperazine has enabled mechanisms to be proposed in which 7-ADCA degrades at pH 7.4 via its cephalosporoic acid to this thiophenone, whilst degradation at pH 3.3 and pH 7.4 of cephalexin occurs via its cephalosporoic acid and diketopiperazine derivatives.

A. INTRODUCTION
THEORY OF POLAROGRAPHY

Voltammetry is the branch of electroanalytical chemistry that deals with the effect of the potential of an electrode, in an electrolysis cell, on the current that flows through it. The term polarography is used chiefly for voltammetry when the electrode of interest (the working electrode) is the dropping mercury electrode (DME) but the distinction is not always followed.

The magnitude of the current that flows through the electrolysis cell as a function of the voltage on the working electrode can be plotted as a curve. Polarography derives its analytical importance from two characteristics of these current-potential curves. Firstly, the half-wave potential (the potential at which the current is one-half the diffusion value) may indicate the identity of the substance which undergoes electron transfer. Secondly, the magnitude of the diffusion current is governed by the concentration of the electroactive substance. Therefore there is a basis for qualitative and quantitative analysis.

In ordinary electrolytic cells with solid electrodes the current-voltage curves are not reproducible. This is due to (a) the effect of accumulation of the products on or near the electrodes and (b) the cell current may be affected by convection currents within the solution. These problems were overcome in 1922 in Czechoslovakia when J. Heyrosky introduced direct current (DC) polarographic analysis[1].

In this technique a dropping mercury electrode is used as the working electrode. This DME consists of a very fine-bore glass capillary. One end of the capillary is connected to a reservoir of mercury and the other end is immersed in the solution being investigated. Due to the hydrostatic pressure of the mercury column the mercury emerges as a succession of droplets. This has several major advantages to offset the inconvenience of handling such a toxic material as mercury.

Advantages of the DME

(i) The electrode surface is continuously being renewed, and hence cannot become fouled or poisoned.

(ii) The mercury electrode has a high hydrogen overvoltage at negative potentials. At these potentials hydrogen is liberated ($2H^+ + 2e^- \longrightarrow H_2$ or $2H_2O + 2e^- \longrightarrow H_2 + 2OH^-$) from the water of solvation. This means that the DME can be utilized as far as -1.8 V in acid or about -2.3 V in basic media, enabling the investigation of processes that can only occur under extremely reducing conditions.

(iii) In comparison with solid electrodes the DME has the advantage that the increasing area of the electrode during the lifetime of a drop more than offsets the decreasing current observed with an electrode of fixed size and makes quantitative analysis much more practicable.

(iv) As successive drops are born into solutions of identical composition and grow at the same rate and reach the same maximum size, each drop therefore duplicates the behaviour of the one that preceded it. Consequently, the currents are accurately reproducible from one drop to the next.and are independent of the previous history of the experiment.

Disadvantages of the DME

(i) Mercury is easily oxidised so that very positive (i.e. strongly oxidising) potentials cannot be secured. Because of this ease of its anodic dissolution the DME cannot be made more positive than +0.25 V.

(ii) Dissolved oxygen is reducible at the DME. Two waves are observed. The first ($E_{\frac{1}{2}}$ = -0.05 V versus SCE) corresponds to the reduction of oxygen to hydrogen peroxide; the second ($E_{\frac{1}{2}}$ = -0.9 V versus SCE) is due to oxygen being reduced to water. As these waves appear in the potential range that is important in polarography they are interferents of high

nuisance value. Oxygen therefore must be removed prior to measurements being made. This is usually done by bubbling an inert gas (normally nitrogen) through the solution, then passing this over the surface of the solution while recording the current-voltage curve to prevent re-solution of air.

(iii) The continuous variation of electrode area gives rise to significant currents even in the absence of a reducible or oxidizable substance.

(iv) As a toxic heavy metal mercury should be handled with great care, the Threshold Limit Value in air being 0.05 mg/m^{-3}. Polarographers must ensure that their laboratory is adequately ventilated.

THEORY OF DIRECT CURRENT POLAROGRAPHY

In classical DC polarography, a very slow moving (about 5 mV/sec) DC potential ramp is applied between the DME and a reference electrode, usually the Saturated Calomel Electrode (SCE). The direction of scan is usually in the negative direction (i.e. the DME is negative with respect to the SCE). As the potential of the electrode moves into a region in which an electrode reaction takes place, a current is obtained, and the characteristic S-shaped DC polarographic wave is developed (see fig. 1). The residual current at the first part of the wave is due to the reduction of traces of impurities and a so-called charging current, corresponding to the capacitance effect of the mercury-solution interface with its sheath of irreducible ions. The current then increases sharply due to the reduction of the species in solution. This takes the form of the current depleting ions of that species from the layers of electrolyte in contact with the surface of the DME by reduction. The ion is then replaced by diffusion from the bulk of the solution, setting up a concentration gradient. After a certain increase in voltage, the current shows a saturation effect due to total depletion of ions in the near vicinity of the DME and

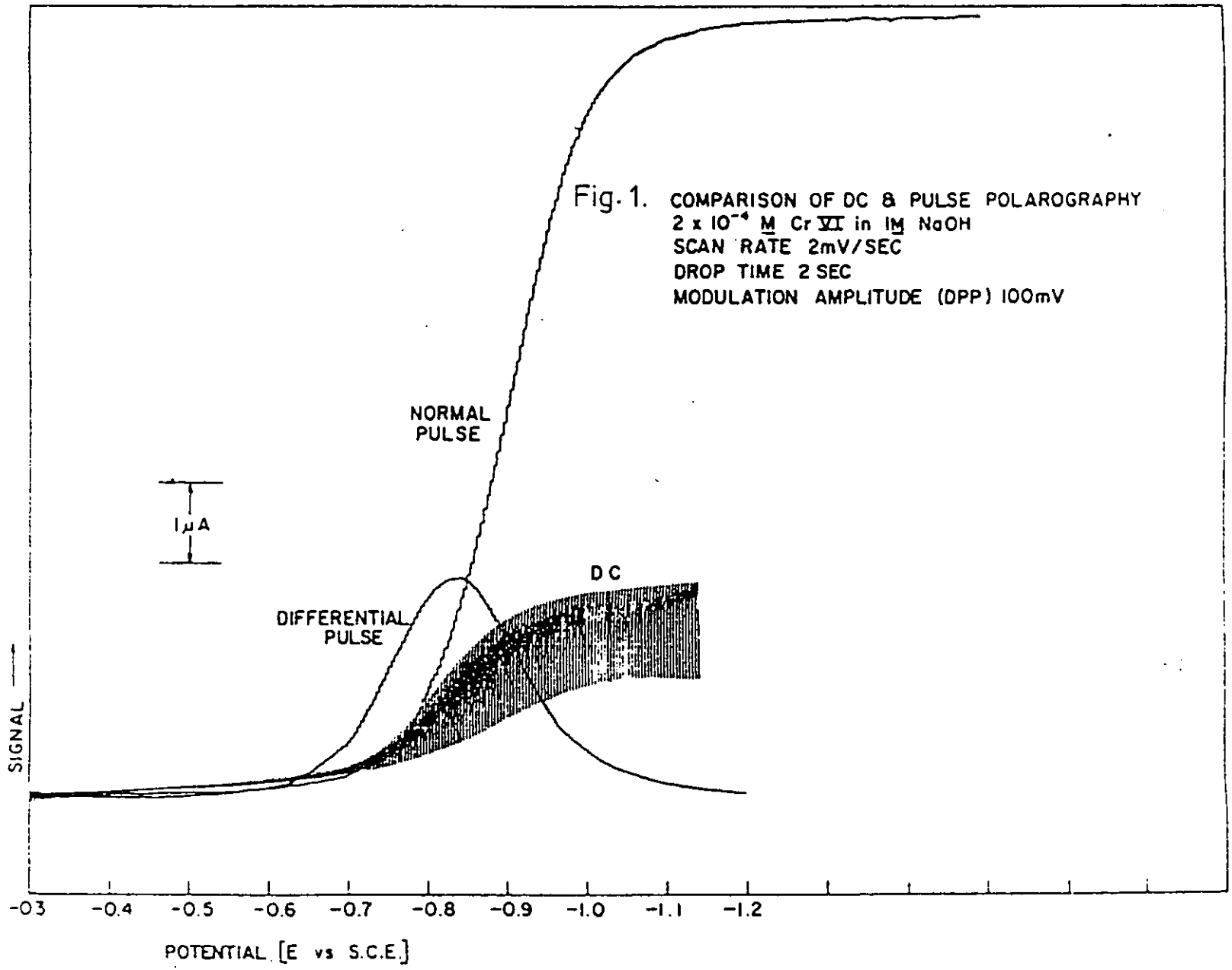


Fig. 1. COMPARISON OF DC & PULSE POLAROGRAPHY
2×10^{-4} M Cr VI in 1M NaOH
SCAN RATE 2mV/SEC
DROP TIME 2 SEC
MODULATION AMPLITUDE (DPP) 100mV

is not noticably affected by any increase in applied voltage. The reduction is happening so fast that the ions are being reduced as rapidly as they can diffuse from the bulk of the solution up to the surface of the electrode. The rate of diffusion is determined solely by the difference between the bulk concentration of the solution and zero, the concentration at the electrode surface. The value of this limiting current, called the diffusion current i_d, is directly dependent on the concentration of the reducible species and is the basis for nearly all quantitative polarographic analysis.

The diffusion current observed consists of two quite different components, (a) the faradaic current which is due to an electron-transfer process and (b) a capacitance current due to the DME-solution interface exhibiting capacitive characteristics.

(a) The faradaic current increases with droplife, unless complicating phenomena like adsorption are present. An expression for the variation in the faradaic current with droptime can be derived if it is assumed that the flow of mercury is constant and the drop is spherical in shape right up to the moment of separation. This leads to the Ilkovic equation (1) describing the mean limiting current i_d, which is diffusion controlled for a reversible reaction

$$i_d = 607n\, CD^{\frac{1}{2}}\, m^{2/3}\, t^{1/6} \qquad \ldots (1)$$

where i_d is measured in μA; n is the number of electrons involved in the electron-transfer process; C is the bulk concentration of electroactive species (m mol cm^{-3}); D is its diffusion coefficient (varies with viscosity of the medium) ($cm^2\ s^{-1}$); m is the rate of mercury flow (mg s^{-1}); and t is the droptime (s). This equation illustrates the relationship between current and concentration, $i_d \propto C$ or $i_d = kC$.

(b) Capacitance current

The capacitance current will only result from a change in the potential of the electrode or a change in the area of the electrode. As the change in voltage at the DME is slow enough to be negligible during the lifetime of a single drop, the capacitance current observed is largely the result of the change in area of the DME. The capacitance current, I_c, resulting from the growth of a mercury drop is directly proportional to the rate of change of drop area, dA/dt.

$$I_c = k \frac{dA}{dt} \cdot \frac{dE}{dt} \qquad \ldots (2)$$

where the constant k includes the dielectric constant and the thickness of the double layer of ions around the DME; dE/dt is the change of potential at the DME. The variation of I_c with time, $(I_c)_t$, is proportional to minus one-third the power of time, i.e. it is relatively large at the start of a drop and then decreases. The faradaic current increases as the one-sixth power of time, starting at zero following the birth of a new drop. Growth of I_f (faradaic current) and decay of I_c (capacitance current) as instantaneous readings during the lifetime of a single drop are shown below in fig. 2.

Near the end of the lifetime of the drop the effect of the capacitance current will be least and the faradaic current greatest.

Problems arise with DC polarography when determinations at levels below 10^{-5} M are attempted. The faradaic current at these low concentrations is reduced to the same order of magnitude as the capacitance current. Also resolution of two waves becomes poor in the presence of relatively large concentrations of a more easily reduced species. Even at concentrations such as 10^{-3} M DC polarography has problems. Evaluation of the mean limiting current, i_d, and the characteristic half-wave potential $E_{\frac{1}{2}}$, is often difficult due to distortion of the ideal-shaped curve by maxima.

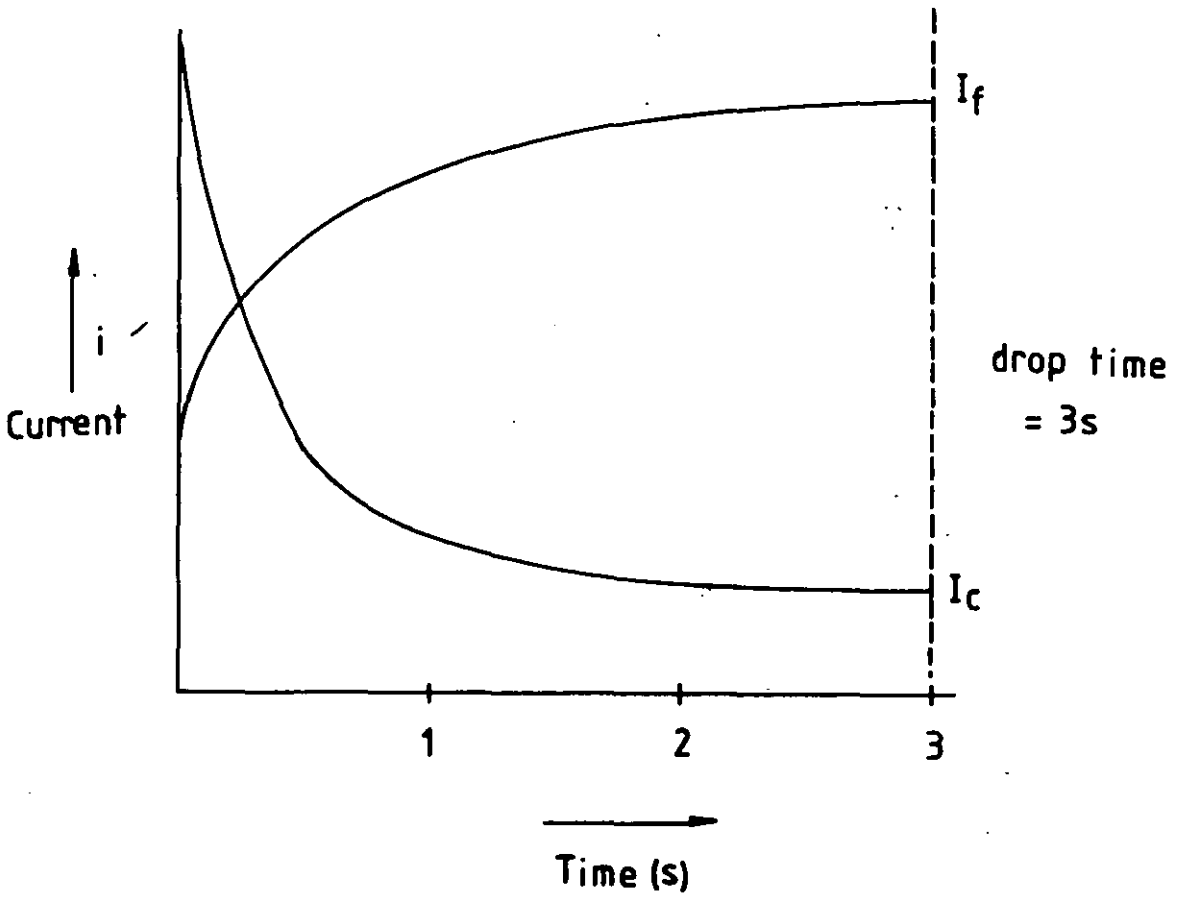


Fig. 2

This usually manifests itself as a slight hump or a sharp peak which may exceed the true wave height by a factor of 2 or more. These maxima seem to be related to the tangential streaming motion of solution past the surface of the drop.

In order to improve the sensitivity and resolution a number of variations on classical DC polarography have been introduced. The techniques relative to the work herein are two pulse polarographic techniques, Normal (or Integral) Pulse Polarography (NPP) and Differential (or Derivative) Pulse Polarography (DPP).

Pulse polarography was first developed in 1960 by Barker[2]. In this method, the potential is only applied periodically in short time intervals (typically 60 msecs) to the working electrode. This pulse is applied once per drop and near the end of the drop lifetime. The resulting current is

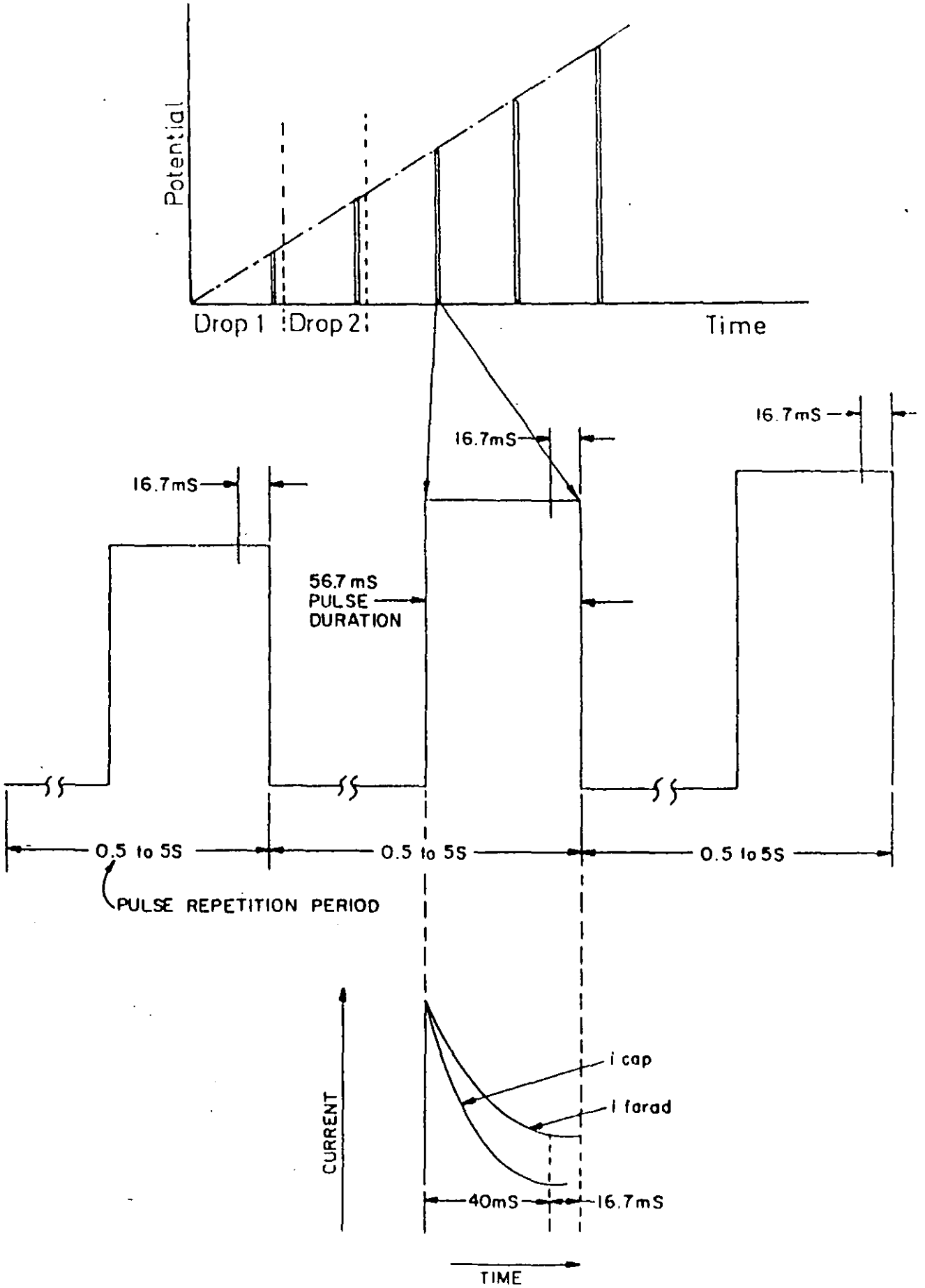
measured at a fixed time (usually 40 msec) after pulse application.

THEORY OF NORMAL PULSE POLAROGRAPHY

In normal or integral pulse polarography (NPP) potential pulses of successively increasing amplitude from a fixed initial potential are applied to the working electrode. The pulses are applied at a fixed time in the life of the drop, so a constant electrode area is maintained. Also, as the pulse is applied towards the end of the drop life, when the rate of change of the drop area, dA/dt, is small, the electrode may be treated as a stationary electrode.

Current values (either "instantaneous" or averaged) are measured in the last 20 msecs of the pulse. The time between pulses is usually 0.5 to 5 seconds. An electronic integration procedure is used in the case of measuring currents over a finite period towards the end of pulse duration and hence the name 'integral pulse polarography' is sometimes used instead of normal pulse polarography. The output is the difference between the current measured over the selected interval after the pulse duration and the current application when the electrode is at the initial potential. The potential waveform and the resulting current time behaviour for NPP is shown in fig. 1.

As with classical DC polarography, NP polarography has two sources of current resulting from pulse application, the faradaic current, I_f, and the capacitance current, I_c. As can be seen from fig. 3, after 40 msec of pulse application, I_c has decayed to almost zero, and is negligible compared to I_f. Therefore the measured current has more faradaic current than DC polarography. The limiting current of NPP is diffusion controlled as in DCP. For a reversible reaction the normal pulse current on the diffusion plateau, $i_{d_{NPP}}$, given by the Cottrell equation[3]



Fig·3 POTENTIAL WAVEFORM & RESULTING CURRENT FOR PULSE POLAROGRAPHY

$$i_{d_{NPP}} = n F A D^{\frac{1}{2}} C / (\pi t_m)^{\frac{1}{2}} \quad \ldots (3)$$

where $i_{d_{NPP}}$ is in μA; n is the number of electrons involved in the electron transfer process; F is the Faraday (96500 coulomb/equivalent); A is the electrode area in cm^2; D is the diffusion coefficient of the reacting species in cm^2/sec; C the bulk concentration in mM; and t_m the time in seconds, measured from pulse application, at which current is measured.

As in the case of DC polarography the limiting diffusion current is directly proportional to C, the bulk concentration. Instead of the faradaic current increasing with $t^{1/6}$ as in the Ilkovic equation (equation 1), $i_{d_{NPP}}$ decreases as $t_m^{\frac{1}{2}}$. Therefore the measurement time should be made as small as possible in order to maximise the signal, but large enough so that I_c is negligible.

The sensitivity of normal pulse polarography with respect to DC polarography is found by calculating the ratio of the normal pulse current, $i_{d_{NPP}}$, from the Cottrell equation (equation 3) to the DC current obtained from the Ilkovic equation (equation 1), substituting for the area term A (term for growing drop)

$$A = (4\pi)^{1/3} (3 \times 10^{-3}/d_{Hg})^{2/3} m^{2/3} t_d^{2/3} \quad \ldots (4)$$

where d_{Hg} is the density of mercury, 13.554 g/cm^3 at 25°C; m is the mercury flow rate in mg/sec; and t_d is the droptime in sec, and including the factor $(7/3)^{\frac{1}{2}}$ of the Ilkovic equation, the Cottrell equation is changed to

$$i_{d_{NPP}} = (462 n D^{\frac{1}{2}} C m^{2/3} t_d^{2/3}) / t^{\frac{1}{2}} \quad \ldots (5)$$

$$i_{d_{DC}} = (7/3)^{\frac{1}{2}} 462\ n\ D^{\frac{1}{2}}\ C\ m^{2/3}\ t_d^{1/6} \qquad \ldots (6)$$

$$\frac{i_{d_{NPP}}}{i_{d_{DC}}} = \left(\frac{3t_d}{7t_m}\right)^{\frac{1}{2}} \qquad \ldots (7)$$

For reasonable values of t_d and t_m this ratio lies in the range 6-7[4].

THEORY OF DIFFERENTIAL PULSE POLAROGRAPHY

In differential pulse polarography a normal DC potential ramp is applied to the system. Near the end of the drop life a small amplitude pulse of fixed height (typically 5-100 mV) is superimposed onto the ramp. The current is sampled twice during the life time of each drop; once just before applying the pulse and once just prior to the end of the pulse (when the capacitance current has decayed). Both these sample periods are the same and in the PAR 174A which has been used throughout this study the time period is 16.7 msec (see fig. 4). As the measured signal is the difference in current, zero difference being obtained when the currents are equal, a peak-shaped curve is obtained, with the peak maximum occurring near $E_{\frac{1}{2}}$ (see fig. 1).

In differential pulse polarography, a constant current on a plateau, as in normal pulse polarography, is not being measured but the ratio at which the current increases to the plateau value as the potential is changed is. In NPP the current-potential relationship (for a reversible reaction) may be expressed as

$$E = E_{\frac{1}{2}} + RT/nF \cdot \log\left[(i_d - i)/i\right] \qquad \ldots (8)$$

If this equation is differentiated with respect to i and the Cottrell equation $i_d = n\ F\ A\ D^{\frac{1}{2}}\ C\ /\ (\pi t_m)^{\frac{1}{2}}$, is substituted for the limiting

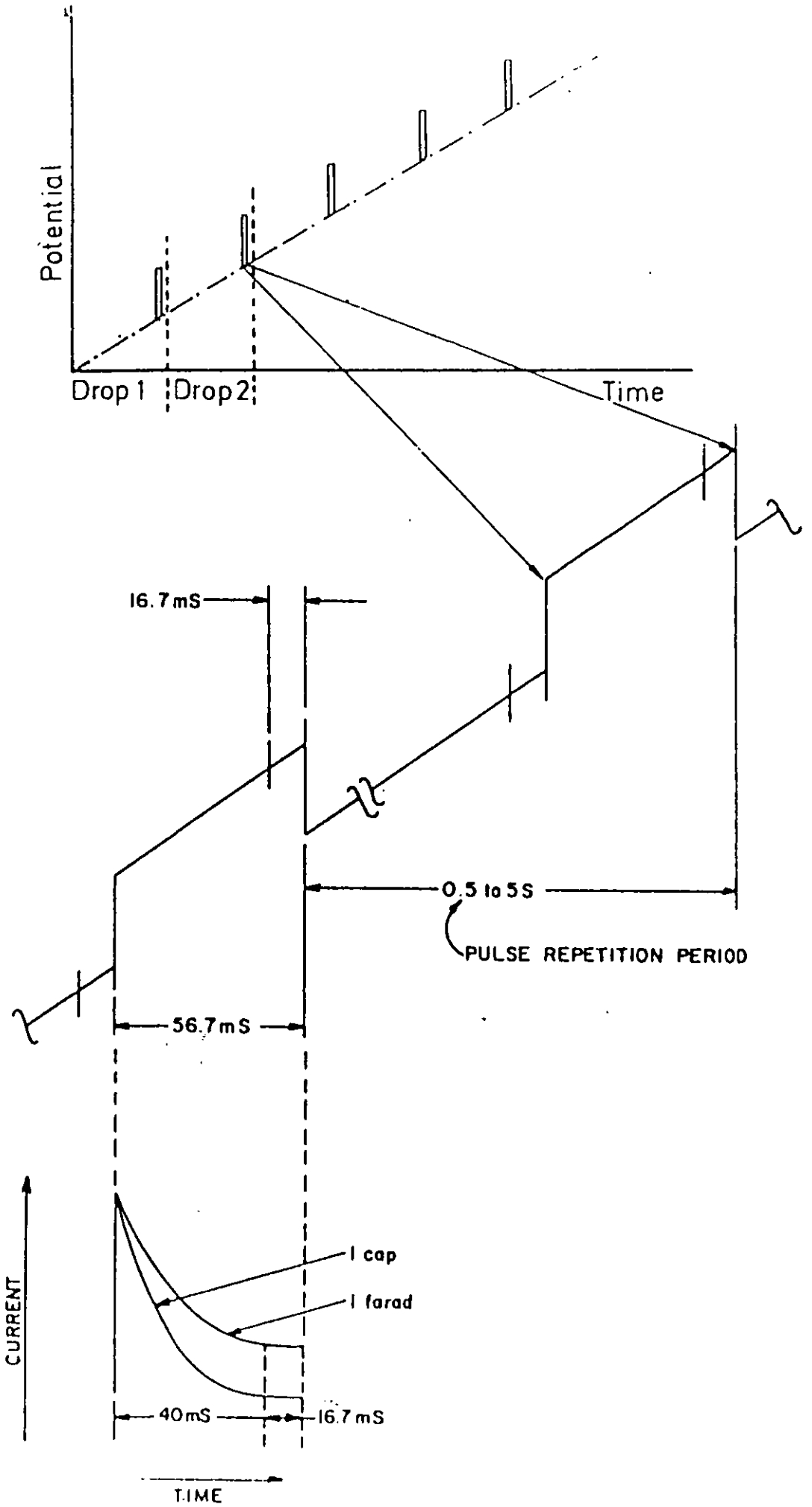


Fig. 4 . DIFFERENTIAL PULSE EXCITATION WAVEFORM & RESULTING CURRENT – TIME BEHAVIOR

diffusion-controlled current, i_d, an expression for the differential pulse current for a reversible reaction, Δi, is obtained.

$$\Delta i = (n^2 F^2 / RT) \cdot AC (+\Delta E^*) \cdot (D/\pi t_m)^{\frac{1}{2}} \cdot \frac{P}{(1+P)^2} \quad \ldots (9)$$

where ΔE = pulse amplitude; and $P = \exp.(E - E_{\frac{1}{2}}) \frac{nF}{RT}$. Maximising Δi with respect to E by differentiating and equating to zero leads to P = 1 for the differential pulse current being the peak or maximum differential pulse current $(\Delta i)_{max}$. Thus

$$(\Delta i)_{max} = \frac{n^2 F^2}{4RT} \cdot AC \cdot \Delta E (D/\pi t_m)^{\frac{1}{2}} \quad \ldots (10)$$

This equation is only valid for a small pulse application because a differential method is being approximated by the derivative.

When large pulses are used,

$$(\Delta i)_{max} = n F A C (D/\pi t_m)^{\frac{1}{2}} \left(\frac{\delta - 1}{\delta + 1}\right) \quad \ldots (11)$$

where $\delta = \exp nF/RT\ (-\Delta E/2)$. If $(-\Delta E/2)$ is smaller than RT/nF, then the small amplitude case prevails

$$(\Delta i)_{max} = (n^2 F^2/4RT)\ AC \cdot \Delta E \cdot (D/\pi t_m)^{\frac{1}{2}} \quad \ldots (12)$$

If $(-\Delta E/2)$ becomes very large with respect to RT/nF, then $(\delta - 1/\delta + 1)$ approaches unity, and the equation for the maximum current is simply the Cottrell equation (equation 3). The relationship between the peak

* (For reduction ΔE should be negative, but in practice the sign is often erroneously omitted in discussion of DPP. On other occasions it is omitted for simplicity.)

difference current in differential pulse, i_{DPP}, and the diffusion current in normal pulse, i_{NPP}, can then be written as

$$i_{DPP} = i_{NPP} \left(\frac{\delta - 1}{\delta + 1}\right) \quad \ldots (13)$$

The ratio (δ - 1/ δ + 1) depends only on the number of electrons, n, transferred and on pulse amplitude, ΔE, and is always less than unity. The pulse amplitude has a great effect on the DPP current. For small pulse amplitudes i_{DPP} increases roughly linearly with ΔE, and therefore gives better sensitivity. However at large values of ΔE the relative increase in current obtained by increasing ΔE is less, and the problem of capacitance currents gets worse. In practice the optimum pulse amplitudes to maximise sensitivity lie in the range 50-100 mV.

In NPP the current increases linearly with n (modified Cottrell equation)

$$i_{NPP} = (462\ n\ D^{\frac{1}{2}}\ C\ m^{2/3}\ t_d^{2/3}) \ /\ t^{\frac{1}{2}} \quad \ldots (14)$$

while in DPP, i_{DPP} increases linearly with n^2 for a small pulse amplitude

$$\Delta i = (n^2\ F^2/RT).AC.\Delta E.(D/\pi t_m)^{\frac{1}{2}} \cdot \frac{P}{(1 + P)^2} \quad \ldots (15)$$

This suggests the higher sensitivity of DPP over NPP for compounds in which the number of electrons involved in the electrochemical reaction is large.

From

$$(\Delta i)_{max} = n\ F\ A\ C\ (D/\pi t_m)^{\frac{1}{2}} \left(\frac{\delta - 1}{\delta + 1}\right) \quad \ldots (16)$$

it is seen that $(\Delta i)_{max}$ is a linear function of concentration. This is

true for electron transfer processes that have a fast reaction rate (reversible) and many other electrode processes other than the reversible case. However, if the electrochemical reaction rate of the substance sought is slow, even a small change or changes in matrix from sample to sample can cause major changes in reaction rates and therefore major changes in the peak height-concentration ratio.

Resolution

The peak-half width, $W_{\frac{1}{2}}$, which is a measure of resolution is dependent on the pulse amplitude and the number of electrons involved. As $\triangle E$, pulse amplitude, is increased so is the value of $(\triangle i)_{max}$, from

$$(\triangle i)_{max} = n\ F\ A\ C\ (D/\pi t_m)^{\frac{1}{2}}\ (\frac{\delta - 1}{\delta + 1}) \qquad \ldots (17)$$

However as the pulse amplitude increases so does the peak-half width, which leads to a decrease in resolution which is undesirable. At small $\triangle E$, the peak-half width is independent of pulse amplitude and inversely proportional to the number of electrons transferred.[5] In practice values of $\triangle E$ between 10 and 100 mV are used as a compromise between adequately large values of $(\triangle i)_{max}$ and adequate resolution.

Peak Potential-Half-Wave Potential Relationship

The relationship between peak potential, E_{peak}, and half-wave potential, $E_{\frac{1}{2}}$, is given by

$$E_{peak} = E_{\frac{1}{2}} - \triangle E/2 \qquad \ldots (18)$$

For infinitely small pulses the peak potential will occur at the polarographic half-wave potential. Thus for a reduction process ($\triangle E$ is negative),

the peak potential is shifted in a positive (anodic) direction.

Relative Sensitivities and Limits of Detection of NPP and DPP

The relationship between the faradaic current in DPP (peak difference current), i_{DPP}, and the faradaic current in NPP, i_{NPP}, is

$$i_{DPP} = i_{NPP} (\delta - 1/ \delta + 1) \quad \ldots (19)$$

As $(\delta - 1/ \delta + 1)$ is always less than unity it follows that i_{DPP} is always less than i_{NPP}. Nevertheless the detection limit in DPP is lower than that of NPP. In NPP each pulse has a greater amplitude than the previous one and during the course of the scan the pulses become quite large. This results in a large capacitance current, which in turn produces a steeply sloping base-line which is the main limitation on detection limit. In DPP each pulse has the same amplitude (50-100 mV) which results in smaller capacitance background currents. The lower capacitance currents in DPP permit detection limits about ten times lower than NPP, also the resulting current is relatively noise-free, making it amenable to signal processing; i.e. amplification. Further, DPP provides peaks which are more easily measurable at low species concentrations instead of steps as in the case of NPP.

All the equations previously quoted are for a reversible reaction, in which the diffusion of electroactive material to the DME solely governs the electrode process. For the irreversible case, in which the diffusion process is not the fastest step in the electrode process, the waveforms are quite different (see fig. 5).

This can be particularly noted in the Differential pulse case, the net result being that peaks are broader and smaller.

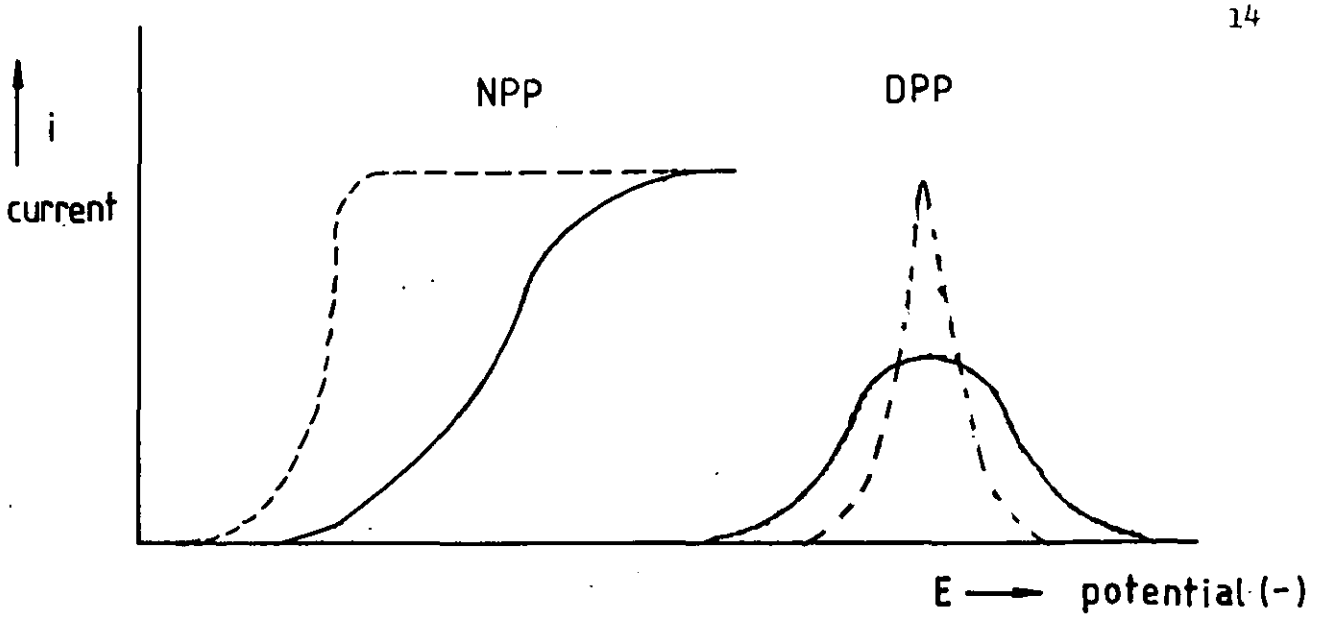


Fig. 5

---------- reversible case

———————— irreversible case

The theory and application of NPP and DPP have been reviewed extensively by A.M. Bond (1980).[4] Other important polarographic and related techniques are:

1. Rapid DC polarography, in which very short controlled droptimes are used, typically t = 0.16 sec (regulated by a mechanical drop knocker). This enables the application of scan rates of potential up to several hundred millivolts per second, and the suppression of maxima without the addition of surfactants. Reproducibility and precision of results is maintained with the increase in scan rate, and time saved. However the larger capacitance-to-faradaic current ratios obtained, due to the short droptime, increase the limit of detection.

2. Current-sampled DC (also known as Strobe and Tast polarography)
This technique follows on from the fact that the longer the drop lifetime, the more favourable the faradaic-to-capacitance current ratio. Hence by recording only the current flowing through the cell near the end of the drop life e.g. the last 5 to 20 msec of a 5 second drop, optimization of sensitivity is obtained in DC polarography and the lower the concentration

limit that can be detected. Polarograms obtained are simpler as the virtual elimination of the serrations due to the continuous mercury drop-fall-drop growth sequence is achieved, current measurements can be evaluated more exactly. However the increase in sensitivity is only marginal and several superior methods for discriminating against the charging current are available, e.g. pulse techniques.

3. Derivative DC polarography As the name indicates, this technique consists of a plot of di/dE versus E and a peak-shaped curve rather than a sigmoidal curve should result. Satisfactory first- or second-derivative DC polarograms can be recorded at concentrations as low as 10^{-7} M for reversible electrode processes[6], which is about one order of magnitude improvement over that offered by normal DC polarography. Resolution between compounds with similar $E_{\frac{1}{2}}$ values is improved over classical DC polarography. Its main disadvantage is that if the DC wave obtained exhibits kinks, inflections or other irregularities, then the derivative may have more than one peak and be unsuitable for analytical work.

4. Linear sweep voltammetry (LSV) is based on utilizing a rapid linear sweep of the potential range under consideration (usually 100 to 200 mV s^{-1}). When applied to the DME, this technique enables the entire potential range to be covered in one drop. Also in LSV the assumption that constant potential-current curves are being recorded is lifted, and the theory is solved under conditions of a continuously changing potential. For theoretical purposes the potential is considered to have been applied to a stationary electrode, because during the recording of the i-E curve the growth of the drop is negligible. Therefore theory presented for LSV at a DME can be applied to any stationary electrode, e.g. platinum, carbon and gold. The typical curve obtained by this method is represented below in fig. 6.

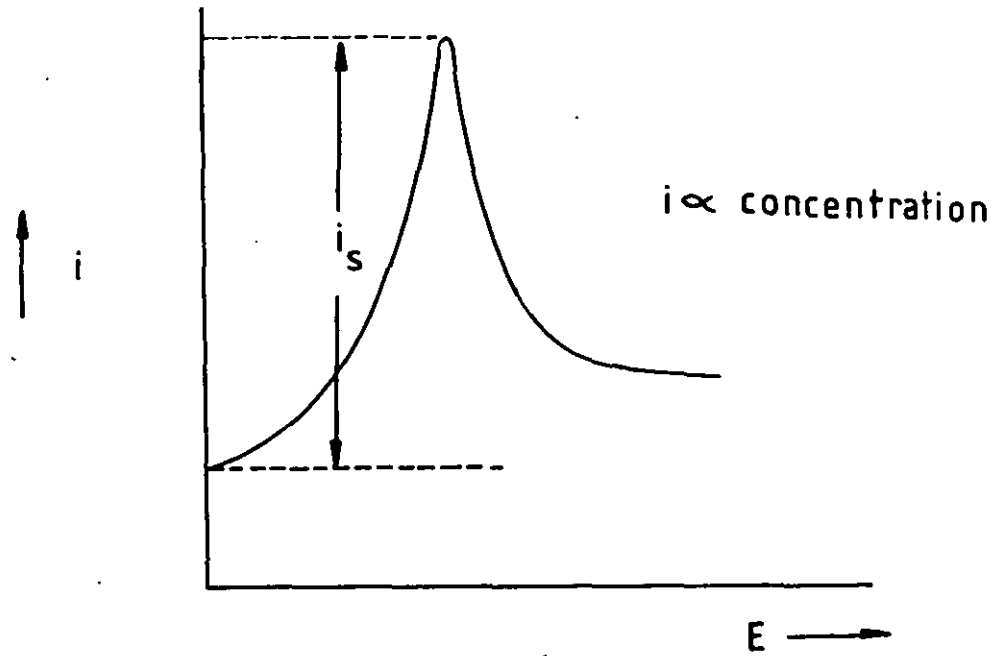


Fig. 6

(i_s = sample current)

The reason for the peak shape is due to depletion of reducible material in the vicinity of the electrode surface.

In analytical work LSV is most frequently applied at mercury electrodes, because of the constantly renewed surface and the high reproducibility of the electrode area obtainable, particularly at a DME. Other variations of mercury electrodes that have been used are the Hanging Mercury Drop Electrode (HMDE) and mercury pool electrodes. A plethora of solid electrodes are also available. Apart from the inert metals such as platinum, gold, silver and iridium, electrodes based on carbon materials have been widely used, e.g. carbon paste, glassy carbon, boron carbide and wax-impregnated graphite. These electrodes are used when the electroactive species oxidises or interacts in some way with mercury or strong adsorption of material onto the surface of the electrode produces unsatisfactory waves.

In routine analytical work the advantage of LSV is (i) in its sensitivity which is in aqueous media 10^{-7} to 10^{-6} M, about one order of magnitude better than DC polarography, and (ii) in the speed of recording the voltammogram.

5. A modification of this rapid-scan technique is Cyclic Voltammetry. The linear potential ramp is applied as in LSV, however following the reduction of interest the potential scan direction is reversed and the voltage proceeds back towards its initial value with the same rate at which it had previously increased. Material reduced in the forward sweep, if stable, may be oxidized back to starting material on the return sweep. In analytical work, cyclic voltammetry is not often used since the forward sweep usually gives the required analytical data, but in the elucidation of electrode mechanisms, the extension to reverse sweep is extremely valuable. The degree of reversibility of the electrode reaction may be determined as the complete cyclic voltammogram may include cathodic and anodic current components. If a reversible process of the $A + ne \rightleftharpoons B$ type occurs, the peak heights of the reduction and oxidation processes are equal.

6. Alternating Current (AC) Polarography A significant improvement in signal-to-noise ratio and hence in the sensitivity of DC polarography can be realised by superimposing a small sinusoidal alternating voltage (10 to 100 mV) on the DC potential ramp, then measuring the AC component of current. The rapid, small variations in potential produce a corresponding rapid variation in the ratio of oxidized to reduced form of the electroactive species present, which results in an alternating current. For a reversible system the magnitude of this alternating current is greatest at the half-wave potential, $E_{\frac{1}{2}}$, when the concentrations of the oxidized and reduced forms are equal. The importance of this method lies in the possible discrimination against capacitance currents. In any AC network, alternating currents possess a particular phase relationship to the input voltage, and the magnitude of the currents at different angles to this are related to vector algebra. Therefore if the capacitance and faradaic

currents possess a different phase angle relationship to each other it is possible to measure AC current at selected phase-angles and at a particular phase-angle to measure pure faradaic currents only.

7. Stripping Voltammetry This technique is used when the reduction product of the electroactive species in solution forms (i) an amalgam with the mercury electrode, or (ii) a plated film on a solid electrode. On reversing the scan, as in cyclic voltammetry, the metal is stripped from the amalgam and oxidized back into the solution. Practically, a controlled-potential electrolysis step precedes the stripping step, the potential being held 300 to 400 mV more negative than the polarographic half-wave potential. This step ensures production of either a metal deposit on a solid electrode (plating) or an amalgam with mercury of a mercury drop. The resulting stripping current is larger than that obtained for the reduction process. This is because the electroactive species is concentrated on the working electrode (mercury drop or solid electrode), unlike normal polarography when the signal is the result of only a small amount of electroactive material being reduced from the bulk.

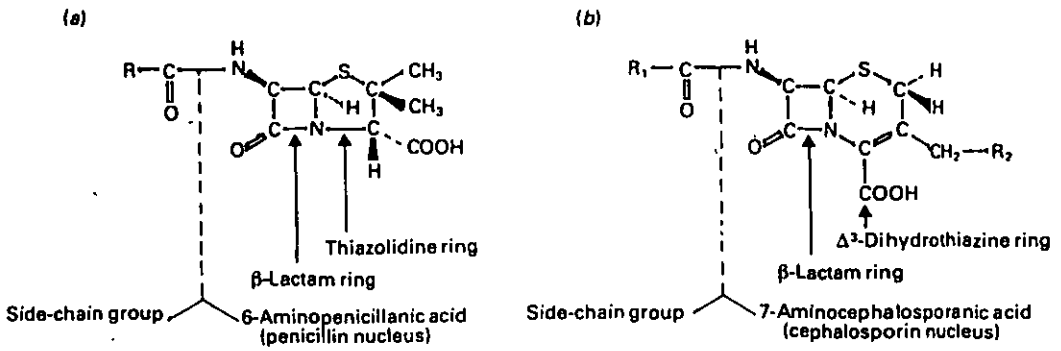
The result is a high faradaic-to-capacitance current ratio. For anodic stripping voltammetry the most widely used electrodes are the HMDE and a mercury thin-film electrode (MTFE).

A cathodic variety of stripping voltammetry is also possible. Mercury is more easily oxidised in the presence of certain anions, such as chloride bromide and sulphide to give insoluble mercury salts which can be stripped from the electrode by applying a negatively directed potential scan. As an analytical method stripping voltammetry is applied to concentrations in the 10^{-6} to 10^{-10} M range, and is therefore widely used in trace analysis.

B.

STRUCTURE OF β-LACTAM ANTIBIOTICS

All β-lactam antibiotics are derivatives of a bicyclic ring system. The penicillin molecule consists of a nucleus and a condensed side-chain group. The nucleus contains a four-membered β-lactam ring fused with a five-membered thiazolidine ring. The basic structure of penicillin is shown below.



Basic structures of (*a*) penicillins and (*b*) cephalosporins.

The cephalosporin ring system differs in so far as the four-membered ring is fused with an unsaturated six-membered dihydrothiazine ring. The basic structure of cephalosporins is shown above.

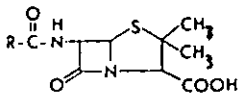
The following tables show the structure of some β-lactams of clinical importance.

Table B lists some cephalosporins of clinical importance.

Table A lists some penicillins of clinical importance.

Table C lists some non-classical β-lactams of clinical importance.

Table A The structure of some penicillins of clinical importance.



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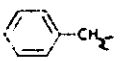
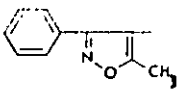
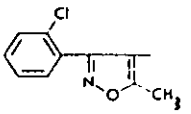
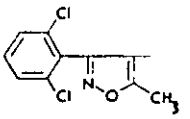
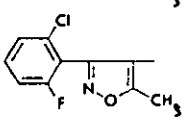
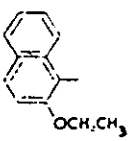
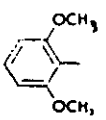
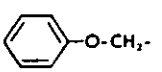
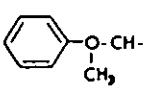
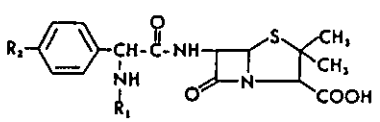
	Penicillin G
	Oxacillin
	Cloxacillin
	Dicloxacillin
	Flucloxacillin
	Nafcillin
	Methicillin
	Penicillin V
	Phenethicillin



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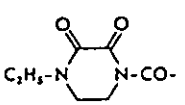
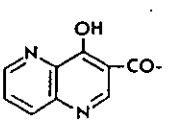
	H	Piperacillin
	H	Apalcillin

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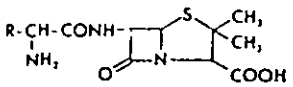

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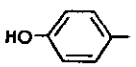
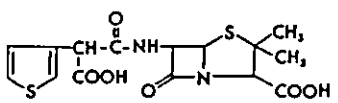
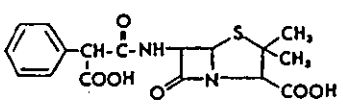
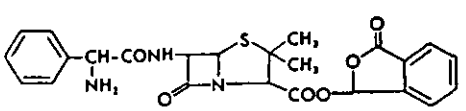
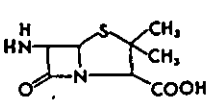
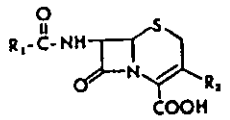
	Amoxicillin
	Epicillin
	Ticarcillin
	Carbenicillin
	Talampicillin
	6-APA

Table 8 The structure of some cephalosporins of clinical importance.



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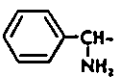
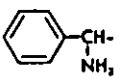
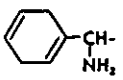
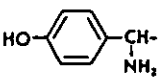
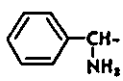
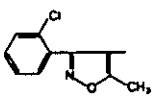
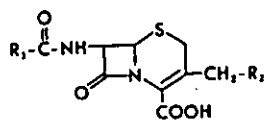
	$-CH_2OCOCH_3$	Cephaloglycin
	$-CH_3$	Cephalexin
	$-CH_3$	Cephradine
	$-CH_3$	Cefadroxil
	$-Cl$	Cefaclor
		Cephoxazole



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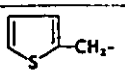
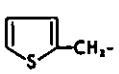
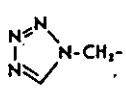
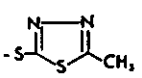
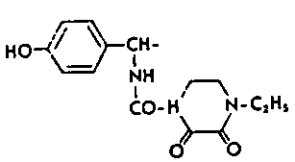
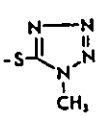
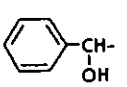
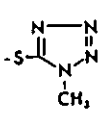
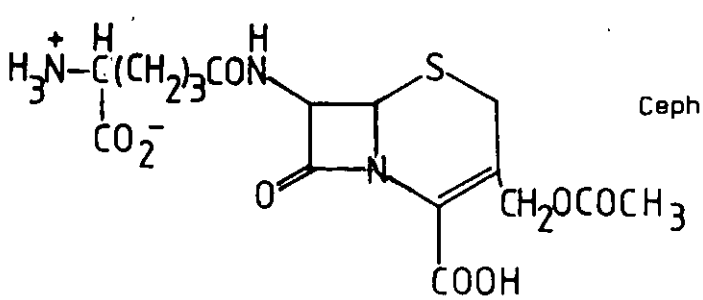
	$-OCOCH_3$	Cephalothin
		Cephaloridine
		Cefazolin
		Cefoperazone
		Cefamandole



Cephalosporin C

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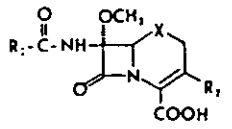

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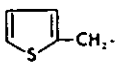
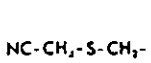
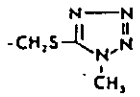
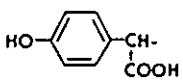
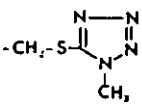
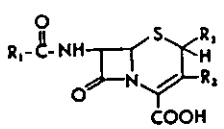
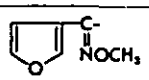
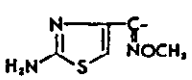
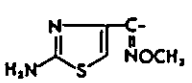
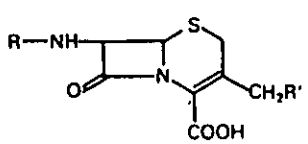
		S	Cefoxitin
		S	Cefmetazole
		O	Moxalactam



R_1	R_2	R_3	Name
	$-CH_2OCONH_2$	-H	Cefuroxime
	$-CH_2OCOCH_3$	-H	Cefotaxime
	-H	-H	Ceftizoxime



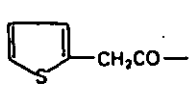
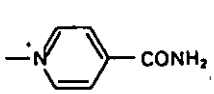
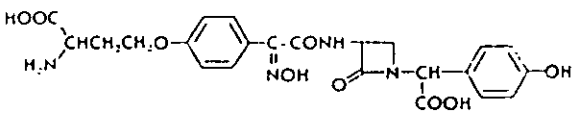
R	R′	
		Cephalonium
H–	–$OCOCH_3$	7-ACA
H–	–H	7-ADCA

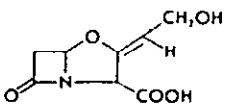
Table C The structure of some non-classical β-lactams of clinical importance.

R

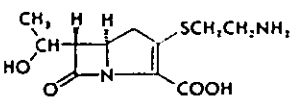
Name



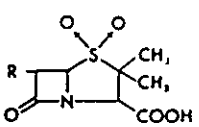
Nocardicin A



Clavulanic acid



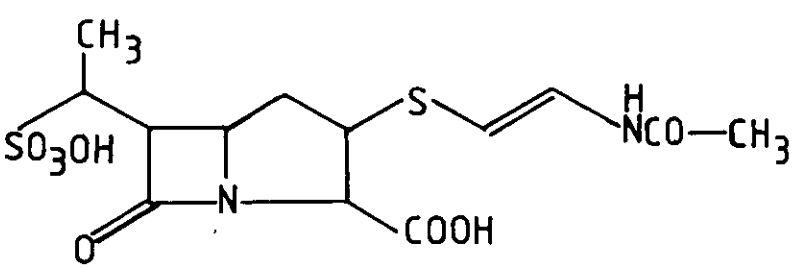
Thienamycin



Sulphone

R = H, Penicillanic acid sulphone.

R =Cl, 6α-chloro-penicillanic acid sulphone.



Olivanic acid

C.
HISTORY AND DEVELOPMENT OF β-LACTAM ANTIBIOTICS

In 1929[7] Sir Alexander Fleming made his historical observations of the antibacterial effect of Penicillium on Staphylococcus aureus at St. Mary's Hospital, London. Attempts to isolate the active material were frustrated due to its instability, until work by Florey and Chain led to the purification of "penicillin" and to the demonstration of its remarkable therapeutic effect against infectious bacteria. These discoveries proved to be the forerunners of others which led to the development of a large family of antibiotics with clinical value, the penicillins and cephalosporins[8].

The history of the cephalosporins is clearly connected with that of the penicillins. In 1945, when the therapeutic properties of penicillin became known, in Sardinia, Giuseppe Brotzu isolated an antibiotic-producing organism from sewage outfall. This was later identified as Cephalosporium acremonium. Further work on this fungus by Abraham and

Newton led to the discovery of cephalosporin C, the parent substance from which the first cephalosporins to find clinical use were derived[9].

These naturally occuring antimicrobial agents have a single structural feature in common, the possession of a β-lactam ring and are commonly referred to as the β-lactam antibiotics. The importance of these antibiotics is their inhibition of the terminal step in bacterial wall synthesis, the β-lactam structure is mistaken for a dipeptide, D-alanyl-D-alanine, a vital component of the cell wall, which leads to bacteriostasis, inhibition of cell growth[9]. Penicillins and cephalosporins are active against a broad spectrum of infectious bacteria, and in addition cephalosporins are generally resistant to one of the main mechanisms of defence of the bacteria cell, the β-lactamase enzyme, and have a low frequency of allergic response. Isolation of the penicillin nucleus, 6-aminopenicillanic acid (6-APA) in 1959[10] and the cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA), in 1961[11] has enabled the production of many semisynthetic β-lactams (by varying the sidechain groups) which could not be produced by fermentation (biosynthesis).

These semisynthetic cephalosporins and penicillins have unique characteristics that have made them especially useful in clinical medicine. These properties are primarily of two types: (a) more desirable antibacterial activity, and (b) more desirable pharmacological properties, e.g. improved acid stability, decreased allergenicity and improved metabolic efficiency.

It has now been over fifty years since the discovery of the first β-lactam antibiotic, penicillin, and forty years of clinical experience has been accumulated. Despite the emergence of these new semisynthetic drugs there are still numerous bacterial infections for which naturally produced penicillin G, the "original penicillin" isolated from a bacterial broth (also known as benzylpenicillin,) (general tables), remains the "drug

of choice". A list of the achievements of using penicillin G are in Table I.

Table I. Achievements of using penicillin G[29]

1. Permitted cure of frequently fatal infections, e.g. pneumonia, meningitis, endocarditis.
2. Reduced morbidity, mortality, length (or need) of hospital stay, need for surgery, and permanent complications of many infections.
3. Permitted true cures, without need for continued administration of the drug.
4. Caused inhibition of many species of bacteria.
5. Worked synergistically with other antibiotics (e.g. aminoglycosides).

The major shortcomings of penicillin G are listed in Table II.

Table II. Major shortcomings of penicillin G[29]

1. Not effective against "resistant" organisms.
2. May cause adverse effects on patients, such as allergy or "toxicity". Immune-related side effects to penicillin G occur in about 3% of the population.
3. Relatively poor oral absorption.
4. Short half-life.
5. Irritates veins when given in high doses, causing pain and obstruction.
6. Relatively poor penetration into the central nervous system.

Faced with these problems the medicinal and organic chemists have succeeded in producing an enormous number of variations on Nature's theme, motivated by a continuing need for both broad and narrow spectrum β-lactams of low toxicity for the treatment of disease in man and animals.

First the development of the penicillin group of β-lactams can be considered. Another biosynthetic penicillin is penicillin V, or phenoxymethylpenicillin (general table). Its major advantage over penicillin G was that it had better oral absorption and was more acid stable. However, these two pharmacological properties were counterbalanced by decreased activity against certain gram-positive organisms.

Semisynthetic antistaphylococcal penicillins revolutionized the in- treatment of infections due to Staphylococcus aureus, which was a problem that tormented patients and their physicians in the 1950s. The fact was that over 90% of such infections were caused by penicillin G resistant strains, which produced enzymes capable of attacking the β-lactam ring and thus rendered the antibiotic ineffective. These enzymes were named β-lactamases. The problem of the chemist was to produce a compound that was resistant to the β-lactamases but still retained antibacterial activity. With the commercial availability of the penicillin nucleus, 6-aminopenicillanic acid, 6-APA (general table), the production of clinically useful products by acylation proved to be highly successful. Examples of semisynthetic penicillins highly resistant to staphylococcal β-lactamase and of proven use in staphylococcal infections are methicillin, naf cillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin (general table). Methicillin, for example, is highly resistant because of its poor affinity for the active site in the enzyme and its slow rate of hydrolysis. Acylation with a hindered side chain provides a β-lactamase stable penicillin.

Recognizable shortcomings of this group of semisynthetic anti- staphylococcal penicillins are shown in Table III.

19

Table III. Shortcomings of antistaphylococcal semisynthetic penicillins

1. Narrow spectrum of activity.
2. Increased likelihood of causing interstitial nephritis.
3. Extensive protein binding occurs and therefore there is not as much drug available for antimicrobial activity.
4. Increased likelihood of causing bone marrow suppression (especially leukopenia).
5. Possibility of staphylococcal resistance emerging.

Further alterations in the C-6 sidechain of penicillins produced the next group of penicillins, the broad spectrum semisynthetic penicillins. This group comprises two main series: (a) oral preparations and (b) parenteral preparations. Prime examples of orally active compounds are ampicillin and amoxicillin (general tables). These compounds have basic sidechain groups, are acid stable and are very active orally. This phenylglycine sidechain is common to all good orally active β-lactam antibiotics.

The other series are known as the "antipseudomonal" semisynthetic penicillins. Their clinical achievements have been primarily exhibited by carbenicillin, developed in 1967, and its thienyl analogue, ticarcillin (general tables). They both contain a free carboxylic acid group adjacent to the sidechain. The observation that an acidic group on the sidechain imparted antipseudomonal activity to penicillins dominated synthetic efforts for a considerable time.

The major clinical achievements of these two series was in providing relatively safe, bactericidal, often very broad spectrum, antibiotics that not only worked but that could be used in place of more toxic antimicrobial agents, such as the aminoglycosides. Their major disadvantage is bacterial resistance.

Examples of new penicillins recently introduced into the market are apalcillin, piperacillin and talampicillin (general tables). Piperacillin, with the introduction of the 2,3-dioxopiperazine moiety, has a potent antibacterial activity and a broad antibacterial spectrum, and shows excellent activity against Pseudomonas aeruginosa, and is widely believed to be the best penicillin yet produced. Talampicillin is an ester of ampicillin, particularly well absorbed and rapidly hydrolysed to give high blood levels of ampicillin. It is indicated for the oral treatment of a wide range of bacterial infections caused by commonly occurring pathogens. Apalcillin has a potent antipseudomonal activity. It is rather acid stable but binds heavily with human serum protein (98%).

The development of the cephalosporins somewhat parallels that of the penicillins. Cephalosporin C was the first microbially active cephalosporin to be isolated. Its level of activity was unfortunately too low for clinical usage to be contemplated. However, its wide range of antibiotic activity, its resistance to β-lactamases from penicillin resistant organisms and its innocuity made it remarkable. It took twenty years from the discovery of cephalosporins in 1945 to the point when the first effective drug of this series was launched onto the market in 1965 by the Lilly group. This was cephalothin, a parenteral drug, with a thienylacetyl side-chain (see general tables). It was effective against a variety of gram-positive bacteria including the penicillinase producing Staphylocci, and against a number of gram-negative bacilli. Cephalothin was followed by Glaxo's cephaloridine, another intravenously administered compound (see general tables), in which the acetoxy group at C-3 of cephalothin was replaced by a pyridinium group. The latter was not removed by acetyl esterases which converted cephalothin to the less active deacetylcephalothin in vivo.

The early cephalosporins such as cephalothin and cephaloridine were not absorbed from the gastrointestinal tract despite their relative stability to acid. However, cephalosporins with a D-phenylglycyl side-chain (like that of ampicillin) at C-7 were readily absorbed when given by mouth. The first of these compounds, introduced by Eli Lilly, was cephalexin, which also contained a methyl group in place of an acetoxy-methyl group at C-3 (see general table). A related compound, cephradine, containing a cyclohexadienylglycyl sidechain was then produced by Squibb (see general table). The main use of these drugs was against penicillin resistant organisms, mainly from gram-positive bacteria. In some cases, and particularly with Pseudomonas aeruginosa, resistance to the semi-synthetic cephalosporins that appeared was partly due to the poor ability of the β-lactams to penetrate the cell wall and reach their sites of action on the cytoplasmic membrane. But another important factor was the ability of many of the resistant organisms to produce a cell bound β-lactamase.

The 1970s have seen the appearance of a second generation of semi-synthetic cephalosporins with a high resistance to hydrolysis by a consider-able number of β-lactamases. They are mostly injectable preparations, lacking the phenylglycine sidechain, e.g. cephamandole (Lilly) and Cefuroxime (Glaxo) (see general table).

Another second generation semisynthetic cephalosporin is cefoxitin from Merck, Sharp and Dohme. It is different from previous cephalosporins in that the hydrogen at C-7 has been substituted giving a 7α-methoxy-cephalosporin (see general table). This class of compounds are known as the cephamycins. They are produced naturally from certain species of Streptomyces. This methoxyl group gives cephamycins great stability in the presence of a variety of β-lactamases. Another example of this group of semisynthetic cephalosporins is cefmetazole produced by Sankyo (Japan)

(see general table).

There is now a third generation of cephalosporins, which is likely to extend the range of infections that can be treated successfully by members of this family. Of these, cefotaxime, developed in 1975, marketed by Roussel, is the first to be launched on the market in the U.K. (see general table). This drug, with a methoximino group and an aminothiazole ring in the sidechain, shows an exceptionally wide spectrum of activity. It is administered intravenously and has exhibited activity against Pseudomonas and Bacteroides species. Others of this third generation are ceftizoxime, which is very similar to cefotaxime , cefoperazone (Toyama, Japan) and moxalactam (Shionogi and Lilly, Japan and U.S.A.) (see general table). Both are on clinical trial in the U.K., although moxalactam has been given a limited launch in W. Germany. Cefoperazone has a similar sidechain to the most successful penicillin to date, piperacillin, namely the 1,-ethyl-2,3-dioxopiperazine moiety. It shows strong antibacterial activity against gram-negative bacilli including P. aeruginosa in comparison with second generation cephalosporins. Moxalactam (6059-S) contains oxygen in place of sulphur in its six-membered ring and also a 7α-methoxy group. This 1-oxa cephalosporin has a complete gram-negative spectrum and is highly resistant to β-lactamases. Replacement of 1-thia to 1-oxa in the cepham nucleus generally results in a 4- to 16-fold increase in the antibacterial activity of cephalosporin derivatives.

Anti-infective drugs account for the largest fraction (usually about one-third) of pharmacy expenditures in large general hospitals, and within this group, in many hospitals, cephalosporins account for a larger fraction of expenditure than any other group of antibiotics. Although a large fraction of their use is for prophylaxis related to surgery, the fact that most cephalosporins have very broad spectra, and are quite safe, accounts for their wide usage. The specific advantages of cephalosporins are listed

in the next table.

Table IV. Advantages of cephalosporins [29]

1. They are useful in some patients allergic to penicillins, especially when there has been a delayed reaction.
2. They are active against a wider range of gram-negative organisms than are most penicillins.
3. Some cephalosporins (especially cefoxitin and to a lesser extent cefamandole) have activity against some gram-negative anaerobic organisms.
4. Some have delayed renal excretion permitting fewer doses and higher blood levels with a given dose.

Cephalosporins and penicillins are referred to as "classical β-lactams" as they possess a cepham and penam nucleus respectively. Those with an unusual nucleus are termed "non-classical β-lactams". Biologically valuable deviations from these nuclei include 1-oxacaphalosporins (such as moxalactam); the carbapenam group, including thienamycin and olivanic acid; sulphones; clavulanic acid and nocardicins (see general tables). Moxalactam has been discussed previously in the cephalosporin section, due to its biological similarity to the cephamycins. All the others are useful for their β-lactamase inhibitory properties.

The carbapenam derivatives include thienamycin, a natural product isolated from Streptomyces cattleya. Discovered by the Merck company in 1976, it has a highly potent spectrum. Its β-lactamase stability against pseudomonads surpasses cefotaxime and moxalactam. Unfortunately it is not being considered as a candidate for clinical trials owing to its unacceptable stability in concentrated solution and solids. The search for a thienamycin analogue, which is equivalent biologically and stable at high

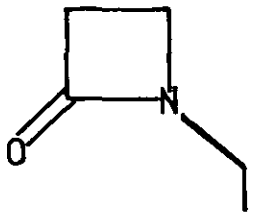
concentrations is now underway. The other carbapenam derivative is olivanic acid. A natural product from Streptomyces olivaceous, it has a broad antibacterial spectrum and it is a potent β-lactamase inhibitor. Clavulanic acid has been discovered in the last 5 years, being isolated from a broth of Streptomyces clavuligarus. Its structure is characterized by the lack of an amide sidechain and by the 1-oxopenam nucleus. It exhibits weak antibacterial activity, but its main property is its progressive and irreversible inhibition of β-lactamases from gram-positive and gram-negative bacteria. It exhibits synergistic effects with classical β-lactams and formulated with amoxicillin it is being marketed by Beechams as the orally taken "Augmentin". Clinical trials are also being carried out on a compound containing clavulanic acid and ticarcillin.

Another natural isolate is norcardicin A, extracted from a species of Norcardia, Norcardis uniformis sub. tsuyamensis has a unique monocyclic structure. It possesses weak intrinsic biological activity against gram-negative bacteria, especially P. aeruginosa, Proteus and Neisseria groups. It is fascinating because of its mode of action. Unlike the classical β-lactams it does not have an effect on cell wall linkages but rather sensitises the bacterium to phagocytotic killing by the host's white cells.

The last of these non-classical β-lactams are the sulphones, which act as irreversible β-lactamase inhibitors. They exhibit an advantage over clavulanic acid in having prolonged stability in buffer and body fluids. Examples of compounds being investigated are penicillanic acid sulphone and 6α-chloro-penicillanic acid sulphone.

Before 1970, it seemed that only those compounds based on the cepham or penam nuclei would be successful in antibacterial activity. However the discovery and research of the last twelve years has changed this preconception, with the advent of, for example, moxalactam, the carbenapenams, clavulanic acid, norcardicin A and the sulphones. It seems that

the minimal structural requirements for activity are as below.



Acidic function

The further requirements of optimal activity are the possession of a sidechain bearing a hydrogen bonding group and some means of increasing the chemical reactivity of the β-lactam group such as incorporating ring strain. Additional β-lactamase stability is achieved by appending a 3α-substituent or using a hindered amide sidechain.

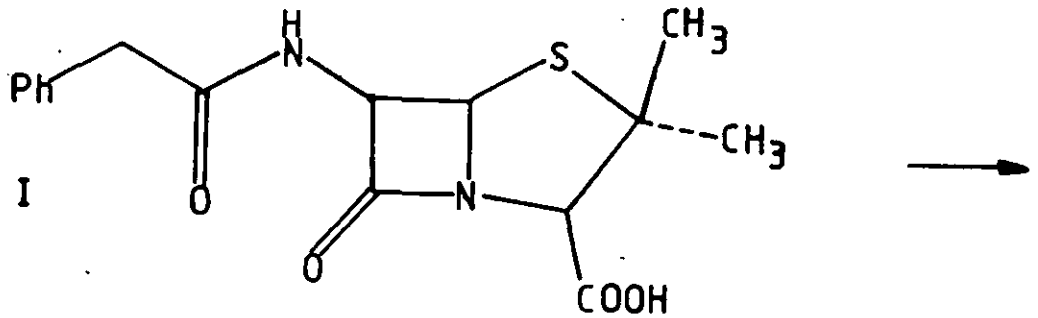
D. SYNTHESIS OF β-LACTAM ANTIBIOTICS

As mentioned previously the isolation of 7-ACA, and 6-APA, the cephalosporin and penicillin nuclei respectively, enables the synthesis of many biologically valuable compounds that could not be produced by biosynthetic techniques. Both of these parent compounds are cleavage products of antibiotics produced by fermentation. 6-APA is manufactured from penicillin G and 7-ACA from cephalosporin C. However cephalosporin C and its cleavage product 7-ACA are several times more expensive than penicillin G and its cleavage product 6-APA. This is because isolation of penicillin G from fermentation broths is simple, whereas that of cephalosporin C is laborious and costly. This price difference between the raw materials is reflected in the cost of therapeutic treatment itself. Oral treatment is less expensive than injections due to the fact that even for the same product the pharmaceutical manufacturing costs differ. Injectable cephalosporins, however, are manufactured by chemical transformation starting from cephalosporin C. Orally active cephalosporins are prepared by rearranging a penicillin, the cost price of which is much lower than that of cephalo-

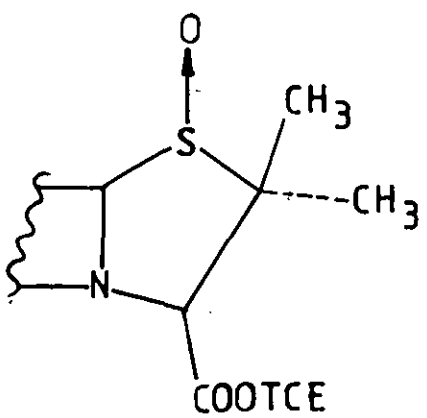
sporin C. However, although oral cephalosporins are less expensive than other cephalosporins their cost price is still higher than that of penicillins, the reason being that their preparation involves a larger number of chemical steps. It takes about six steps to produce the orally active cephalexin from penicillin G. In contrast the preparation of penicillins needs only two or three steps. The number of steps gives a good indication of the relative prices of products obtained.

Prompted by these large cost differentials favouring penicillins and by the clinical success of the cephalosporins, different hemisynthetic preparative routes leading to cephalosporins have been examined. Since the initial work by Morin[12,13] *et al.* in 1963 on the conversion of penicillin sulphoxides to deacetoxycephalosporins, an avalanche of papers has followed. These have recently been reviewed[14-20].

Ring expansion from the thiazolidine, penicillin G, (I) is achieved after protection of the acid function by trichloroethyl ester (TCE) and oxidation of the penicillin sulphur atom to the sulphoxide, (II).

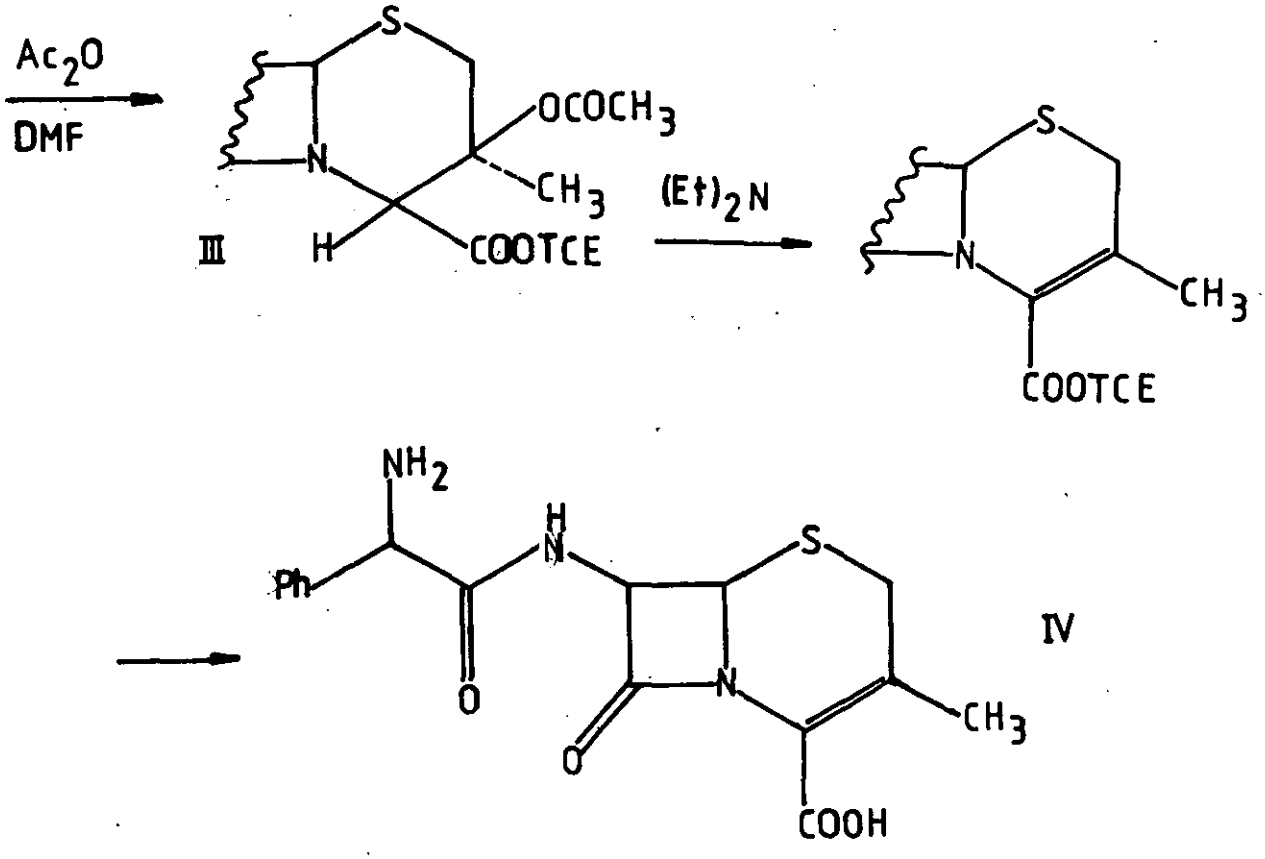


I



II

Refluxing in 5% acetic anhydride (Ac_2O) in dimethylformamide (DMF) gives 60% yield of the 6-membered ring compound (III). Treatment with base results in elimination of acetic acid. Sidechain rearrangement and deprotection of the carboxyl group results in cephalexin (IV).



With regard to total synthesis, no great progress has been made since Woodward in 1966[21]. This fifteen step route cannot compete with natural sources for preparing the classical compounds at present on the market.

To demonstrate the intense activity of research into β-lactams, 150 independent research groups put forward patent applications between 1975 and 1978. The American Chemical Society publishes through its Chemical Abstract Service around 300 abstracts of papers on all aspects of β-lactam antibiotic chemistry (analysis, synthesis, patents, pharmacology, reviews) each month in special " β-Lactam Antibiotics" C.A. select pamphlets.

The broader achievements of the use of β-lactam antibiotics and in particular their impact on society are listed below in Table V.

TABLE V [29]

1. Decreased fear of illness.
2. Decreased fear of childbirth.
3. Increased sexual freedom (less fear of venereal disease).
4. Increased life expectancy.

In recent years excellent reviews of the chemistry[19,20,22-30], pharmacology[31-37,9] and structural-activity relationships[38,39] and clinical aspects[40,41] of these drugs have been published.

E. ANALYSIS OF β-LACTAM ANTIBIOTICS

The development phase of any new potential drug from the first biological screening that demonstrates interesting pharmacological activity to full-time marketing is an expensive and time consuming process. The analytical chemist is involved at all stages of this development, developing methods for formulation, stability indication, quality control, drug metabolism studies and identity of related substances and degradation studies.

Many analytical technqiues have been described for β-lactam antibiotics. In many instances these methods were first developed for penicillin, but owing to the presence of the β-lactam ring, a feature common to both cephalosporins and penicillins, many of these techniques have also been utilized for cephalosporins.

In clinical analysis the β-lactam antimicrobics as a class are

measured frequently, but individually are measured only infrequently. Techniques used up to 1970 have been reviewed by Marelli[42]. Methods used up to 1975 have been reviewed by Hughes et al.[43]. A list of the major techniques and a discussion of their advantages and disadvantages are given below.

1. Microbiological assays

These methods, which include plate assays and photometric assays, provide quantitative results. They are the British Pharmacoepia standard methods for the determination of drug potency. These methods are normally used for β-lactam determination in complex formulations and at the low levels encountered in biological fluids. This is because of the high blanks obtained by chemical methods, caused by sample impurities. Microbiological analysis is very sensitive, going to the nano-gram level but more often at the micro-gram level. However, its lack of selectivity means that similar antibiotics cannot be distinguished from each other. The techniques are also very time consuming, taking from 12 to 16 hours for an assay and therefore lacking the capability to function as a rapid screening test.

2. Chemical methods

Although more rapid than microbiological assays, chemical methods suffer from limited specificity. Similar antibiotics and their degradation products cannot be distinguished from each other and hence the chemical purity of the compound cannot be ascertained.

These chemical methods are usually used for serial analysis, examples of which are below.

(a) The Iodometric method

A titrimetric method in which the alkaline hydrolysis product of the β-lactam antibiotic, the penicilloic acid or the cephalosporoic acid is

reacted with iodine, excess iodine being titrated with thiosulphate. First reported by Alicino in 1946[44] and modified for semisynthetic compounds in 1961[45], this method is still the standard assay for cephalexin, cephalothin, cephaloridine, cephradine, penicillin G and penicillin V in the British Pharmacoepia (1980). Degradation products with intact β-lactam ring also react with iodine, therefore giving a false assay.

(b) The Hydroxylamine method

Another chemical method is the hydroxylamine method[46,47], a colorimetric assay in which the β-lactam molecule reacts rapidly with hydroxylamine to give hydroxamic acid which forms a purple complex with iron(III) ions. It is however very non-specific.

Other chemical methods are (c) the ninhydrin colorimetric method[48], and (d) the coulometric determination of β-lactams with mercury(II) solution[49,50]. A method specific to the intact molecule is (e) the Spectroscopic imidazole assay for penicillins and cephalosporins[51]. The β-lactam molecule reacts with imidazole and mercury(II) chloride to form penicillenic acid type mercuric mercaptides. This method is stability indicating and levels down to 0.5 μg/ml can be detected. It has been adopted as the official British Pharmacoepia assay for amoxicillin, ampicillin, cloxacillin, flucloxacillin and phenethicillin[52].

3. Chromatographic methods

Chromatographic techniques have served as an indispensible aid in the understanding of the chemistry of β-lactams. Paper partition chromatography and Thin Layer Chromatography (TLC) have played the largest role so far. This is because the original work carried out used these techniques and they allow greater migration distances thus achieving greater physical separations than some of the more modern techniques such as gas liquid chromatography and high performance liquid chromatography. However

the long development time needed for these separations does not lend itself to routine analysis.

Electrophoresis and in particular paper electrophoresis has enjoyed a long history of usefulness, but due to the prevalence of biological contamination, leading to difficulty in bioautographic detection, and insignificant charge differences between degradation products and impurities at a given pH, this method has limited analytical usage.

In general β-lactam antibiotics are not sufficiently volatile or thermally stable to be suitable for analysis by gas liquid chromatography (GLC). While GLC provides short analysis times, the separations require derivatization and are limited by the labile nature of the compounds.

Recent literature indicates that high performance liquid chromatography (HPLC), especially reversed-phase or ion-pair reversed-phase HPLC on micro-particulate ($\leqslant 10\ \mu m$) bonded packing materials, is amenable to the analysis of β-lactam antibiotics[53,54]. HPLC is the method of choice in the analysis of many antibiotics because of demonstrated accuracy, speed and ability to provide ancillary information relative to the nature and identity of purity-suppressing substances, such as reaction by-products or natural degradation products present in the sample. In addition the analysis can often be accomplished when the drug is part of a complex mixture such as a biological fluid or blend formation. The chromatographic separation required can usually be achieved in less than five minutes. Detection is usually by UV, for example Twomey [55] has reported a HPLC method for determining carbenicillin with UV detection. For ten samples the relative standard deviation was 0.79%. Electrochemical detection is also possible. For the HPLC determination of amoxicillin with a carbon paste electrode and detection in the 100 ng region the average standard deviation was 2.5%[56], comparing well with results from iodometric assay.

4. Physicochemical Techniques

A great battery of physicochemical methods are available to monitor the purity of the β-lactam antibiotics and are used routinely. The British Pharmacoepia uses UV absorption, infrared spectroscopy, an auxillary method, Optical Rotatory Dispersion, and non-aqueous titrations using perchloric acid in a glacial acetic acid medium. Nuclear Magnetic Resonance (NMR) spectroscopy is used routinely for the identification of β-lactams. Analytically the limits of detection of NMR are rather poor, being 3 to 5%. X-ray diffraction patterns are, as with NMR spectra, used for identification purposes.

Fluorimetry is a highly sensitive technique comparable with microbiological assays. The procedures are reasonably specific usually based on controlled degradation of the β-lactam to a fluorescent product. A sensitive fluorimetric assay for ampicillin using acid hydrolysis has been developed[57]. A general method specific for α-aminobenzyl-β-lactams has been developed by producing pyrazine derivatives, permitting analysis down to 0.1 μg/ml in plasma or serum[58].

Polarography is a useful specific technique of analysis providing the compounds sought are polarographically active. Benner[59] reported determination of β-lactams after alkaline hydrolysis. Squella et al.[60] have reported the selective determination of α-aminobenzyl-β-lactams by the production of pyrazine derivatives. Fogg et al.[61] have developed a selective procedure for the determination of cephalexin down to the μg/ml level.

F. POLAROGRAPHY OF β-LACTAM ANTIBIOTICS

Electrochemical techniques, and in particular polarography, are now being widely used to assay for drug purity, degradation products and possible contaminants. For electrochemical methods to be of use it is necessary that compounds being determined possess oxidisible or reducible

groups or respond potentiometrically. This condition is often fulfilled due to the wide potential range that can be scanned using the mercury electrode. In addition some substances, although electroinactive themselves, can be transformed by introducing a reducible substituent (the most general method is to use nitration, nitrosation and oxidation reactions).

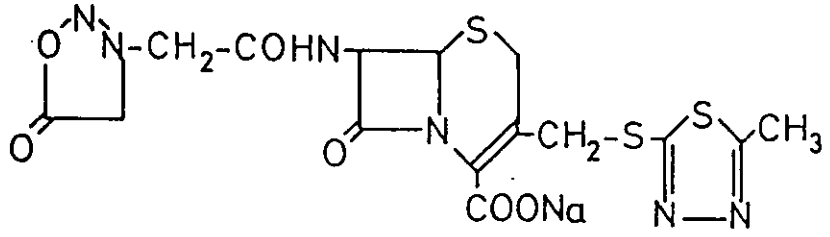
Page[62] was the first to report polarographic activity from a β-lactam antibiotic. Penicillin G was degraded and the catalytic wave of penicillamine, a degradation product, was observed. Penicillins themselves do not give polarographic waves and have to be degraded in order that some of their electroactive degradation products are produced. Krejčí[63] used polarography in a study of the kinetics of penicillin degradations in acidic aqueous solutions. Doan et al.[64] reported the DC polarographic characteristics of several antibiotics but obtained negative results with the nitrosated product of procaine penicillin G. However, using DPP Siegerman[65] obtained a detection capability of 1 ppm for the nitrosated product of penicillin G. Use of rapid scan DC (or oscillographic) polarography by Dušinský et al.[66] allowed the measurement of the inactivation of penicillin G by a β-lactamase enzyme at pH 7. The peak measured corresponded to penicilloic acid, a product of enzymatic attack and alkaline hydrolysis of penicillin.

Widespread use of cephalosporins as antibiotics brought interest in their electrochemistry and in 1968 Jones et al.[67] showed that cephalosporin C, cephalothin and cephaloridine were electroactive at the DME without the need of prior functionalization. The DC wave from cephaloridine allowed it to be determined quantitatively between 2×10^{-3} M and 1×10^{-5} M, but the waves of cephalosporin C and cephalothin were restricted to qualitative purposes.

The application of polarography to the determination of several

penicillins and cephalosporins in serum was described by Benner[59] using rapid scan voltammetry. This two hour assay of serum ultrafiltrate gave linear ranges for methicillin, oxacillin, cloxacillin, dicloxacillin, penicillin G and penicillin V for 1 - 50 μg/ml. The peak obtained from cephalexin was very stable allowing its determination with an accuracy of ± 1 μg/ml in the range 1 - 10 μg/ml.

In 1973 Hall[68], from the DC polarography and coulometric study of the degradation of a cephalosporin C derivative assigned the reducibility of the compound to the C-3 substituent, the thiol-thiadiazole group being reductively eliminated.

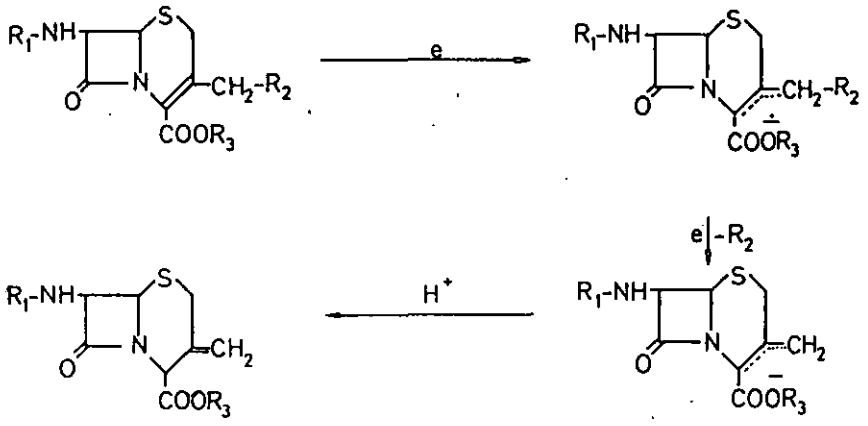


3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-7-2-(3-synone)acetamido)-3-cephem-4-carboxylic acid, sodium salt.

He also suggested that the polarographic waves exhibited by cephalosporin C, cephaloridine and cephalothin were also due to the reductive elimination of the C-3 substituents, differences in reduction potentials between the compounds occurring because of structural differences.

Ochiai et al.[69,70] investigating the electrochemical reduction of cephalosporanic acid derivatives containing various substituents at the C-3 position found that cephalosporin C upon macroelectrolysis at a mercury pool electrode at pH 6.6 is converted to the desacetoxy derivative, that is, the acetoxy group at the C-3 position is reductively eliminated. (see Scheme I). They concluded that in general cephalosporanic acids with C-3 substituents gave the corresponding 3-methylene-cepham derivative; that variation in the sidechain at C-7 does not effect this reaction and

that the presence of a substituted methyl group at the C-3 position is the essential requirement for the electroreduction of these compounds.



Scheme I

In 1977, Hall et al.[71] studied the electrochemical behaviour of cephalothin by AC and DC polarography, cyclic voltammetry and coulometry in both aqueous and non-aqueous media and found that it gave both 3-methylene-cepham and 3-desacetoxy-cepham derivatives on reduction, the ratio of these compounds to one another being dependent on experimental conditions.

Rickard et al.[72] described a DC polarographic assay for cefamandole and its formyl ester, cefamandole nafate to determine the purity of the samples by comparison to a reference. Controlled potential coulometry was used as an absolute measure of purity of these compounds without the need for a reference material. Results were identical with those obtained from microbiological and iodometric assays.

Jemal et al.[73-76] studied the polarography of a degradation product of penicillin G, benzylpenicillenic acid, which has been implicated in penicillin allergy by DP polarography. Penicillamine was found to be a degradation product of benzylpenicillenic acid at neutral pH[73] and its electrochemistry was investigated by DC and DP polarography[76].

Schröder et al.[77] reported a DC polarographic method for ampicillin after acidic hydrolysis at 100°C. Fogg et al.[78] have studied the DP polarography of cephalosporins and their degradation products. The neutral degradation of the orally active cephalexin was studied in detail by DPP[79] and a reliable DPP method for the determination of this compound at the 2 μg/ml level after hydrolysis in neutral phosphate buffer at 100°C was reported[61]. The acidic degradation of ampicillin to form penicillamine and a pyrazine derivative has also been reported[80].

Siegerman[83] has reported a DPP method for the determination of cephaloglycin at the 1 ppm level. A DC polarographic assay for cephalexin at the 10^{-3} M level in HCl (5 M) at 80°C has been determined by Squella[82] et al. DC polarographic determination of other α-aminobenzyl-β-lactams by acid hydrolysis to pyrazines has been carried out by Squella et al.[83-89,60].

The acidic degradation of flucloxacillin to a thiol has been made the basis of a DC polarographic assay at the 10^{-3} M level[90]. Faith[91] has used square-wave polarography in the qualitative determination of semisynthetic penicillins in dosage form and urine. The alkaline degradation product of penicillin G, benzylpenicilloic acid, was investigated by DC and DP polarography[92]. Benzylpenicilloic acid and penicillamine could be determined in each other's presence at the 10^{-5} M level. Cathodic stripping voltammetry was used to determine penicillamine in the presence of cupric ions[93]. The limit of detection was 2×10^{-9} M.

The application of polarography to the determination of β-lactam antibiotics has recently been reviewed[94-99].

G. DEGRADATIONS OF PENICILLINS AND CEPHALOSPORINS

The chemical stability of β-lactam antibiotics in aqueous solutions has received much attention in recent years. It was hoped that an understanding of their degradation mechanisms would help in the search for new β-lactam antimicrobial agents that would not be degraded *in vivo*.

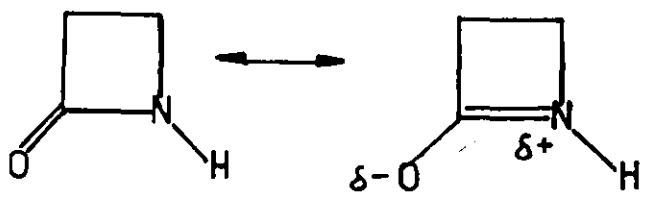
Penicillins and cephalosporins have been known to undergo facile cleavage of the β-lactam bonds in aqueous solution and the chemical reactivity of the β-lactam moeity is linked with antimicrobial activity[100], the antibacterial activity of β-lactams being for all practical purposes destroyed by β-lactam bond cleavage.

It was also thought that such degradation studies might lead to an understanding of allergenic responses to β-lactam antibiotics which might be related to formation of complexes between proteins and β-lactam degradation products. It is known that allergy is mediated by antigen-antibody reactions, and drug molecules of low molecular weight, such as β-lactam antibiotics, are non-immunogenic themselves. The ability of these compounds to induce an immune response depends largely on the capacity of these molecules, or their degradation products, or metabolites formed *in vivo* to react covalently with protein. Once the antibody is formed in response to exposure of an individual to the immunogenic substance, it can react with antigen to initiate the allergic reaction. These problems have been extensively reviewed[22,41,101]. It is safe to say that a knowledge of the chemical degradation reactions of β-lactam antibiotics is an essential requirement for the successful study of their allergenicity.

PENICILLIN DEGRADATIONS

The bicyclic β-lactam thiazolidine structure in penicillin is more susceptible to base- or acid-catalyzed hydrolysis than simple β-lactams and linear amide structures. This lability was attributed to (i) ring

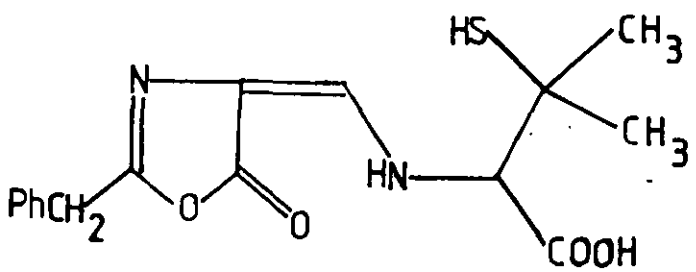
strain and (ii) the high reactivity of the β-lactam moeity of penicillins due to the non-planarity of the β-lactam-thiazolidine structure which causes a great deal of suppression of the usual amide resonance as compared with that caused by the dipolar stabilized forms in the normal β-lactam structure (see below).



This reactivity of the penicillin drugs is characterised by the high sensitivity of penicillin to attack on the β-lactam ring by acid-base reagents, metal ions, penicillinases, organic catalytic agents, nucleophilic, electrophilic and oxidising agents and even water molecules[102,103].

In the early work on penicillin degradations it was established that penillic acid (see fig. 7) is a major rearrangement product of penicillin G[103]. Dennen et al.[104] proposed that benzylpenicilloic acid (see fig. 7) is also an important penicillin G degradation product in acid solutions. The concurrent formation of penillic acid and penicilloic acid with the maximum yield of penillic acid in the pH range 2 to 3 was reported by Florey[105].

From a consideration of the results of Krejčí[106], Schwartz[107] proposed that benzylpenicillenic acid (below) forms from the reaction of the



anionic penicillin with a proton or from the spontaneous rearrangement of undissociated penicillin. It was suggested that penillic acid forms from penicillenic acid and that in a second parallel reaction penilloic acid

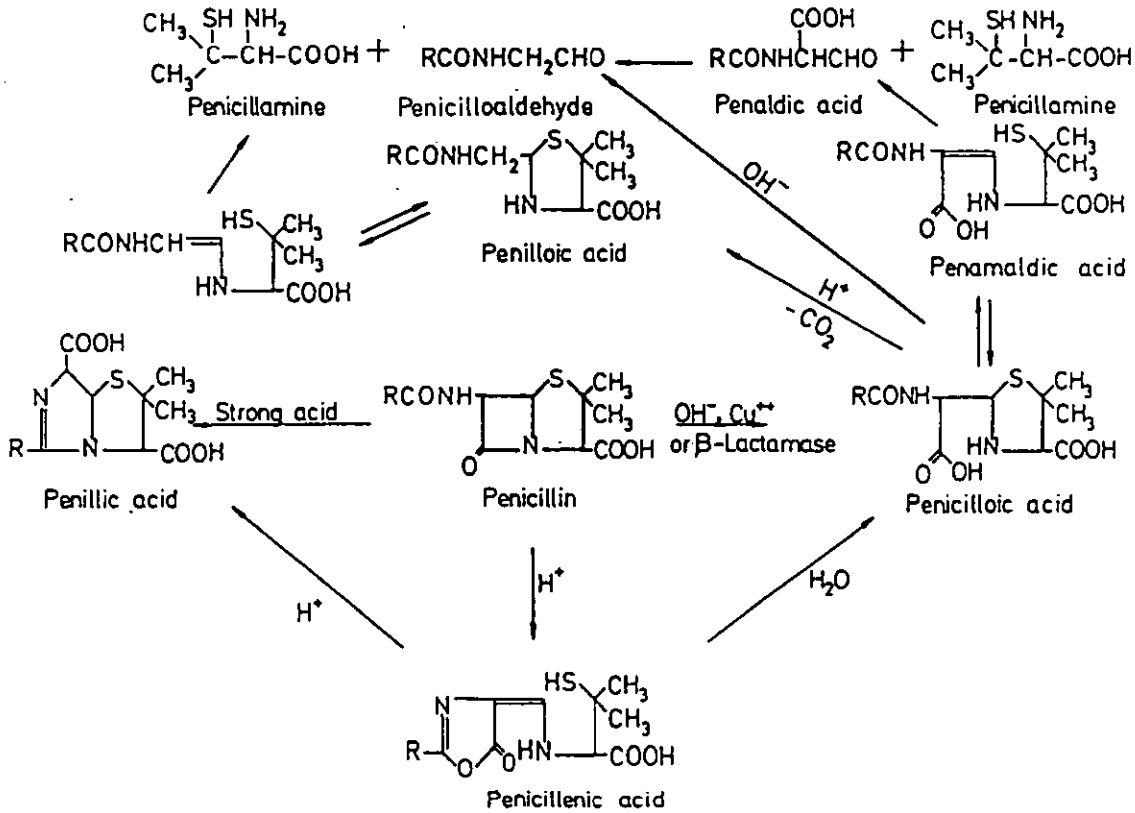
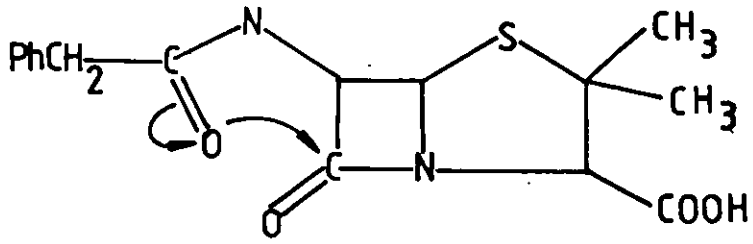

Penicillamine
Penicilloaldehyde
Penaldic acid
Penicillamine
Penilloic acid
Penamaldic acid
OH⁻
H⁺
-CO₂
Strong acid
OH⁻, Cu⁺⁺
or β-Lactamase
Penillic acid
Penicillin
Penicilloic acid
H⁺
H⁺
H₂O
Penicillenic acid

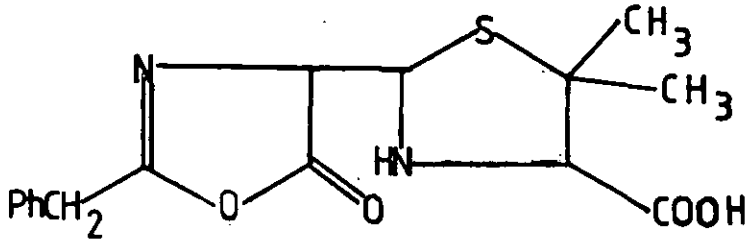
Fig.7

(see fig. 7) (the decarboxylate of penicilloic acid)is the product from the acid catalysis of undissociated penicillin, with penicilloic acid as the intermediate, the formation of penicillenic acid by spontaneous arrangement occurring via attack of amide carbonyl oxygen on the β-lactam carbon .



A scheme for the acidic degradation of benzylpenicilloic acid was put forward by Schwartz[41]. Benzylpenicilloic acid decarboxylates to benzylpenilloic acid which goes into equilibrium with a penamaldic acid which further degrades to penicillamine and benzylpenicilloaldehyde (see fig. 7).

Bundgaard[108] hypothesized the presence of an oxazolone -thiazolidine meta-stable intermediate (below) in the formation of benzylpenicillenic acid.

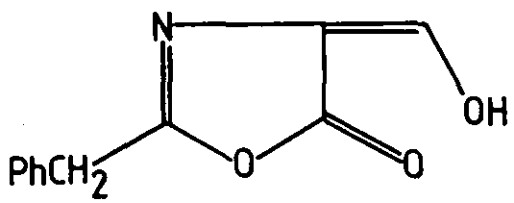


Regna[109] suggested the formation of penicillamine and penaldic acid (see fig. 7) as degradation products of penicillin in acidic media. Yamana et al.[110] postulated that penicillins are hydrolysed to their penicillenic acids via hydronium (H_3O^+) ion-catalyzed rearrangement of penicillin and spontaneous rearrangement of penicillin.

In 1976 Blaha et al.[111] followed the degradation of penicillin G in acidic media by HPLC. Benzylpenicillenic acid was found to be the key intermediate and was on the main formation pathway of all main degradation products. No benzylpenicilloic acid was detected and penicillamine, benzylpenicilloaldehyde, carbon dioxide and benzylpenilloic acid were found to be the final degradation products. Another degradation scheme

was proposed, based on NMR measurements, of penicillin G ageing in acid by Degelaen et al.[112] in 1979. This differed from the previous HPLC study in that penicillin G was proposed to degrade by three parallel reactions rather than directly to benzylpenicillenic acid and that penicillamine and benzylpenicilloaldehyde are not formed. In 1981 Kessler et al.[113] confirmed benzylpenicillenic acid as a key intermediate and penicillamine as a degradation product, by DP polarography, in the acidic degradation of penicillin G. The scheme of Blaha et al.[111] provided the best fit to experimental data. Penicillamine had been found previously to give an anodic polarographic wave[73,76] and Fogg et al.[80] have found that penicillamine is a product of the acidic degradation of a penicillin, ampicillin.

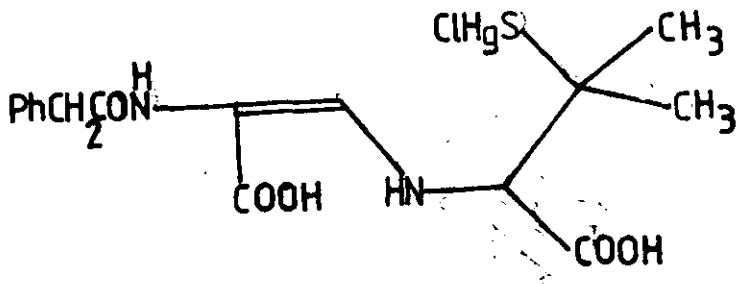
Penicillenic acid has been cited as the principal antigenic determinant of penicillin allergy[41]. It is formed from dilute acid or neutral solutions of penicillin G. In neutral solutions it hydrolyses to penicilloic acid[114] and under a variety of reaction conditions it has been shown to rapidly hydrolyse to penillic acid and 4-hydroxymethyleneoxazol-5(4H)-one[115] (see below) and penamaldic acid[116,117]. It has been found to exist in



equilibrium with penicillins; its equilibrium concentration is small but if a mercury(II) salt is present (which reacts with thiol groups) the equilibrium is forced towards penicillenic acid[118]. It is highly susceptible to attack by nucleophiles. Its kinetics of formation and disappearance in solutions of penicillins has been investigated by Butler et al.[119] who found it to be most stable at pH 6.

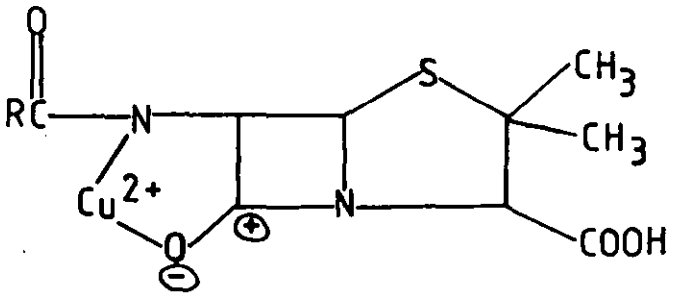
In neutral or alkaline solutions penicillin G is hydrolysed to the biologically inactive benzylpenicilloic acid. This compound is stable in alkaline or neutral solutions in the form of salts or esters. By UV data it has been found that the formation of benzylpenicilloic acid in neutral solutions goes via benzylpenicillenic acid[120,106], whilst in strong alkaline solution benzylpenicilloic acid is formed directly from penicillin G[121]. In 1963 Rapson et al.[122] found that from the evidence of pKa values benzylpenicilloic acid in alkaline solution (pH 12) forms benzylpenamaldic acid. This isomerization was found to be independent of pH in the range 7.3-12[123]. This reaction was described as a reverse Michael addition by Schwartz[41]. Benzylpenamaldic acid so formed then degrades to benzylpenaldic acid and penicillamine (pH 7-13). Benzylpenaldic acid then decarboxylates to give benzylpenicilloaldehyde. In the presence of aqueous mercury(II) chloride, benzylpenicilloic acid at pH 2.5 gave penicillamine and benzylpenicilloaldehyde[124].

In alkaline solutions and in the presence of $HgCl_2$ benzylpenicilloic acid forms an unstable penamaldic acid derivative (see below).



This has been made the basis of a UV assay for benzylpenicilloic acid as the penamaldate (above) has an absorbance maximum at 282 nm[125]. The instability of the reaction product of $HgCl_2$ with benzylpenicilloic acid has been suggested as being due to decarboxylation. Stabilization of the penamaldate so formed by reacting it with imidazole reagent has been made a sensitive UV assay for penicillins adopted by the British Pharmacoepia (1980)[51,126].

Using DP polarography Jemal et al.[73] showed that although penicillamine was obtained from benzylpenicillenic acid, it was not formed from benzylpenicilloic acid at neutral pH. Penicilloic acid has also been shown to be formed by enzymic hydrolysis of penicillins with penicillinase[127]. There is approximately 1 μg of copper present in 1 ml of human serum mostly bound to the copper protein ceruloplasmin. The presence of cupric ions (Cu^{2+}) in penicillin solutions, through the pH range 4-6, has been shown to promote degradation to penicilloic acid[128,129]. Cressman et al. in 1969[130] found that rapid complexation of intact penicillin by Cu(II) ion is followed by a rate limiting hydroxide ion attack upon the complex, splitting the β-lactam ring. Presence of a nitrogen atom and a sidechain at the C-6 position promotes degradation through a 5-membered chelate (see below).



Harwood et al.[131] concluded that in the aminolysis of penicillin by glycine in mild acid (pH 5) solution, the presence of Cu(II) ions made penicillin go quantitatively to penicilloic acid, in contrast to the slower reaction between penicillin and glycine without Cu(II) ions where the main degradation product is penicilloaldehyde[132,133]. Penicillin G reacts with certain alcohols and amines to form the esters and amides of benzylpenicilloic acid[103]. Gensmantel et al.[134] has found that in copper(II) ion catalysed hydrolysis and aminolysis of penicillin G, the 1:1 complex is attacked 10^7 times faster by hydroxide-ion and amines than unco-ordinated penicillin G. The mechanisms of these catalysis reactions do not involve benzylpenicillenic acid formation as an intermediate. Gensmantel et al.[135]

have also looked at the effect of metal(II) ions on the degradation of penicillin G. Cu, Zn, Ni and Co(II) ions all make penicillin prone to attack by nucleophiles.

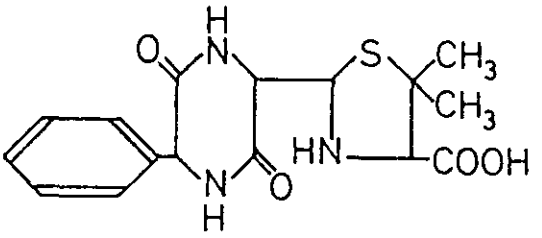
Interest in the aminolysis of penicillins to form penicilloylamides, which may be involved in allergic reactions, has prompted recent investigations[136-139]. Complex formation between pencillin G and sucrose has been reported[142]. Complexation involves the intact penicillin and although the degradation of penicillin at neutral pH is accelerated, the neutral degradation pathway does not appear to change.

A summary of the more common penicillin degradation pathways is shown in fig. 7. In general, as the pH of the degrading solution increases, the penicillin degradations become more simple.

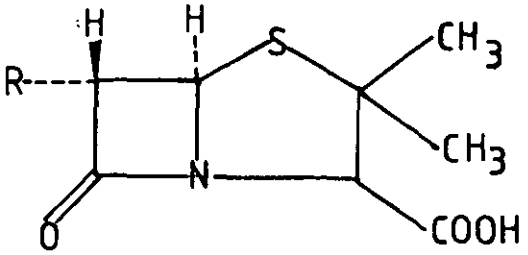
DEGRADATIONS OF α-AMINOBENZYLPENICILLINS

α-Aminobenzylpenicillins have been found clinically to be the most acid stable of the penicillins so far introduced. Their ease of crossing the gut wall is due to the α-aminobenzyl sidechain. The first study of the kinetics and mechanism of the degradation of one of these compounds was that by Hou *et al.*[117] on ampicillin who used iodometry to monitor residual ampicillin. The acid degradation was similar to penicillin G with α-aminopenicillenic acid and α-aminopenillic being formed with α-aminopenaldic acid produced in strong acid solution. Ampicillin was found to be two hundred times more acid stable than penicillin G and it was proposed that this was due to stabilization of the β-lactam ring by hydrogen bonding with the sidechain amino group. The basic degradation pattern was also stated to be similar to penicillin G with the penicilloic and penilloic acids being formed. The amino group sidechain was stated to play a significant role in the rate of degradation but not on the mechanism of degradation.

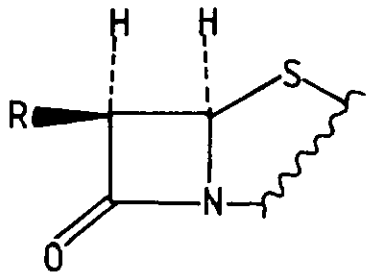
Schwartz[41] in 1969 reported that ampicillin does not readily form the penicillenic acid. Jusko[57] showed that a fluorescent degradation product is obtained following ampicillin acid hydrolysis. The fluorescent compound was also obtained from the alkaline hydrolysis product of ampicillin, α-aminopenicilloic acid, under the same acidic conditions. The fluorescent compound was not produced if the pH was greater than 7. The addition of formaldehyde was found to increase the yield of fluorescent product and the rate of its production. Jusko concluded that (i) either ampicillin degrades to the penicilloic acid during fluorescent compound production (i.e. the penicilloic acid is an intermediate compound) or both can be transformed directly to the fluorescent compound; (ii) both the α-amino sidechain group and the β-lactam group are needed for fluorescent compound production; (iii) formaldehyde reduces the basicity of the amino group, which facilitates the formation of the fluorescent compound which (iv) he tentatively proposed to be a diketopiperazine (DKP) derivative (see below).



Roets[140] obtained a DKP derivative in high yield from 6-epi-ampicillin (see below) in the presence of pyridine and acetic acid.



Formation of the DKP occurs from intramolecular nucleophilic attack of the amino sidechain group on the β-lactam carbonyl from the unhindered exo-side of the molecule. In normal ampicillin (see below) the reaction must come from the endo-side which is hindered sterically.

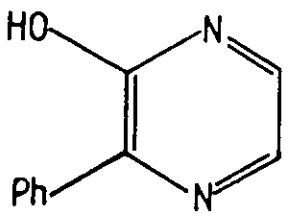


Therefore no DKP can be produced from ampicillin. This was confirmed by Indelicato et al.[141] when cephalosporins with α-amino sidechains form DKP derivatives in refluxing benzene while ampicillin trichloro ethyl ester remained unchanged under the same conditions.

Miyazaki et al.[142] reported a fluorophore being formed from α-amino-penicilloic acid in a neutral solution containing $HgCl_2$, the fluorophore was also formed from ampicillin after its alkaline hydrolysis to form the penicilloic acid. Miyazaki concluded that the α-aminobenzyl sidechain was essential for production of the fluorescent compound.

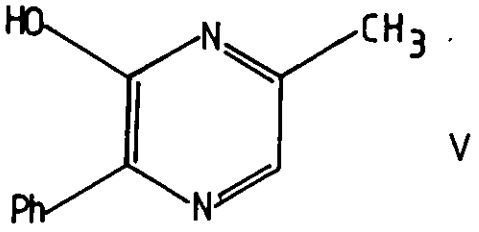
Numerous publications have appeared reporting assay procedures based on the formation of fluorescent degradation products for the analysis of α-amino sidechained β-lactams under different experimental conditions[143-151]. Durr et al.[143] used uranyl acetate for ampicillin determination. Miyazaki et al.[142] used $HgCl_2$ for ampicillin while Davidson[144] used formaldehyde in acid conditions for amoxicillin. This long and tedious process used a potentially explosive solvent extraction system, acetone/chloroform (1:1 v/v). Barbhiaya et al.[145,146] used formaldehyde at pH 4 after alkaline hydrolysis for ampicillin and strong alkaline hydrolysis but no formaldehyde for amoxicillin.

Some investigators reported that the fluorescent compound is 2-hydroxy-3-phenylpyrazine[152,153] (see below). Barbhiaya et al.[58] showed that the



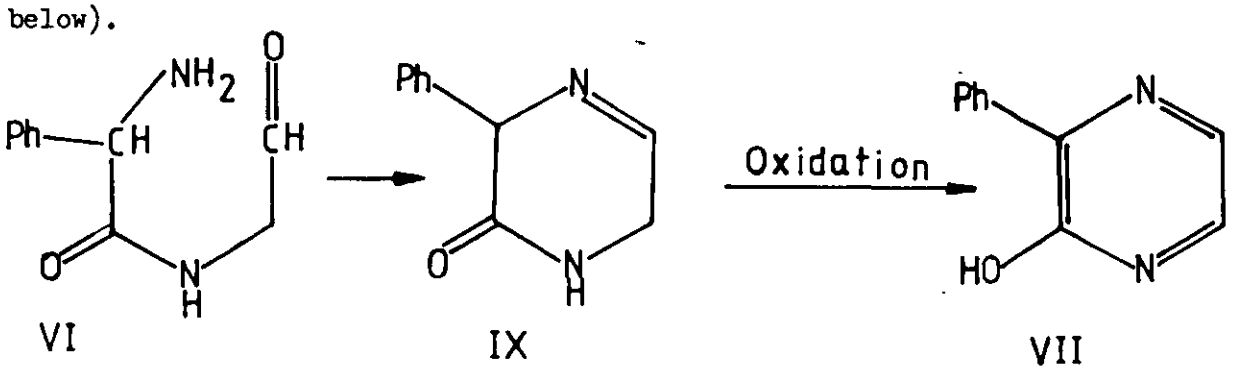
VII

alkaline degradation of various α-amino-β-lactams followed by mild acid hydrolysis in the presence of formaldehyde yielded an identical fluorophore whose spectral and chromatographic properties were identical with those of authentic 2-hydroxy-3-phenyl-6-methylpyrazine which was synthesized from α-phenylglycineamide and methylglyoxal (see below). Barbhiaya et al.[58]



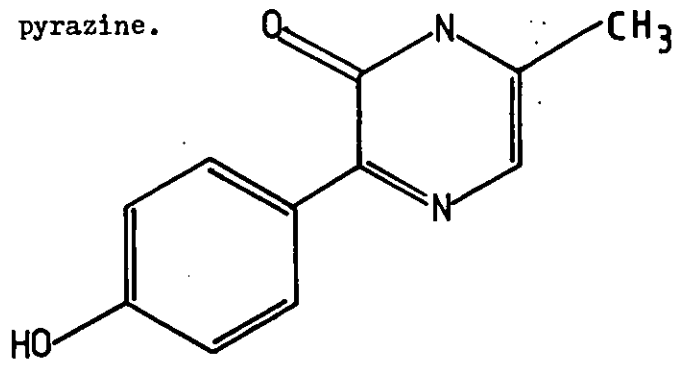
V

suggested that the formaldehyde only acts as a catalyst and does not take part in the reaction. From TLC results it was concluded that the first step to the pyrazine from ampicillin was formation of α-aminobenzylpenicilloic acid. Employing a similar reaction procedure except for the use of formaldehyde, Le Belle et al.[154] showed that the fluorophore from ampicillin was identical with synthetic 2-hydroxy-3-phenylpyrazine. It was suggested that formaldehyde is actually incorporated into the reaction product and is not just a catalyst. A mechanism was put forward involving formation of the penicilloic acid and then its rearrangement to the penamaldic acid (fluorescent compounds have been formed under conditions known to involve formation of the penamaldic acid derivative of ampicillin[143,150]). Degradation of this through the penaldic acid to the penicilloaldehyde may then proceed. Cyclization could occur by formation of the Schiff's base between the sidechain amino group and the aldehyde[155]. Subsequent oxidation of the dihydropyrazine would lead to 2-hydroxy-3-phenylpyrazine[156] (for scheme see below).



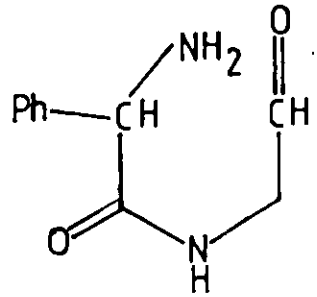
VI IX VII

Moll et al.[157] reports the production of a pyrazine from amoxicillin in acidic solutions, pH 1-3, being accelerated by the addition of formaldehyde. The compound was identified by independent synthesis as 2-keto-3-(para-hydroxyphenyl)-6-methyl pyrazine.



In this production an alkaline hydrolysis step was not required. Fogg et al.[80] and Squella et al.[89] have observed pyrazines as degradation products of ampicillin in weak acid (pH 2.5) and strong acid (HCl 5 M) respectively, both using polarography.

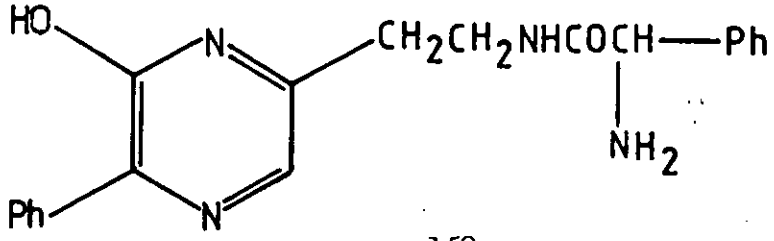
In a HPLC study of the isolation and structural investigation of the fluorescent degradation products of ampicillin Uno et al.[158] elucidated the reaction mechanisms of combined alkaline hydrolysis and mild acid hydrolysis, with and without the presence of aldehyde, in terms of a key penicilloaldehyde intermediate which was on the pathway to all fluorescent products. The penicilloaldehyde (see below) in low ampicillin concentrations



VI

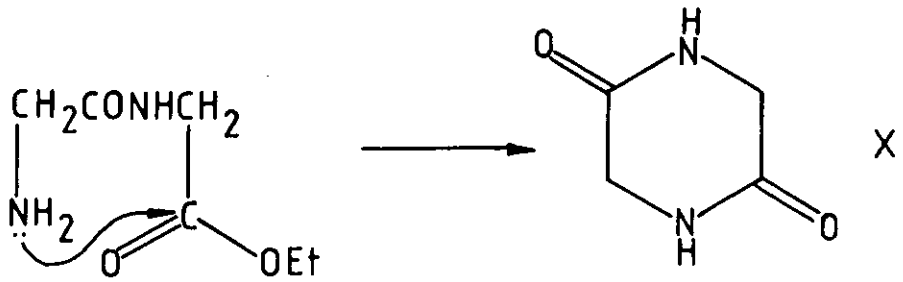
(<100 ppm) cyclizes in the absence of aldehyde to give 2-hydroxy-3-phenylpyrazine. In the presence of aldehydes, such as formaldehyde or acetaldehyde, the penicilloaldehyde undergoes an addition reaction and then cyclization to the 2-hydroxy-3-phenyl-6-methylpyrazine with formaldehyde and the -6-ethylpyrazine with acetaldehyde present. If the initial ampicillin concentration is high (>200 ppm), one penicilloaldehyde

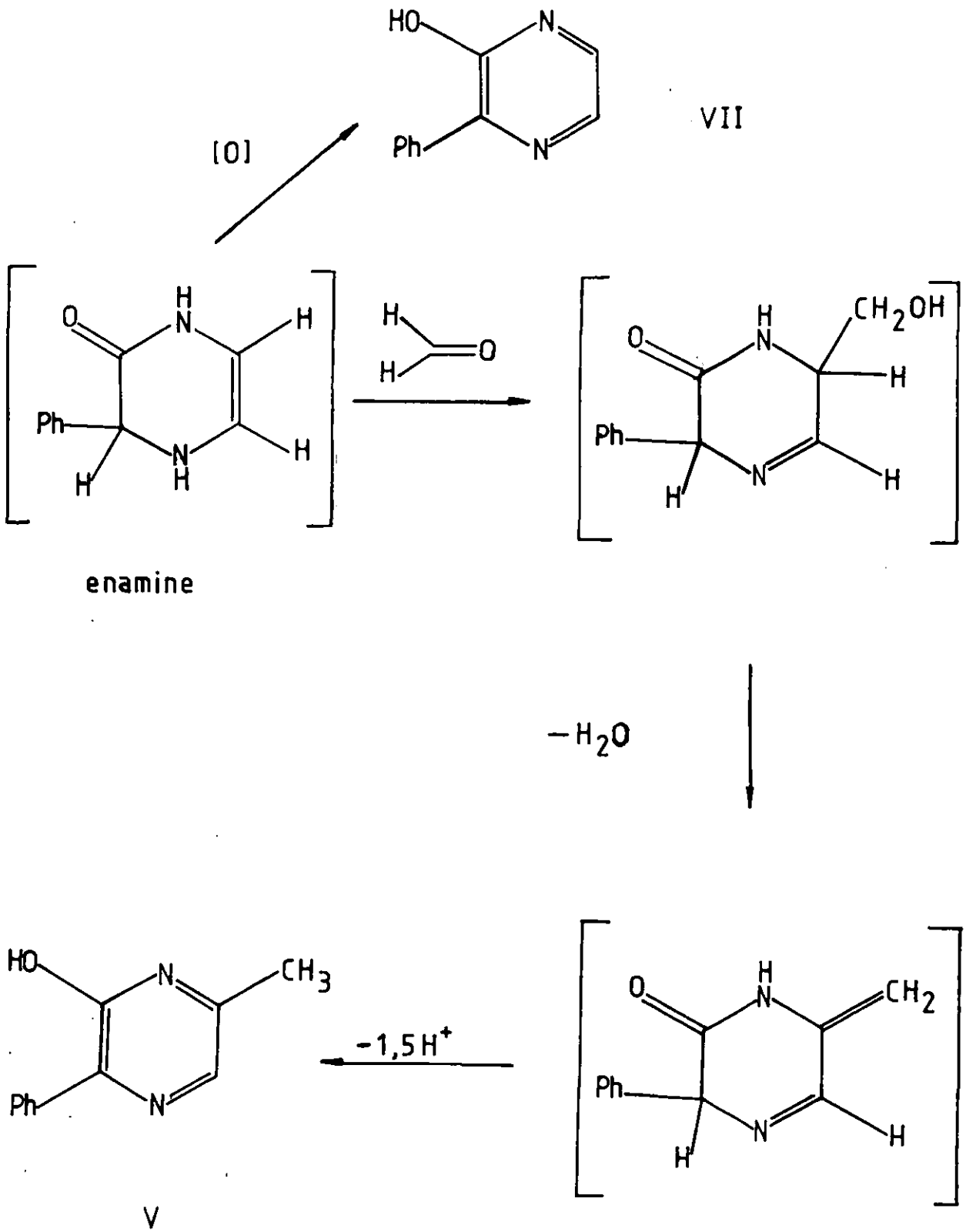
molecule will undergo addition with another and on cyclization 2-hydroxy-3-phenyl-6-penillopyrazine is formed (see below).



In an NMR study Indelicato et al.[159], using labelled formaldehyde (90% ^{13}C), confirmed the mechanisms of Le Belle et al.[154], and proposed a common intermediate, an enamine, that on condensation with formaldehyde forms the -6-methyl derivative, and in the absence of formaldehyde is oxidised to 2-hydroxy-3-phenylpyrazine (see fig. 8).

Kuchinskas et al.[160] found that at neutral pH ampicillin readily formed polymers. The polymer linear combinations consisted of an opened β-lactam carboxyl group linked to the free amino sidechain group of another molecule. Bundgaard et al.[161-166] have studied the polymerization of ampicillin and amoxicillin by HPLC. The polymers are cited to be antigenic. Intermolecular aminolysis to form polymers ranging from the dimer to the octomer is reported. Linkage is by the β-lactam carboxyl group joined to the α-amino sidechain of another molecule. Bundgaard et al.[167] report the formation of a DKP derivative from ampicillin at pH 9.2 in the presence of 10% glucose. Nucleophilic attack of the carbohydrate on the β-lactam ring produces an α-aminopenicilloyl ester, 80% of which then undergoes intramolecular nucleophilic aminolysis and desterification to form the DKP, the other 20% forming α-aminopenicilloic acid by simple hydrolysis. The intermolecular aminolysis is analogous to the reaction of simple dipeptide esters in weak alkaline aqueous solutions to form 2,5-diones (see below).




HO
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H
N
H
enamine
−H2O
HO
N
CH3
Ph
N
−1,5 H+
O
H
N
CH2
Ph
N
H
V

Fig. 8

The steric hindrance to direct formation of DKP from ampicillin does not operate in this case as it did as reported by Roets et al.[140].

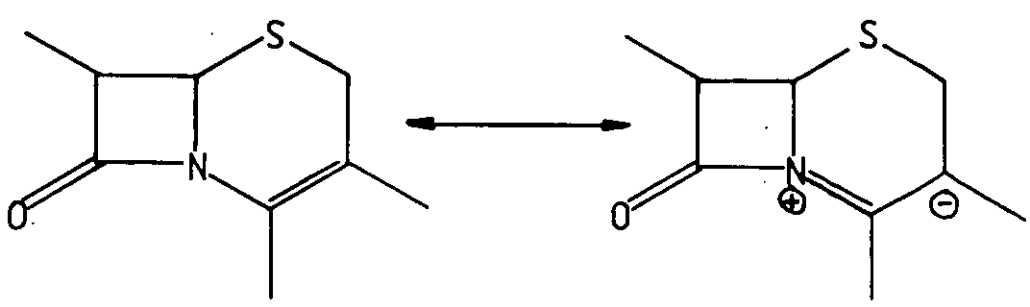
DEGRADATIONS OF CEPHALOSPORINS

Compared with the extensive studies on the degradation reactions of penicillins, relatively little work has been done on the degradation reactions of cephalosporins. This was the case until work done by Schwartz in 1969[44], concerning the immunochemical mechanisms underlying allergic reactions to β-lactam antibiotics, caused a renewal of interest in the stability of these antibiotics.

The degradation studies of the first cephalosporin to be isolated, the naturally occurring cephalosporin C, played an important role in assigning the structure of this compound. These degradation studies were, in general, combinations of hydrogenation, desulphurization and hydrolysis[168-171].

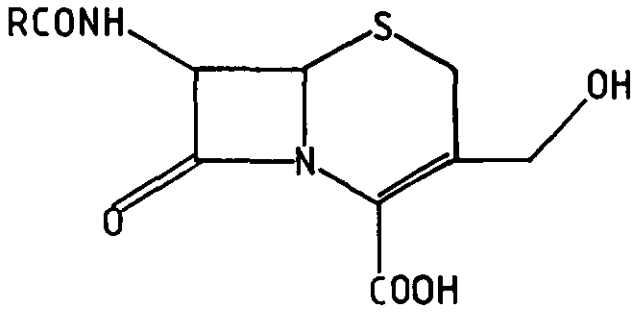
The cephalosporin C molecule (see general tables) has three sites susceptible to attack by hydrogen ions (i) the ester group of the acetyl sidechain; (ii) the amide group of the α-aminoadipyl sidechain; and (iii) the β-lactam ring.

The great susceptibility of Δ^3-cephalosporins to attack by nucleophiles has, as in the case of penicillins, been attributed mainly to suppression of the usual amide resonance. In cephalosporins this amide resonance may further be decreased by delocalization of the unshared electron pair of the nitrogen atom in the adjacent α,β-unsaturated system (enamine resonance[172], see below).

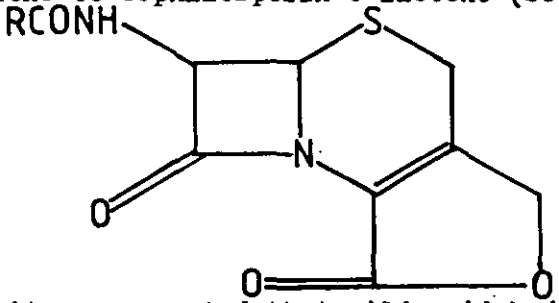


Cephalosporins such as cephalothin[173,174] and cephaloridine[175] have been shown to have the same chemical properties as cephalosporin C. Although acids have been reported to catalyse the removal of the acetyl and amino-adipic sidechains[11], Newton *et al.*[176] states that cephalosporin C is relatively stable at room temperature and pH 2.5. In the same study it was reported that acids cleave the cephalosporin nucleus, the end products of this acid hydrolysis being carbon dioxide and α-aminoadipic acid. Cephalosporins do not undergo the penicillin-penillic acid type of rearrangements that destabilises penicillins in acidic solutions even when they have the same sidechain as the penicillins[37]. This has been attributed to the lower anionoid reactivity of the nitrogen in the dihydrothiazine ring[177].

The most reactive group in cephalosporins is the 3-acetoxy group. With dilute acid or an acetylesterase cleavage of the acetoxy group will occur giving the corresponding 3-methyl alcohol, known as desacetyl cephalosporin C (see below).



Desacetyl cephalosporin C is not readily acylated, but can be lactonised under acid conditions to cephalosporin C lactone (see below)[168].



In the same study it was reported that mild acid hydrolysis of this lactone opened the β-lactam ring and yielded a relatively stable product which could be regarded as the corresponding cephalosporoate.

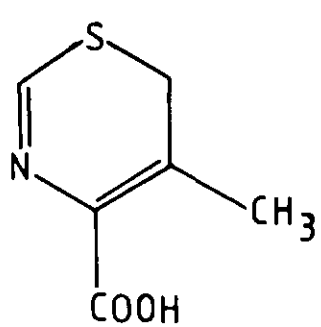
With dilute acid, hydrolysis of the C-7 sidechain of cephalosporin C

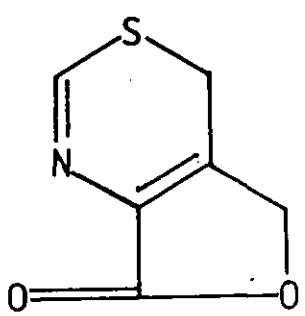
gives a small amount of the cephalosporin nucleus, 7-ACA, which then goes on to 7-ACA-lactone[168,179]. Yamana et al.[180] found that desacetoxy-cephalosporins were fifty times more acid-stable than other cephalosporins and one hundred times more stable than the most acid-stable penicillin, ampicillin, at pH 1.0. In another study Yamana et al.[186] found that in acid the degradation of acetoxymethylcephalosporins was not dependent on the C-3 side chain and was a simple hydrogen-ion catalyzed hydrolysis of the β-lactam bond. In weak acid their β-lactam bonds were seven times more stable than that of ampicillin. The acetyl function at C-3 also hydrolysed eight times faster than the β-lactam ring to the desacetyl compounds which were easily converted to the lactones[187].

The degradation of the desacetoxy compounds was independent of pH even in concentrated acid, where the desacetoxy derivatives were twenty-five times more stable than the 3-acetoxy compounds due to the absence of the inductive effect of the acetoxy group destabilizing the β-lactam ring.

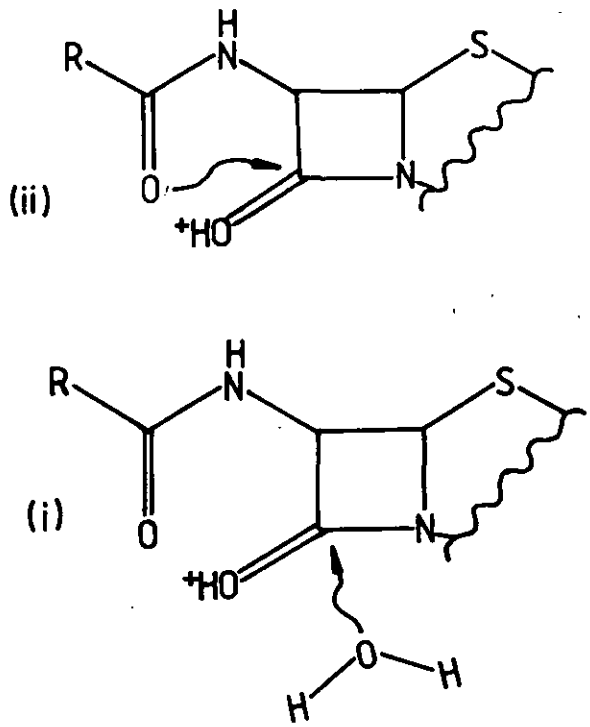
Haginaka et al.[183] in a study of the acidic degradation of cephaloglycin found that desacetylation is the rate determining step for the degradation of cephaloglycin to the lactone. This acidic hydrolysis of 3-acetoxy-methyl cephalosporins has been used as a synthesis for 3-desacetyl derivatives and the corresponding lactones by Kukolja[175]. The hydrolysis of cephalosporins in concentrated acid at 100°C has been reported by Bontchev et al.[184]. Hydrolysis proceeds with a high rate, resulting in the breaking of the β-lactam ring, definite low molecular weight degradation products and breaking of the C-N bond in the amido-group at C-7 giving amino acetic acid and thiazine compounds (see below).

XVI





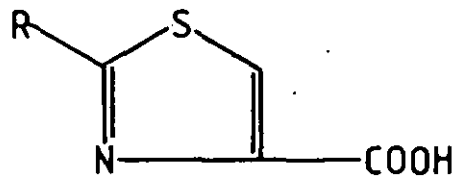
In an assessment of the rates of acid-catalyzed degradation of ampicillin and cephalexin by specific kinetic treatment, Tsuji et al.[185] proposed that in acid two reactions occurred, (i) water attack on the carbon of the protonated β-lactam carbonyl (intermolecular), and (ii) intramolecular acid catalyzed degradation of the protonated species (see below).



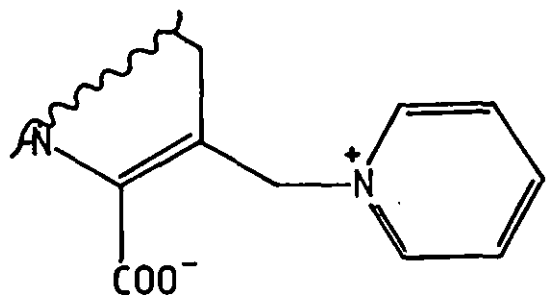
With cephalexin the intramolecular reaction was ten times slower than the almost totally dominating intermolecular reaction. In the case of ampicillin the intramolecular reaction was twice as fast as the intermolecular process, and consequently competition occurred.

Cephalosporins are readily attacked by nucleophilic reagents and cephalosporin β-lactamases. However the initial degradation product corresponding to penicilloic acid, cephalosporoic acid or "cephalosporoate" is not stable and rapidly fragments in aqueous solution[186,187]. However, Kawabara[188] has reported that cephalothin gave a cephalosporoate after β-lactamase hydrolysis.

Degradation of cephalosporin C at 37°C at neutral pH slowly gave a thiazole carboxylic acid in an aqueous pyridine solution.



In neutral solution cephalosporin C reacts with pyridine to form cepahlosporin C_A[189].

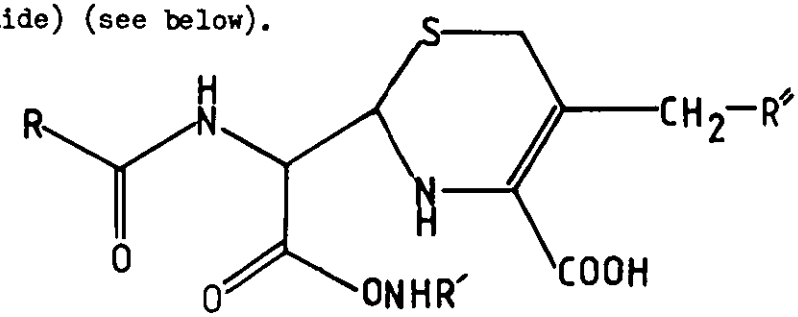


It has been shown that any reagent of greater nucleophilicity than oxygen will displace the acetoxy group at C-3[190], for example, pyridine, tertiary amines and azides will replace the acetoxy group. Yamana et al.[180] observed that in the pH range 3-10, two competing reactions were occurring, (i) hydrolytic cleavage of β-lactam ring by direct water molecule attack and (ii) cleavage of the β-lactam ring by intramolecular participation of the neighbouring sidechain amido-carbonyl group. The reactivity of cephalosporins towards water is reflected by the result that in spontaneous hydrolysis, they react ten times faster than penicillins. In a later paper Yamana et al.[181] proposed that as in the formation of benzylpenicillenic acid from penicillin G in the neutral pH range (3-8), an analogous "penicillenate" was formed from cephalosporins by attack of sidechain amide-carbonyl on β-lactam carbon. The "penicillenate" type compound would be very unstable in aqueous solutions, taking part in allergic reactions, but ultimately degrading to more stable compounds.

Dilute alkali treatment of cephalosporin C at room temperature gave a low yield of desacetylcephalosporin C, while an acetyl esterase in neutral solution gave high yields of this compound. The hydrolysis of the acetyl group was also accompanied to some extent by opening of the β-lactam ring[178]. In weak alkaline solution the 260 nm UV band,

characteristic of the cephalosporin nucleus, disappears rapidly[191].

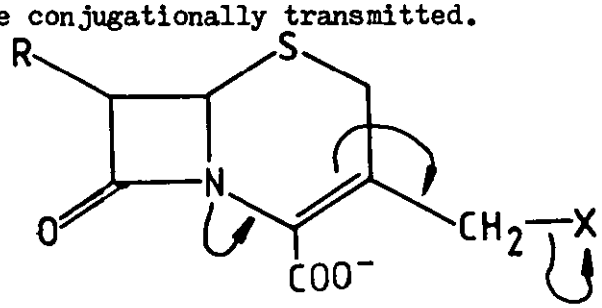
Aminolysis of cephalosporins in weak alkaline solutions was found to give a labile compound with λ_{max} at 230 nm. This compound was not found in the alkaline solutions of desacetyl and desacetoxy cephalosporins but did occur when cephalosporins and their desacetyl derivatives were subjected to β-lactamase. These degradation products were found to degrade further to compounds tentatively identified as penamaldates and penaldates[192]. Hamilton-Miller et al.[193] followed the aminolysis and enzymic hydrolysis of a number of cephalosporins having different substituents at the C-3 position by NMR and UV spectrophotometry. The signäls obtained after aminolysis of the β-lactam ring of a cephalosporin, 7-n-butyramido-cephalosporanic acid, indicated that the reaction is accompanied by expulsion of the acetoxy group as acetate, formation of a double bond in the $\Delta^{4,5}$-position and the appearance of an exocyclic methylene group (λ_{max} 230 nm) as a semistable intermediate. This compound was found to degrade further to give a penaldate (λ_{max} 270 nm). Similar results were obtained with cephalosporin C. Aminolysis of desacetyl- and desacetoxy-cephalosporins can occur without immediate structural changes in the dihydrothiazine ring, i.e. no exomethylene or $\Delta^{4,5}$-double bond, in fact a "cephalosporoate" is formed (or a cephalosporoamide) (see below).



Enzymic hydrolysis of cephalosporin C and desacetyl-cephalosporin C gave an intermediate with λ_{max} 230 nm - an exomethylene compound and different from the aminolysis product. The investigators speculated that enzymic hydrolysis could have converted the hydroxyl group of desacetyl cephalosporins into better leaving groups resulting in behaviour similar to that

of cephalosporin C.

The acetoxy group of a cephalosporin has been known previously to be spontaneously expelled as acetate in a concerted reaction when the β-lactam ring is opened by enzymic hydrolysis of cephalothin and cephalosporin C, similarly with cephaloridine, pyridine is expelled. Bundgaard[195] observed that during aminolysis and alkaline hydrolysis of cephaloridine, expulsion of pyridine ion from the C-3 position proceeded simultaneously with cleavage of the β-lactam ring. It was also reported that differences in reactivity of cephalosporins containing identical 7-acylamido sidechains, but different C-3 sidechains could be accounted for by the purely inductive effects of the C-3 substituents. Indelicato et al.[196] stated that C-3 methylene substituents may inductively exert considerable influence upon the chemical reactivity of the β-lactam moiety although they are four atoms away. The effect may be conjugationally transmitted.



It was observed that the rates of β-lactam hydrolysis showed correlation with the calculated electron densities at the β-lactam carbonyl as the C-3 substituent was varied, but generally substituent effects due to C-7 acylamido sidechain modification resulted in insignificant changes in β-lactam reactivity.

Yamana et al.[181] stated that substituent changes at C-7 appeared to have little effect on the rate constants for H^+ and hydroxide-ion catalyzed hydrolysis. The intramolecular participation of the acyl amido sidechain in penicillins and cephalosporins at high pH was discounted, although substituent changes at C-7 may affect the shape of the rate profile in the neutral pH region. Those cephalosporins with α-amino groups in the C-7 sidechain showed a sharp rise in their pH profile at pH 6-8, with an

inflection point at pH 8.

Rattie et al.[197] stated that phosphate buffer had a catalytic effect on the degradations of the cephalosporins, cephalexin and cefazolin, at pH 6.5-8. As regards the complexity of the mechanism of degradation the initial β-lactam ring structure cleavage appears to be responsible for the overall degradation observed for the cephalosporin in aqueous solutions. As in the case of penicillins the amino group sidechain of cephalexin plays a significant role in the rate but not the mechanism of degradation. The difference in the reactivity of α-amino sidechained penicillins and cephalosporins at low pH being due to the amino group in cephalosporins exerting a stabilizing effect on the molecule in solution.

Recently hydrogen sulphide has been determined as a major alkaline degradation product of cephalosporins[198]. Penicillins were found not to give hydrogen sulphide in similar alkaline conditions. The 4-carboxyl group in cephalosporins, as in penicillins, can be modified to esters or amides. Many esters and amides of cephalothin have been prepared, all of which have been shown to be much less biologically active than the parent compound[199].

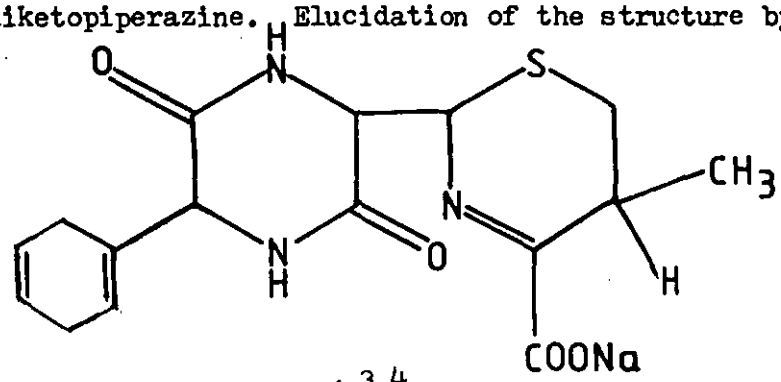
The lability of cephalosporin to UV light was first reported by Demain et al.[200] who showed that cephalosporin C lost 90% of its original biological activity in aqueous solution after being irradiated with ultra-violet light for thirty minutes. Maki et al.[201] studied the mode of photodegradation of cephalosporins in alcohols and observed photo-rearrangement to thiazole compounds by 3-cepham derivatives.

DEGRADATIONS OF α-AMINOBENZYLCEPHALOSPORINS

As with α-aminobenzylpenicillins, the presence of the α-amino-benzyl sidechain of these cephalosporins enable them to be completely absorbed following oral administration. Indelicato et al.[141,196] suggested

that the unusual increase in reactivity of cephalosporins having an α-amino sidechain group in alkaline aqueous solutions at pH 10 was due to an additional reaction competing with hydroxide attack on the β-lactam ring. This reaction was thought to be intramolecular nucleophilic attack of the α-amino sidechain group on the carbonyl of the β-lactam ring forming a diketopiperazine. Diketopiperazines of α-aminobenzylcephalosporin esters were isolated after refluxing in benzene, but they were unable to isolate diketopiperazines from aqueous solutions of α-aminobenzylcephalosporin, as the degradation was carried out at pH 10, where the major degradation route is β-lactam hydrolysis to the cephalosporoic acid. Previously this intramolecular nucleophilic attack was suggested as a decomposition mechanism for cephaloglycin[202].

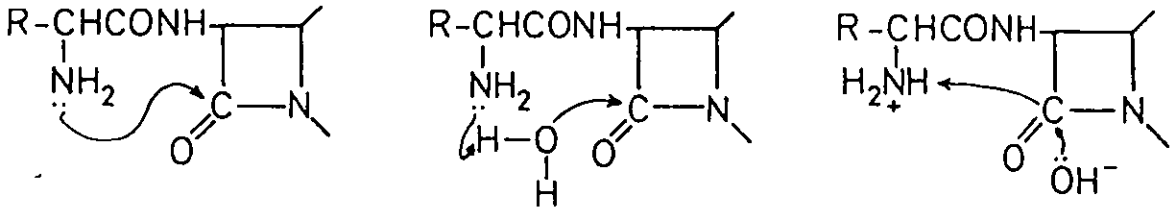
Cohen et al.[203] reported that aqueous alkaline hydrolysis (pH 9.2) of cephradine yields a diketopiperazine. Elucidation of the structure by NMR



revealed that the double bond present at $\Delta^{3,4}$ in the dihydrothiazine ring of the cephalosporin had migrated to $\Delta^{4,5}$ in the diketopiperazine derivative. Extensive kinetic and mechanistic studies of the degradation of cephalosporins have been reported by Yamana et al.[180,181]. They reported that at pH 8, cephalosporins with the α-aminobenzyl sidechain were ten to twenty times less stable than those without. At this near neutral pH the rate profile of cephalexin and cephaloglycin was consistent with mechanisms involving intramolecular catalyzed hydrolysis of the anionic antibiotics, superceded at low pH by direct water catalyzed hydrolysis of the β-lactam ring by hydroxide-ion. A strongly fluorescent compound was isolated from a reaction mixture of cephalexin and cephaloglycin at pH 8 and was proposed

to be a diketopiperazine.

Three plausible kinetically indistinguishable mechanisms for the α-amino groups to take part in the β-lactam ring cleavage of α-amino-benzylcephalosporins were suggested (see below).



Mechanism (A) Intramolecular nucleophilic attack of the unprotonated sidechain amino group on the β-lactam carbonyl moiety.

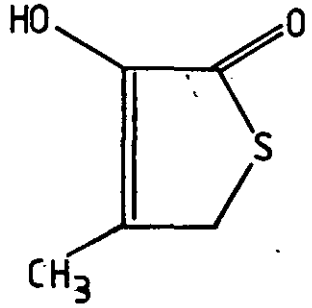
Mechanism (B) Intramolecular general base catalysis by the amino group of the attack of water molecule on the β-lactam bond.

Mechanism (C) Intramolecular general acid catalysis by the protonated amino group of the attack of hydroxide ion on the β-lactam bond.

In a study of hydrolysis and intramolecular aminolysis of cephalexin and cephaloglycin, Bundgaard[204] found evidence of the involvement of mechanism (A) in neutral solutions by isolating the DKP derivative of cephalexin and relating the extent of intramolecular aminolysis by the disappearance of the primary amino group using trinitrobenzenesulphonate[205]. Primary amine analysis indicated quantitative conversion of cephalexin to its DKP derivative at neutral pH. At pH values above 10, only 32% of the cephalexin gave the DKP, produced by hydroxide catalysed intramolecular aminolysis (I.M.A.). The remaining 68% was hydrolysed to the cephalosporoic acid. In similar alkaline solutions cephaloglycin produced 23% DKP, and 17% cephalosporoic acid, the remaining 60% degrading via hydroxide catalyzed desacetylation although some of the deacetoxycephaloglycin went on to its

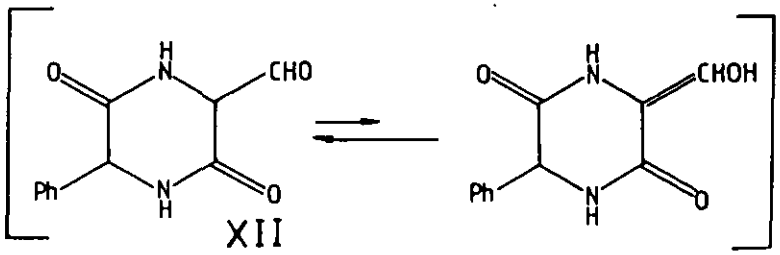
DKP derivative. In the neutral pH range (5-8.5) the observed first order kinetics were taken to mean that hydrolytic desacetylation only makes a small contribution to degradation in this pH range, the degradation being dominated by spontaneous or water-catalysed I.M.A. to the DKP. Primary amine analysis showed a 96% loss at pH 7. The β-lactam ring of cephaloglycin was found to be four times more susceptible to hydroxide-ion hydrolysis and eleven times more susceptible to I.M.A. than cephalexin, owing to the different inductive effects of their C-3 substituents.

In the study of the alkaline hydrolysis of cefaclor, a 3-chloro-3-cepham cephalosporin, Indelicato et al.[206] was unable to isolate the DKP derivative. However a DKP derivative was isolated from the acidic aqueous degradation of cefaclor and a DKP derivative was obtained by refluxing the p-nitrobenzyl ester of cefaclor in benzene. Elemental analysis and NMR studies revealed that the chlorine was absent from the C-3 position. It was suggested that loss of hydrogen chloride from cefaclor may be subsequent to β-lactam opening. Dinner et al.[207] reported that unbuffered degradation of cephalexin at pH 3 afforded two major products, the non-polar 3-hydroxy-4-methyl-2(5H)-thiophenone (see below)



XVII

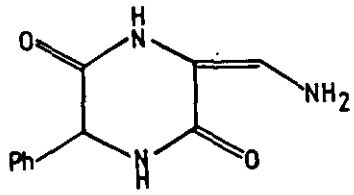
and the polar 3-formyl-3,6-dihydro-6-phenyl-2,5 (1H,4H)-pyrazinedione, which exists as two tautomeric isomers (see below).



XII

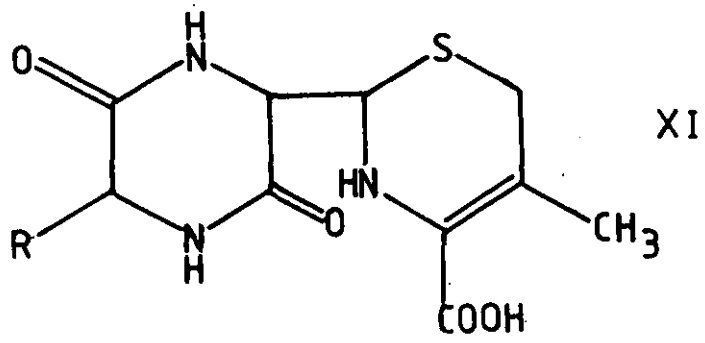
The DKP of cephalexin was suggested, by TLC and UV measurements, to degrade rapidly to both the thiolactone, via an amine, and to the aldehyde, directly.

No DKP derivative could be isolated from degrading solutions of cephalexin at pH 3. Bungaard[208] isolated and characterised a compound which was precipitated during the neutral degradation of cephalexin as 3-aminomethylene-6-phenyl-piperazine-2,5-dione (see below), and the thiophenone.

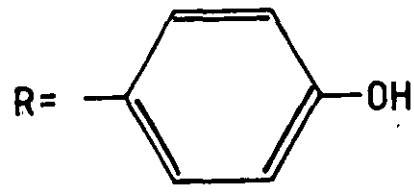


XIII

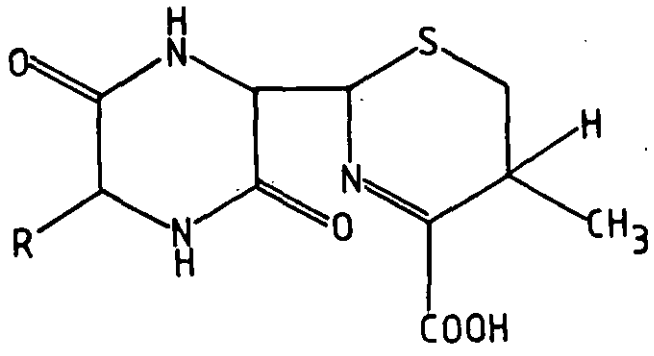
The neutral degradation of the DKP from cephalexin also produced the aminomethylene compound and the thiophenone. This aminomethylene compound was proposed to be a precursor to the aldehyde isolated by Dinner (above), the reaction being analogous to the penamaldate-penaldate conversion in acid[209]. Recently Tsuji et al.[210] isolated the thiophenone from a basic aqueous solution of cef adroxil. Two diketopiperazines wereals isolated, one with the double bond at $\triangle^{3,4}$, minor product A, and the other with the double bond at $\triangle^{4,5}$, major product B.



XI



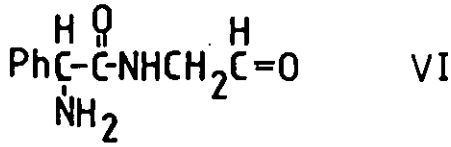
A.



B.

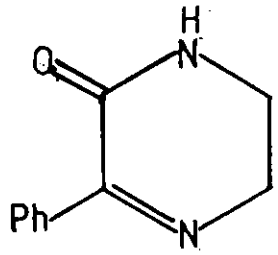
No thiophenone was observed from the degrading basic solutions of the DKP derivatives, therefore suggesting that the thiophenone is only formed after formation of the cephalosporoic acid. At neutral pH, 35°C, 83% of cef adroxil underwent I.M.A. to DKP, the remaining 17% gave the cephalosporoic acid by water-catalysed hydrolysis.

Barbhaiya et al.[58] have shown that the fluorescent degradation product formed by alkaline hydrolysis of cephalexin at room temperature followed by mild acid hydrolysis in presence of formaldehyde is 2-hydroxy-3-phenyl-6-methylpyrazine. Strong acid hydrolysis (5 M HCl) of cephalexin[82] and cephradine[84] gave the same analytically useful polarographic wave, which was later found to be due to 2-hydroxy-3-phenylpyrazine and addition of formaldehyde to the acidic solution of cephalexin produced the polarographically indistinguishable 2-hydroxy-3-phenyl-6-methylpyrazine. In the strong acid hydrolysis of cephalexin (12.5% H_2SO_4, 100°C) Bontchev et al.[184] isolated an aminoaldehyde (penicilloaldehyde).



VI

Changes in the UV spectra of the degrading solution were observed if it was heated for more than ten minutes. It was suggested that this was due to cyclization of the aminoaldehyde, possibly to structure XV,



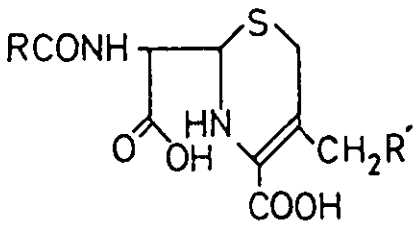
XV

which may then form an aromatic pyrazine (VII).

Fogg et al.[78] followed the build-up of the DKP derivatives of cephalexin and cephradine at neutral pH by polarography. Half the total sulphur available was evolved as hydrogen sulphide and a pyrazine derivative was also found in the degrading solutions[79]. Neutral degradation at elevated temperatures (100°C) afforded a high yield of a polarographically

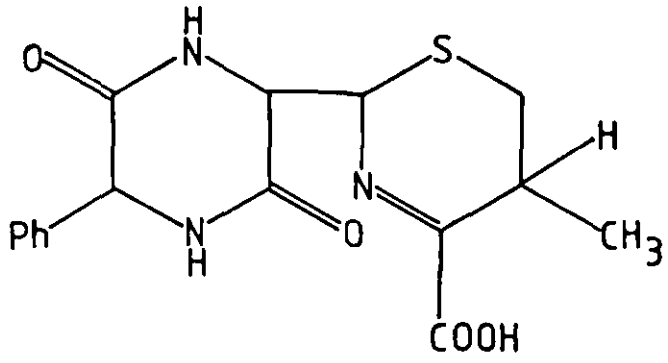
active carbonyl compound[83], thought to be formed from the degradation of the thiophenone isolated by Dinner[207]. Recently hydrogen sulphide has been found to be present in large yields from ∝-aminobenzylcephalosporins when subjected to strong alkaline hydrolysis[198].

Below shows the general structure of cephalosporoic acid derivatives of cephalosporins and the DKP derivative of cephalexin (VIII).



Cephalosporoic acid

VIII



EXPERIMENTAL

Equipment

Polarographic measurements were made with a PAR 174 polarographic analyser (Princeton Applied Research Corporation, Princeton, New Jersey, U.S.A.), equipped with a drop timer. Polarograms were recorded with an Advance HR 2000 x-y recorder. Three electrode operation was used with a dropping mercury electrode as working electrode, a platinum counter electrode and a saturated calomel reference electrode. For differential pulse operation the forced drop-time was 1.0 second, the pulse height 50 mV and the scan rate 5 mV s^{-1}. The glass capillary used with the dropping mercury electrode had an internal diameter of 0.05 to 0.07 mm and was 10 cm in length. The mercury reservoir was kept at a height of 63 cm. The resultant natural drop-time from this system was typically 2 seconds. Traces of oxygen in the nitrogen gas used to deoxygenate the solutions were removed by means of vanadium(II) scrubbers[211].

Spectrophotometric measurements were made with a Pye Unicam SP8-100 spectrophotometer.

Chemicals

All chemicals were of analytical reagent grade except where otherwise indicated.

Samples

Samples of cephalothin, cephaloridine, cephalosporin C, desacetyl-cephalosporin C, 7-aminocephalosporanic acid (7-ACA), 7-aminodesacetoxy-cephalosporanic acid (7-ADCA), cephaxazole, cephalonium, cefuroxime, cephalexin, benzylpenicillin (penicillin G), phenoxypenicillin (penicillin V), benzylpenicilloic acid, benzylpen illic acid and penicillamine were obtained from Glaxo Operations (U.K.) Ltd. Samples of cephaloglycin,

cefaclor and cefazolin were obtained from Lilly Research Centre Ltd. Samples of cephradine and ampicillin trihydrate were kindly provided by E.R. Squibb and Sons Ltd. and Beechams Pharmaceuticals Ltd., respectively.

Preparation of Buffer Solutions

Britton-Robinson buffer solution

A stock solution Britton-Robinson universal buffer solution (B.R. buffer) pH 1.9 composed of a mixture of boric acid, orthophosphoric acid and glacial acetic acid, all 0.04 M, was prepared; pH adjustments were made with 0.2 M sodium hydroxide solution as required.

Phosphate buffer solution (pH 7.4)

The phosphate buffer was prepared by adjusting a 0.5 M disodium hydrogen phosphate solution to pH 7.4 by addition of 0.5 M potassium dihydrogen phosphate solution.

McIlvain's citrate-phosphate buffer solution

A stock solution of citrate-phosphate buffer was prepared by adjusting 0.1 M citrate acid solution to the required pH with 0.2 M disodium hydrogen phosphate solution.

Sorensen's citrate buffer solution

A stock solution of Sorensen's citrate buffer solution was prepared by adjusting the pH of 0.1 M disodium citrate (21.0 g citric acid monohydrate dissolved in 200 ml 1 M sodium hydroxide and made up to 1 litre with distilled water) to the required pH with 0.1 M sodium hydroxide and 0.1 M hydrochloric acid.

EXPERIMENTAL AND RESULTS

A. DP POLAROGRAPHIC STUDY OF THE ALKALINE DEGRADATION OF CEPHALOSPORINS AND PENICILLINS

1. Alkaline hydrolysis of cephalosporins

Little is known of the alkaline hydrolysis of cephalosporins. The cephalosporin molecules are thought to degrade via opening of their β-lactam rings to the unstable cephalosporoic acid derivative which then fragments[188]. A number of cephalosporins were degraded in alkaline conditions and these solutions were then examined polarographically for similarities.

Degradation of cephalexin using NaOH (0.1 M)

Cephalexin (105 ppm) (3.02×10^{-4} M) was degraded at 25°C in sodium hydroxide (0.1 M). Periodically samples were removed and polarographed at pH 2.

Two waves were recorded; at -0.39 V and -0.50 V. Their change of wave height with hydrolysis time is shown below (Table 1).

Table 1.

Time/hrs	Peak height/ μA	
	Peak at -0.39 V	Peak at -0.50 V
0.00	0.0	0.0
0.33	4.9	0.0
0.67	5.8	0.0
1.00	5.8	0.0
2.00	5.5	0.9
4.00	4.2	1.3
15.20	1.2	2.4
23.00	1.2	4.5
48.00	0.0	0.0

DC polarographic scans of the two waves were both cathodic and therefore unlikely to be due to thiol-type compounds. The first degradation product recorded (-0.39 V) reaches its maximum wave height after 0.67 h.

Degradation of cephalexin using NaOH (0.025 M)

Cephalexin (100 ppm) (2.88×10^{-4} M) was degraded and polarographed as above, only 0.025 M sodium hydroxide was used.

As in the NaOH (0.1 M) study only two peaks were recorded (see fig. 9), at -0.50 V and -0.39 V.

The use of a weaker solution of sodium hydroxide has two effects on the cathodic waves; (i) the time taken to reach the maximum heights is greater and their subsequent degradation is slower; (ii) the actual values of the maximum wave heights is lower than in the 0.1 M NaOH study.

In both studies the -0.50 V wave only appears as the -0.39 V wave begins to decrease.

Degradation of cephaloglycin

Cephaloglycin (100 ppm) (2.47×10^{-4} M) was treated as cephalexin (above) using sodium hydroxide (0.025 M).

The -0.39 V and -0.50 V peaks were the only waves observed. There was no sign of the -0.90 V wave that is due to the reductive elimination of the acetyl group from the C-3 position of cephaloglycin meaning that almost immediately no intact cephaloglycin is present. (For a comparison of the change of wave heights with time see fig. 10).

The -0.39 V wave increases with a faster rate than the cephalexin study but has a lower maximum wave height (i_p max). The -0.50 V wave forms at the same time as the -0.39 V wave.

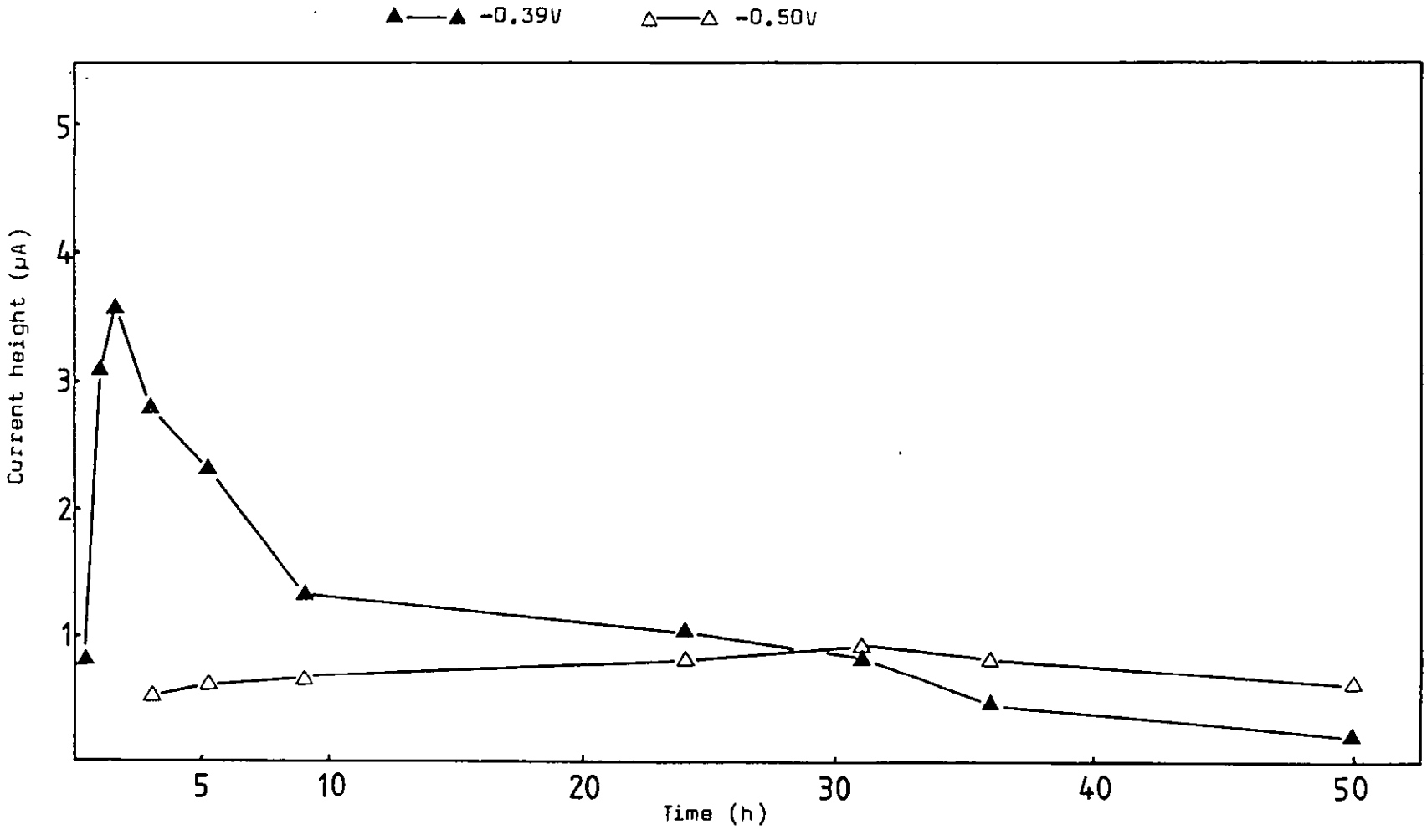


Fig.9 Plot of peak heights of the degradation products of cephalexin after alkaline hydrolysis, NaOH (0.025M), 25°C, polarography at pH 2.

Key as for Fig. 9

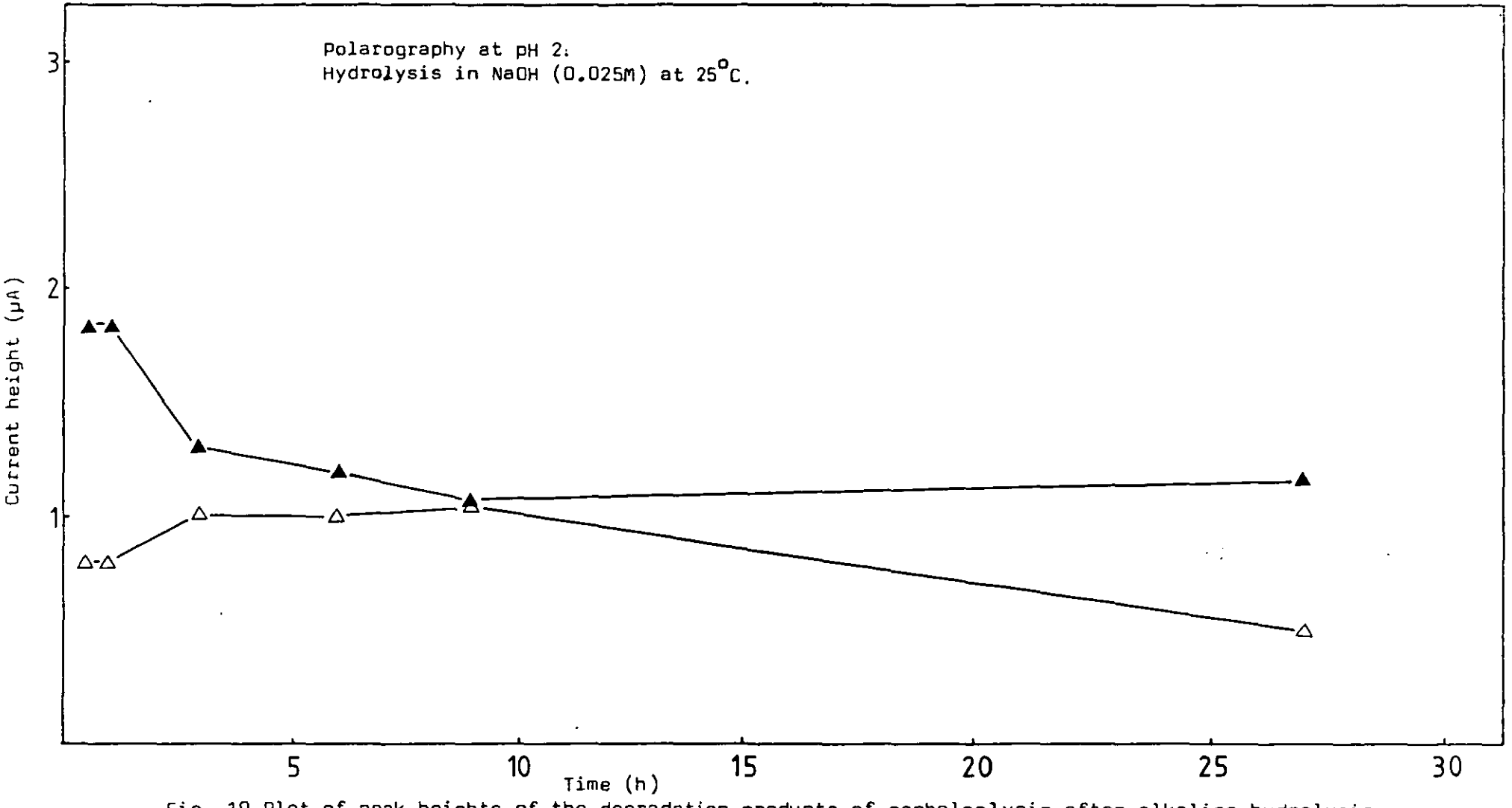


Fig. 10 Plot of peak heights of the degradation products of cephaloglycin after alkaline hydrolysis.

Degradation of cephalothin

Cephalothin (2.39×10^{-4} M) was degraded as cephalexin (above) using sodium hydroxide (0.025 M).

Cephalothin, which has the same C-3 group as cephaloglycin ($-CH_2OC(O)CH_3$), shows a large wave at -1.04 V (3.6 μA) which was observed in the initial solution immediately after being made up. This is due to the reductive elimination of the acetyl group. After 30 minutes hydrolysis this had almost disappeared showing that some intact cephalothin was still present after this time. It was not until 1.5 hours that this wave had completely gone.

The -0.39 V and -0.50 V waves behaved as with cephalexin. The -0.39 V wave increased and reached a maximum then declined as the -0.50 V wave appeared.

Degradation of cephaloridine

Cephaloridine (2.58×10^{-4} M) was treated as cephalexin (above) using sodium hydroxide (0.025 M).

Cephaloridine initially showed a large wave at -0.93 V (3.5 μA) (due to the pyridine group at C-3 being reductively eliminated). This wave had completely disappeared after 30 minutes showing that after this time no intact cephaloridine was present. The -0.39 V and -0.50 V waves were present and behaved as in the cephalexin study.

A further number of cephalosporins were subjected to sodium hydroxide (0.025 M) hydrolysis at 25°C. Periodically samples were removed and polarographed at pH 2.

With cephalosporin C (2.47×10^{-4} M) the two cathodic waves at -0.39 V and -0.50 V are formed very much quicker than in previous studies and the same pattern was followed (build up of the -0.39 V wave, as it declines the -0.50 V wave appears), over the 24 hour study as in previous cases (see fig. 11).

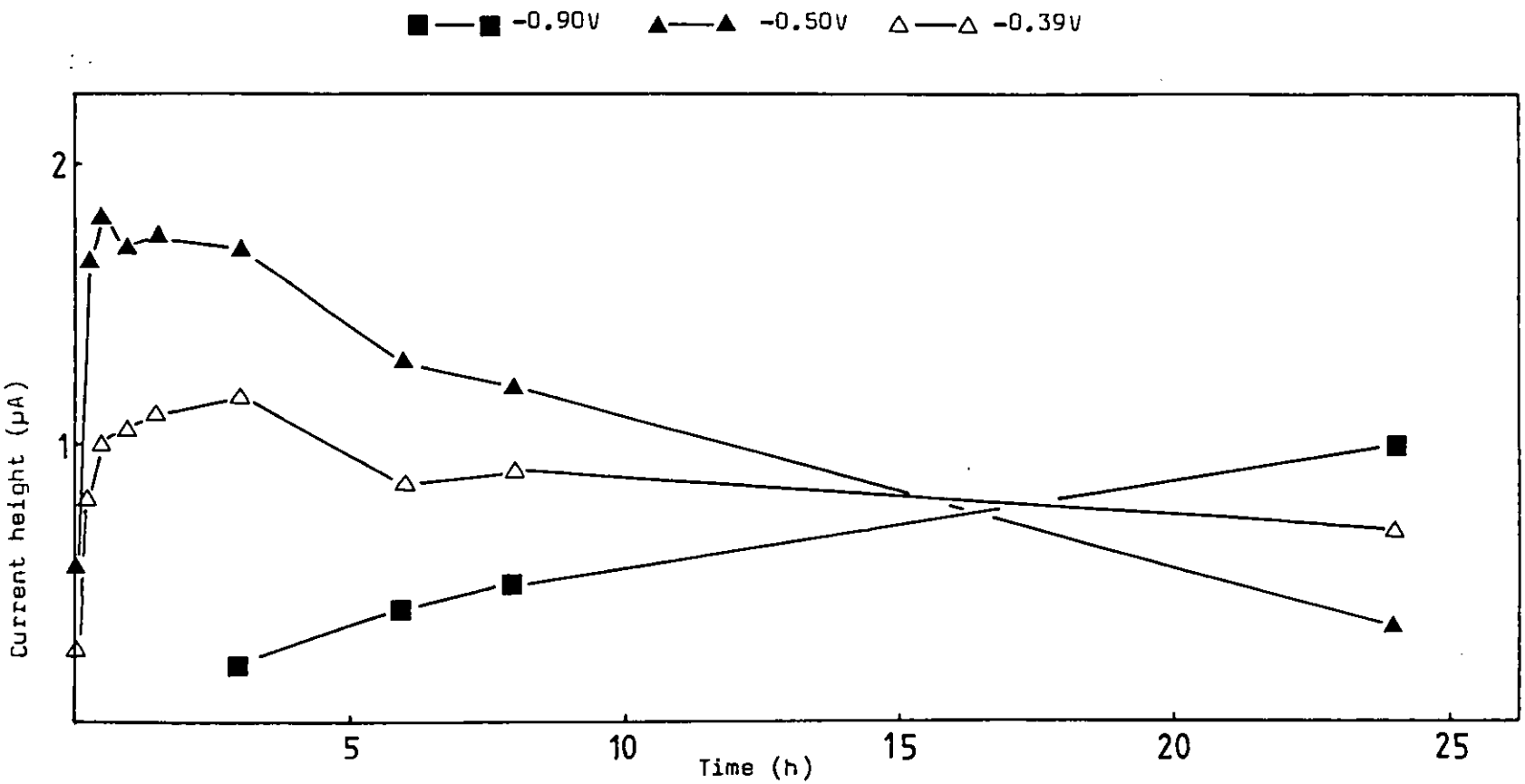


Fig. 11 Plot of peak heights of the degradation products of cephalosporin C after alkaline hydrolysis, NaOH (0.025M), 25°C, polarography at pH 2.

In the case of desacetylcephalosporin C (2.93×10^{-4} M) the absence of the acetyl group had no major effect on the hydrolysis pattern, although the -0.50 V wave reached the same level as the -0.39 V wave (see fig. 12).

Cefuroxime (2.61×10^{-4} M) initially gave a large wave at -1.06 V, (due to the reductive elimination of the C-3 substituent group, $-OC(O)NH_2$). Under these conditions no intact cefuroxime was present after 6 hours. Also initially a large wave at -0.39 V was recorded (12 μA). This reached a maximum height after 0.5 hours, after which it declined. No -0.50 V wave was recorded. A wave at -0.67 V increased steadily throughout the study (see fig. 13).

Cephalonium (2.29×10^{-4} M) gives five waves. The wave at -0.50 V reaches a low level (1.2 μA) then declines slowly. The -0.39 V wave is much larger than the -0.50 V wave, reaching a maximum of 4 μA, afterwards it declines. A small wave corresponding to the reduction of the C-3 substituent was present at -1.07 V. This wave had completely disappeared after 6 hours. Two very large waves at -0.70 V and -0.82 V were present. That at -0.70 V decreases as that at -0.82 V increases (see figs. 14 and 15).

Cephoxazole (2.20×10^{-4} M) showed no sign of the usual reduction wave at approximately -1.0 V that is common with C-3 substituted cephalosporins, suggesting that no intact cephoxazole was present as soon as the drug was made up in the hydroxide solution, as intact cephoxazole gives a wave at -0.95 V in pH 2[78]. Three waves were present. The -0.39 V wave reached a maximum value after 30 minutes and then declined. Concurrently the -0.50 V wave reached a maximum of 1.1 μA, afterwards it declined slowly. As these two waves declined a wave at -0.86 V was being formed. It reached a maximum at 6 h (1.2 μA) after which it slowly declined (see fig. 16).

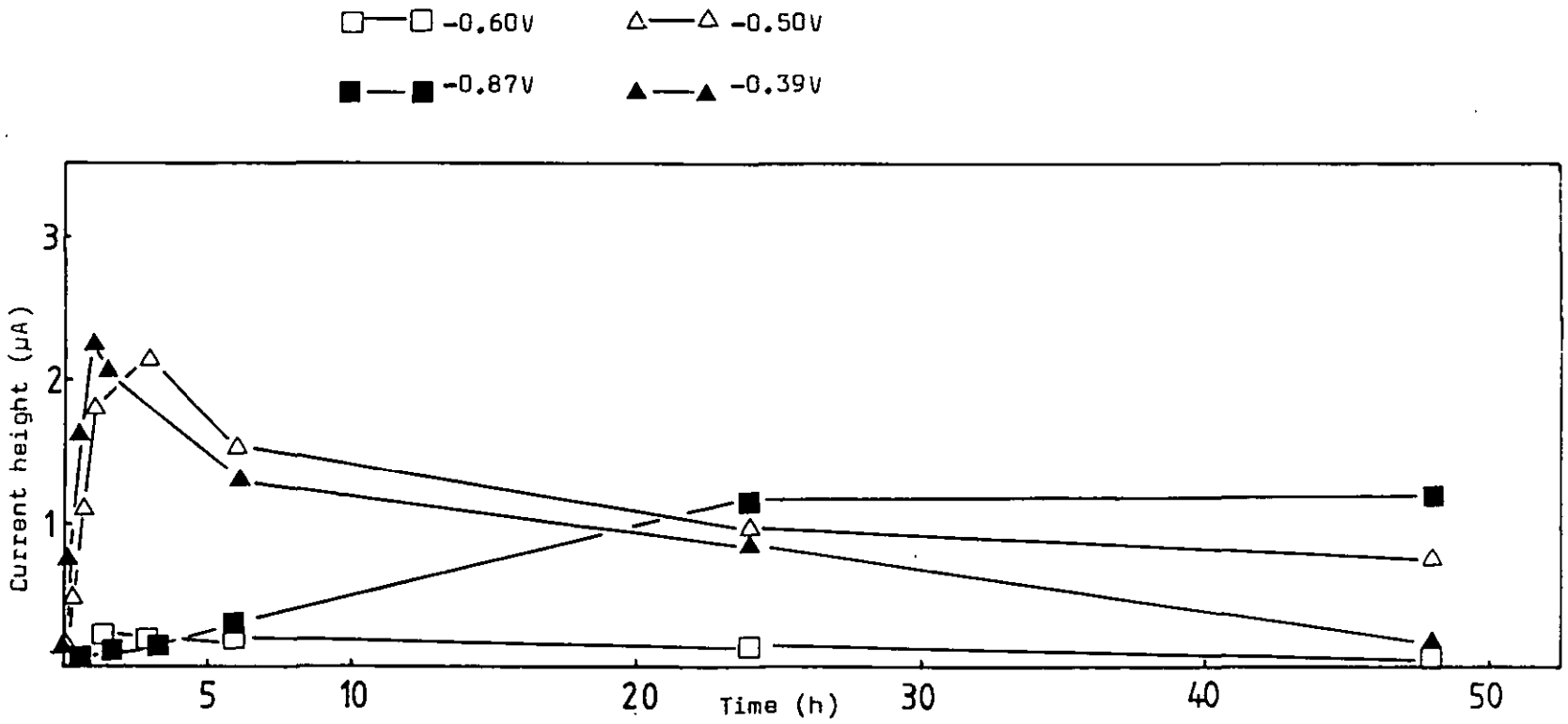


Fig. 12 Plot of peak heights of the degradation products of desacetylcephalosporin C after alkaline hydrolysis, NaOH (0.025M), 25°C, polarography at pH 2.

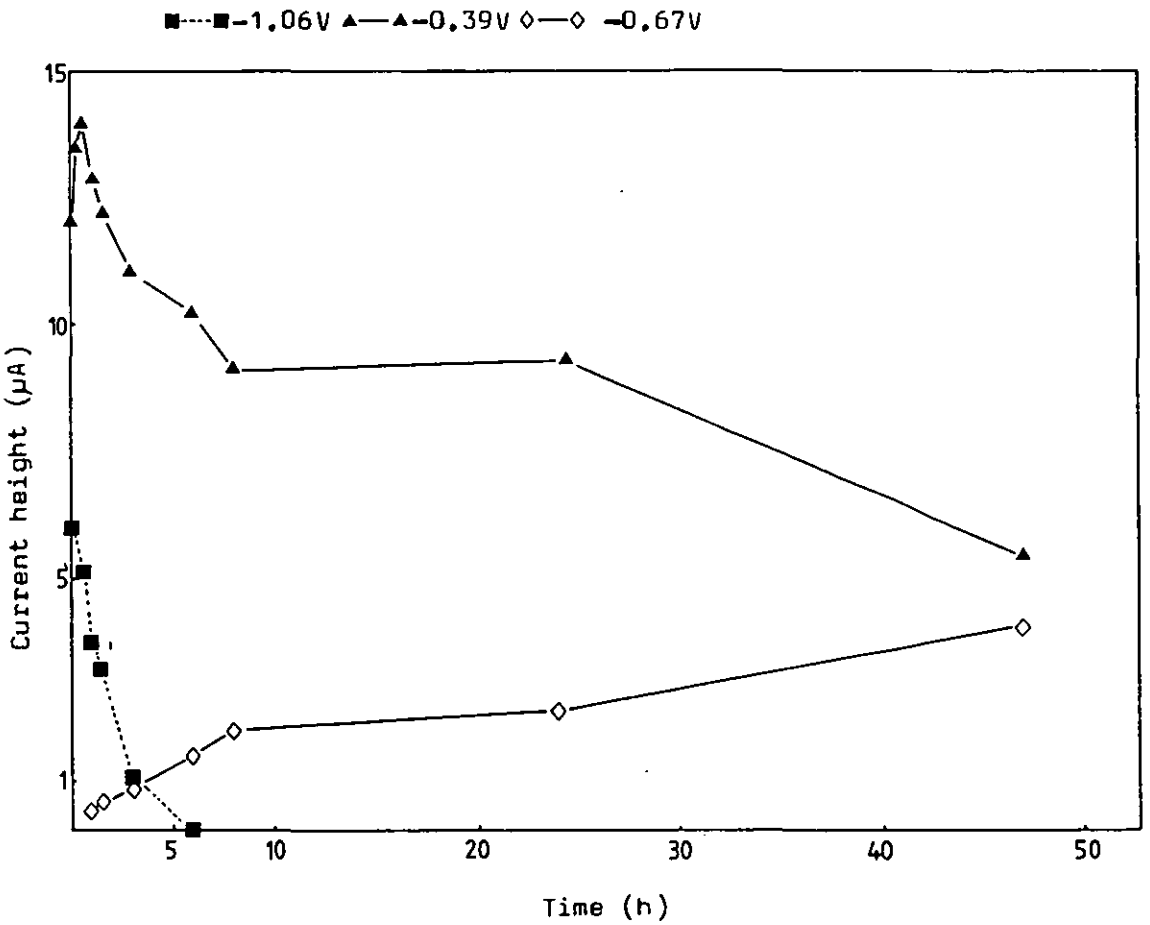


Fig. 13 Plot of peak heights of the degradation products of cefuroxime after alkaline hydrolysis, NaOH (0.025M), 25°C, polarography at pH 2.

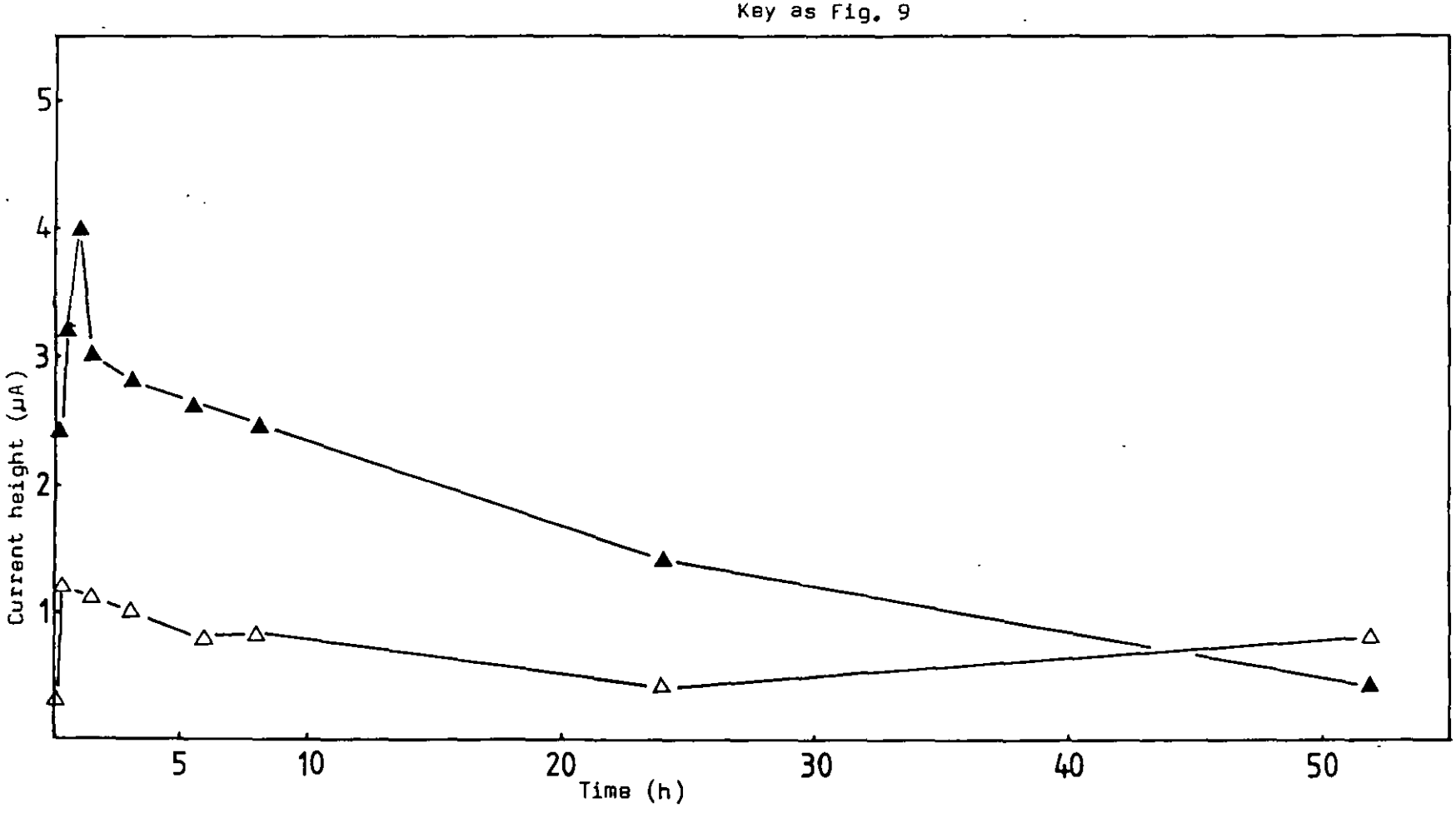


Fig. 14 Plot of peak heights of the degradation products of cephalonium after alkaline hydrolysis, NaOH (0.025M), 25°C, polarography at pH 2.

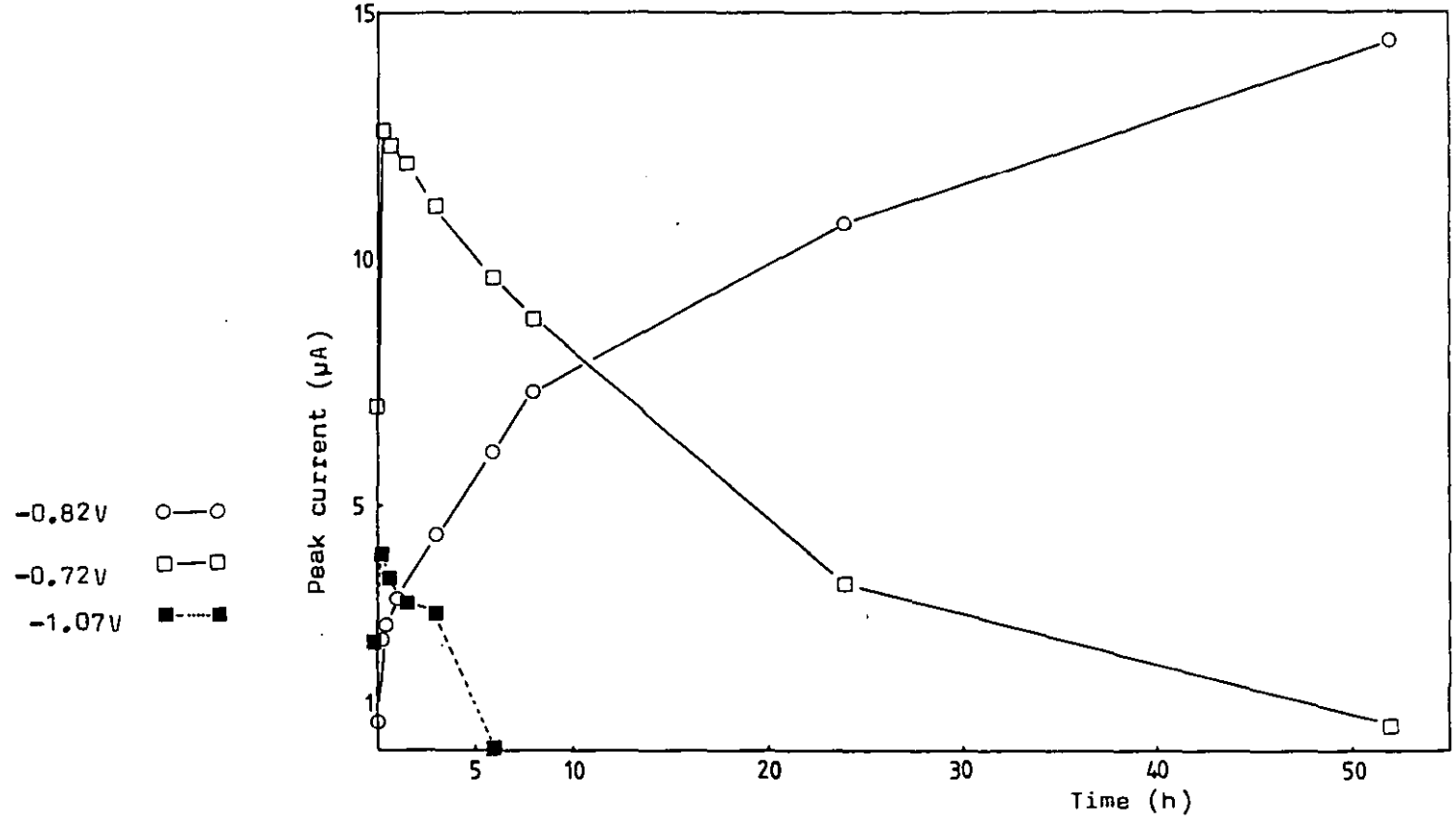


Fig. 15 Cephalonium Plot continued from fig. 14.

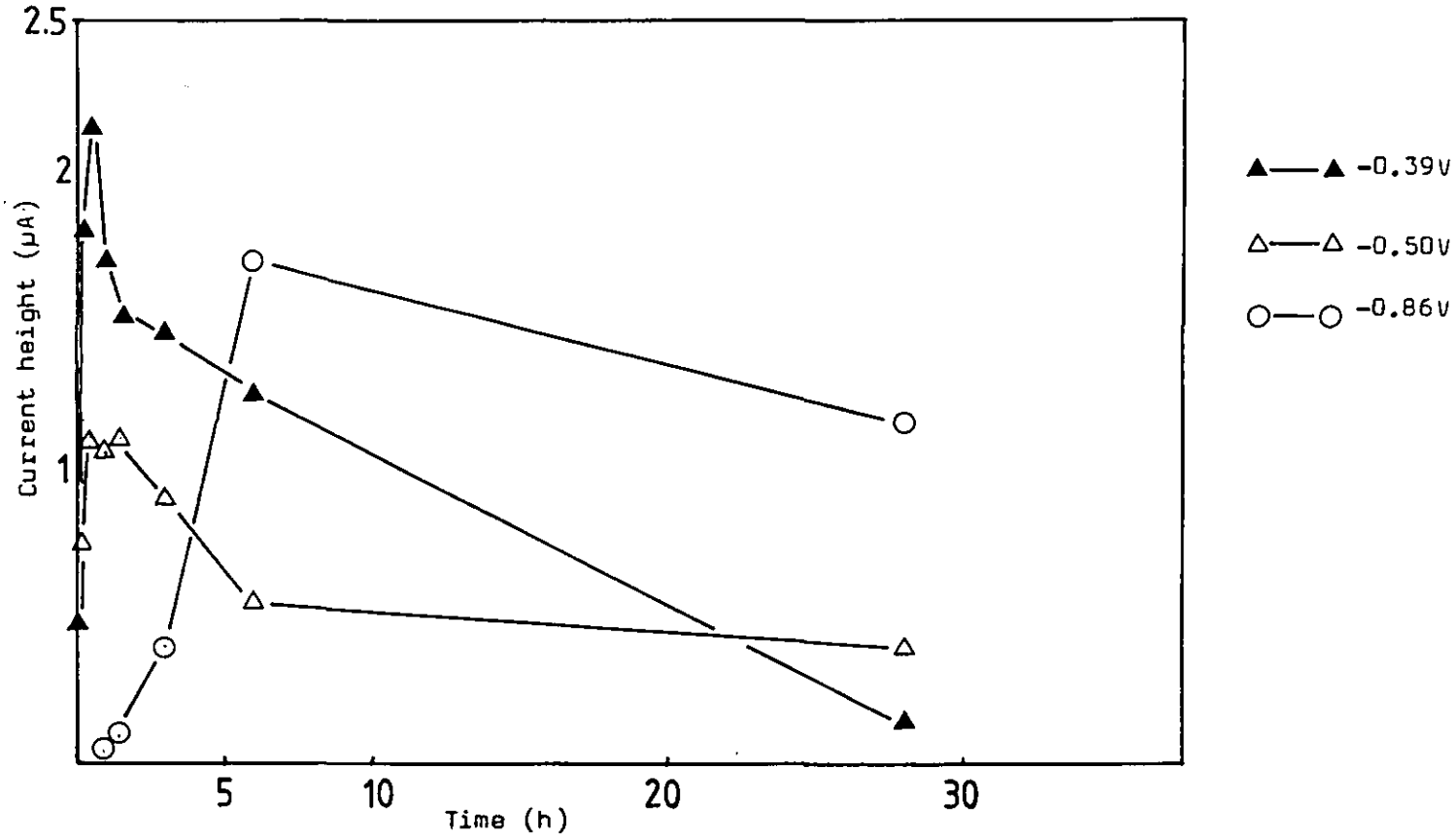


Fig. 16 Plot of peak heights of the degradation products of cephoxazole after alkaline hydrolysis, NaOH (0.025M), 25°C, polarography at pH 2.

2.

The synthesis of the diketopiperazine derivative of cephalexin(VIII)

The DKP was isolated according to Bundgaard[204]. A solution of 2 g of cephalexin in 100 ml of 0.3 M aqueous phosphate buffer solution with pH adjusted to 7.6 was kept at 35°C for 24 hours. After removal of a small amount of a crystalline precipitate formed during the reaction, the solution was brought to pH 2.5 with 5 M hydrochloric acid and extracted with two 100 ml portions of ethyl acetate. After being washed twice with water and subsequently dried over anhydrous sodium sulphate the combined ethyl acetate extracts were evaporated in vacuo below 30°C. The residue of evaporation was suspended in 25 ml of boiling chloroform and filtered off. After drying in vacuo over phosphorus pentoxide for 24 hours a slightly yellow-coloured amorphous solid was obtained. The melting point is quoted as 160-162°C dec. The compound isolated gave a m.p. of 162°C dec.

The dependence on pH of the half-wave potential of the DKP of cephalexin (111 ppm) was determined (see Table 2).

Table 2. Data obtained for the change in pH of a solution of DKP (cephalexin)

pH	Reduction potential V (-)	Current height (μA)
2.00	0.37	6.7
2.95	0.47	4.6
3.70	0.57	4.8
4.60	0.60	5.1
5.00	0.69	4.6
6.10	0.74	4.6
7.10	0.93	4.5
8.10	1.04	4.5
8.50	1.05	4.5
9.10	1.08	2.8
9.50	1.08	2.5
10.00	1.08	1.5

A graph was plotted of these results (see fig. 17). A linear slope was obtained in the pH range 2-9. The $E_{\frac{1}{2}}$ equation obtained was

$$E_{\frac{1}{2}} = (0.15 - 0.103\ pH)\ V.$$

3.

When degraded as cephalexin (p. 71),

The DKP derivative of cephalexin (2.90×10^{-4} M) gave two major peaks, one of which was a doublet (see fig. 18). The doublet gave two signals of equal height throughout the study. One of these signals was at -0.39 V, previously observed, while the other was at -0.35 V and corresponds to the diketopiperazine compound being reduced. Unlike any of the previous studies the -0.39 V wave was present at its maximum value immediately the drug compound was made up in the hydroxide solution with very little hydrolysis. Also unlike any of the previous studies the -0.39 V wave declines rapidly and has completely gone by 10 hours.

As the doublet at approximately -0.38 V declines a wave at -0.50 V increases and reaches its $i_{p_{max}}$ (3.55 μA) after 6 h, after which it degrades until it reaches a steady level of 2.25 μA (see fig. 19).

4.

Alkaline hydrolysis of penicillins (For structures see fig. 7)

Some penicillins and penicillin degradation products were degraded at 25°C in sodium hydroxide (0.025 M). Periodically samples were removed and polarographed at pH 2 unless otherwise stated.

With ampicillin (102 ppm) (2.53×10^{-4} M) no major polarographic activity was observed. These solutions were also polarographed at pH 7.4 and again no polarographic activity was observed. The study was carried out for 75 hours.

In the case of penicillin G no major polarographic peaks were recorded at pH 2. With polarography at pH 7.4 a large peak at -0.23 V (2.7 μA) was observed only after 15 hours, and remained constant for the 75 hours of the study.

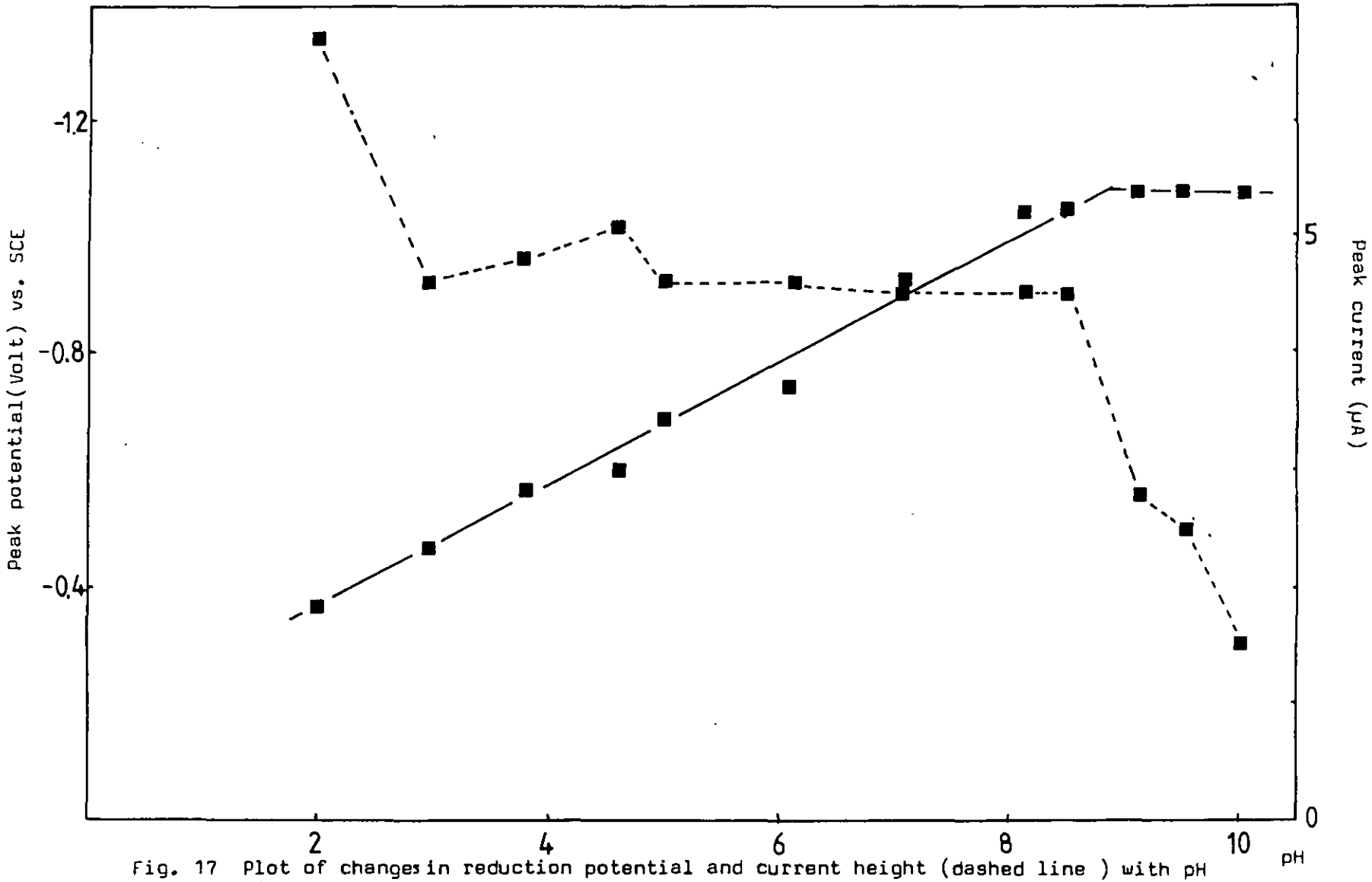


Fig. 17 Plot of changes in reduction potential and current height (dashed line) with pH for the DKP derivative of cephalexin.

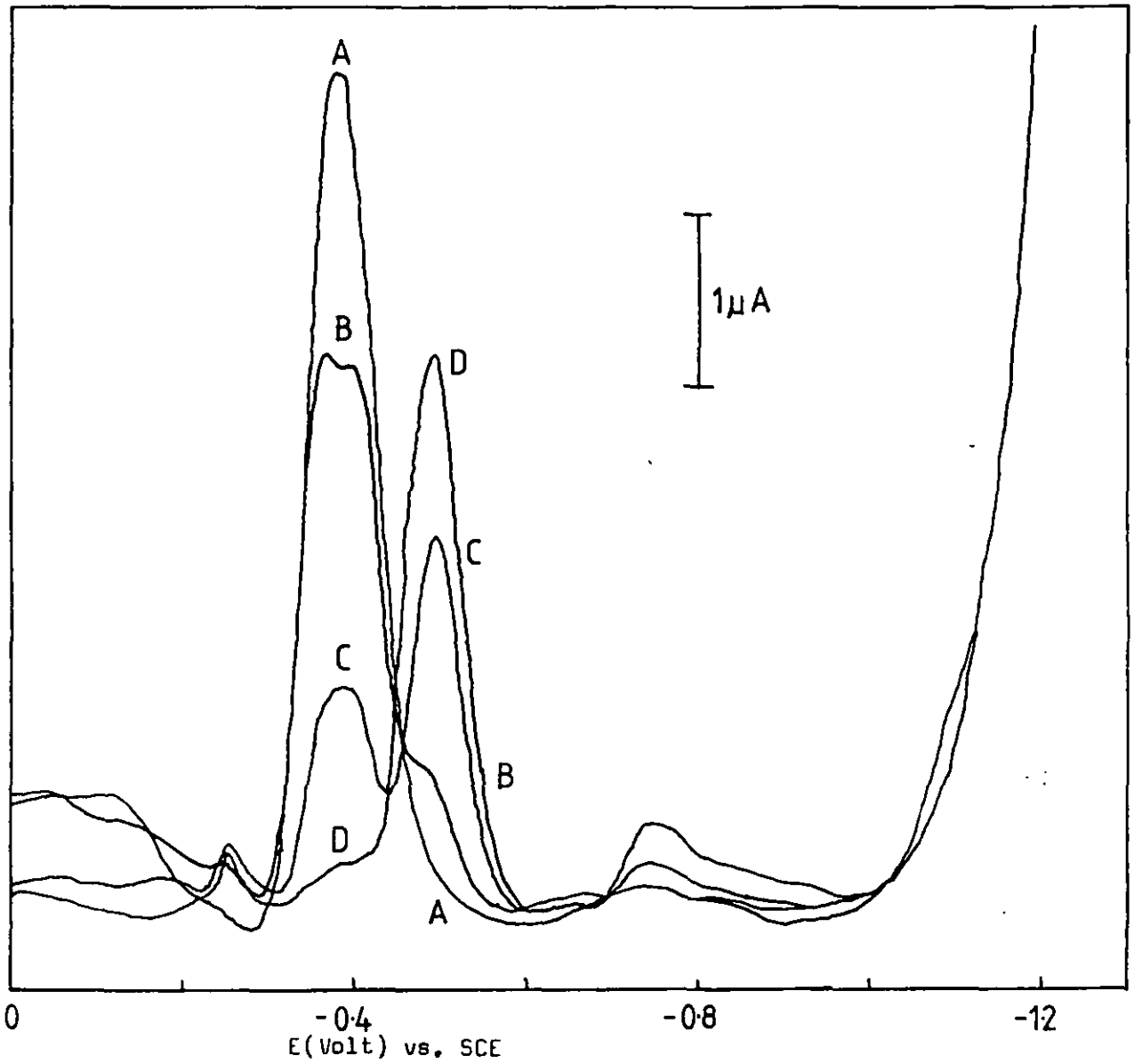


Fig. 18 DP polarograms of the degradation of the DKP derivative of cephalexin at 25°C in NaOH (0.025M), polarography at pH 2. A (0 hours), B (0.5 hours), C (3 hours), D (6 hours).

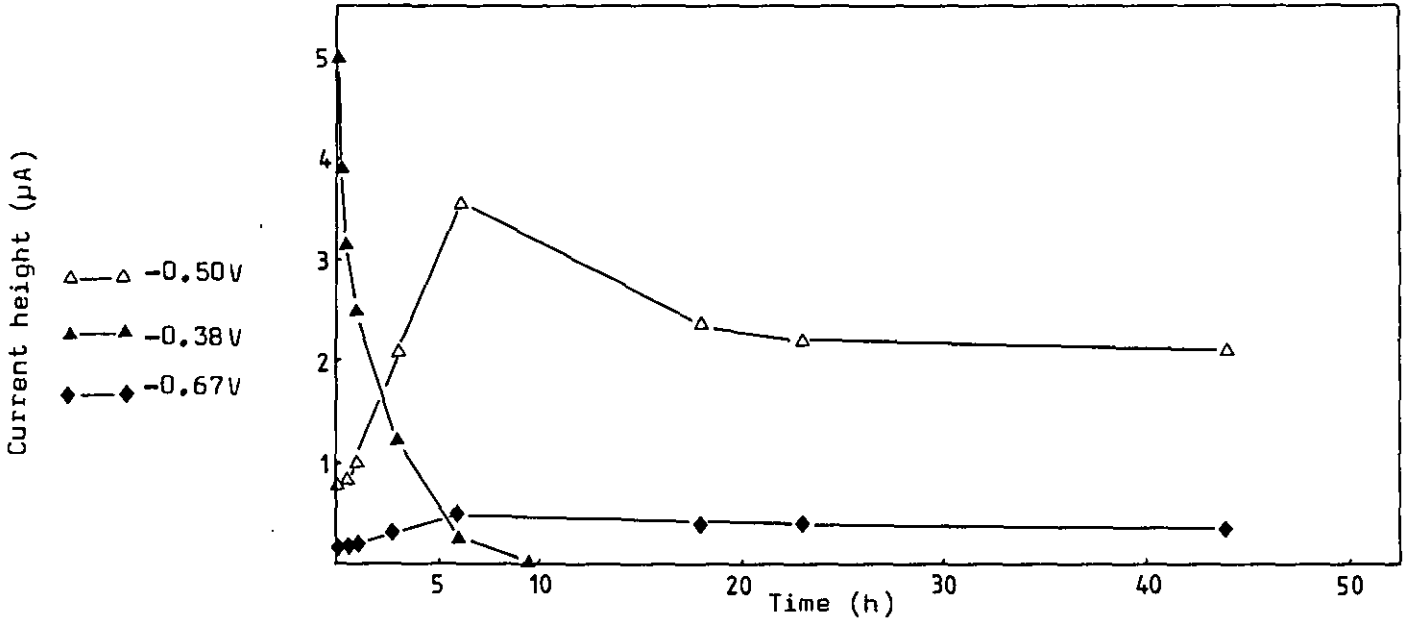


Fig. 19 Plot of peak heights of the degradation products of the DKP derivative after alkaline hydrolysis, NaOH (0.025M), 25°C, polarography at pH 2.

This behaviour suggests a lag period for the formation of the -0.23 V compound from some non-electroactive intermediate. This was the only wave recorded throughout the 75 hours of the study.

With penicillin V (101 ppm) (2.98×10^{-4} M) no waves were recorded for this degradation. The length of the study was 50 hours.

Benzylpenicilloic acid (103 ppm) (3.04×10^{-4} M) showed no polarographic waves with polarography at pH 2.

Benzylpenillic acid (103 ppm) (3.04×10^{-4} M) showed a wave at -0.18 V. This decreased in height throughout the length of the study from a maximum value at zero time of 1 μA (see fig. 20).

Penicillamine (63 ppm) (4.25×10^{-4} M) gave a single wave at -0.13 V. The wave, due to penicillamine, degraded within 10 hours (see fig. 21).

5.

Alkaline degradation of cephaloglycin in the absence of molecular oxygen

Cephaloglycin (102 ppm) (2.52×10^{-4} M) was degraded in sodium hydroxide (0.025 M) at 25°C in the absence of molecular oxygen. Periodically samples were removed and polarographed at pH 2.

As in the study with oxygen (fig. 10), the two major waves observed were at -0.39 V and -0.50 V (see fig. 22). The time of maximum production is not changed with the absence of oxygen. What is markedly noticable is that there is a three-fold increase in the peak height of the wave at -0.39 V and a two-fold increase in the wave at -0.50 V with the study using oxygen.

6.

Strong alkaline hydrolysis of penicillins

A number of penicillins and a penicillin degradation product, penicillamine, were subjected to stronger alkaline hydrolysis than previously. Waves so formed during this hydrolysis were compared by plotting graphs of

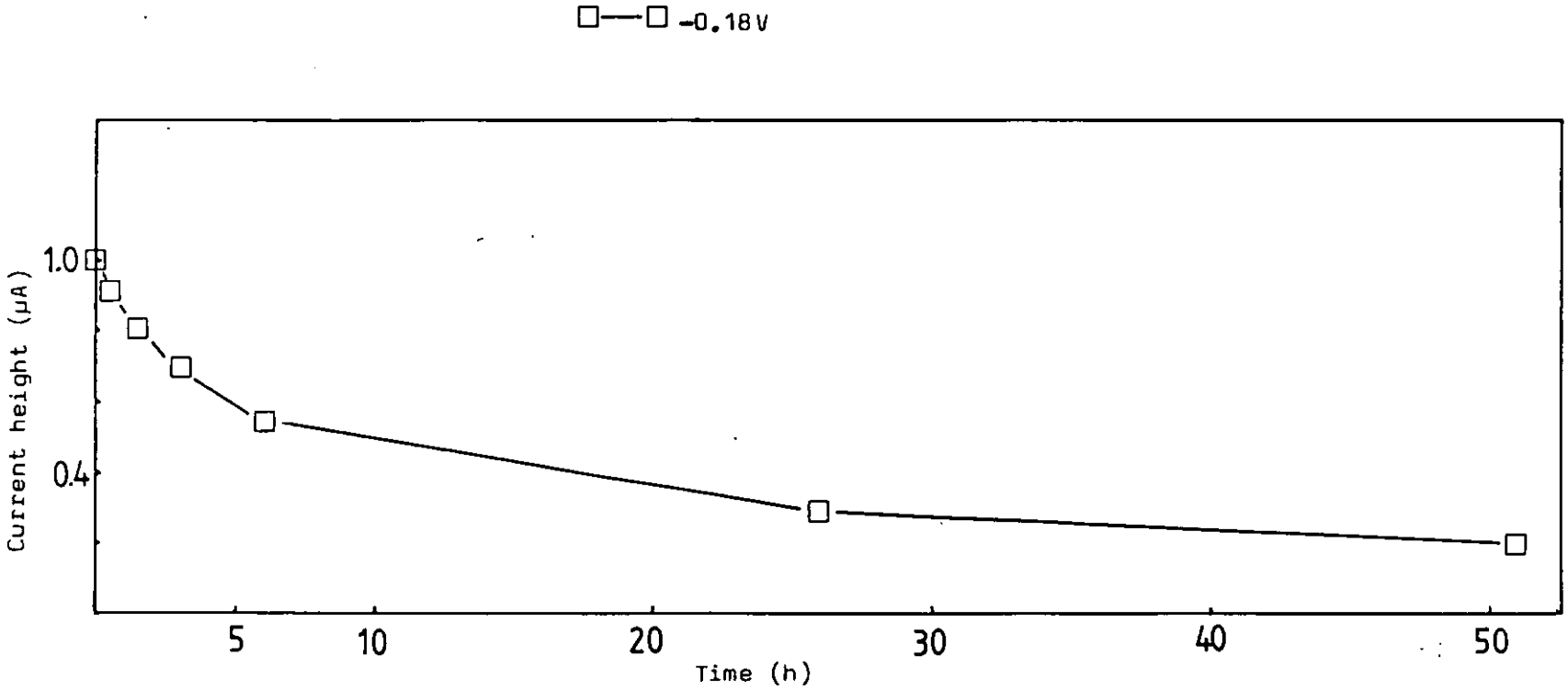


Fig. 20 Plot of peak height of a wave (-0.18V) obtained from the alkaline hydrolysis of benzylpenillic acid, NaOH (0.025M), 25°C, polarography at pH 2.

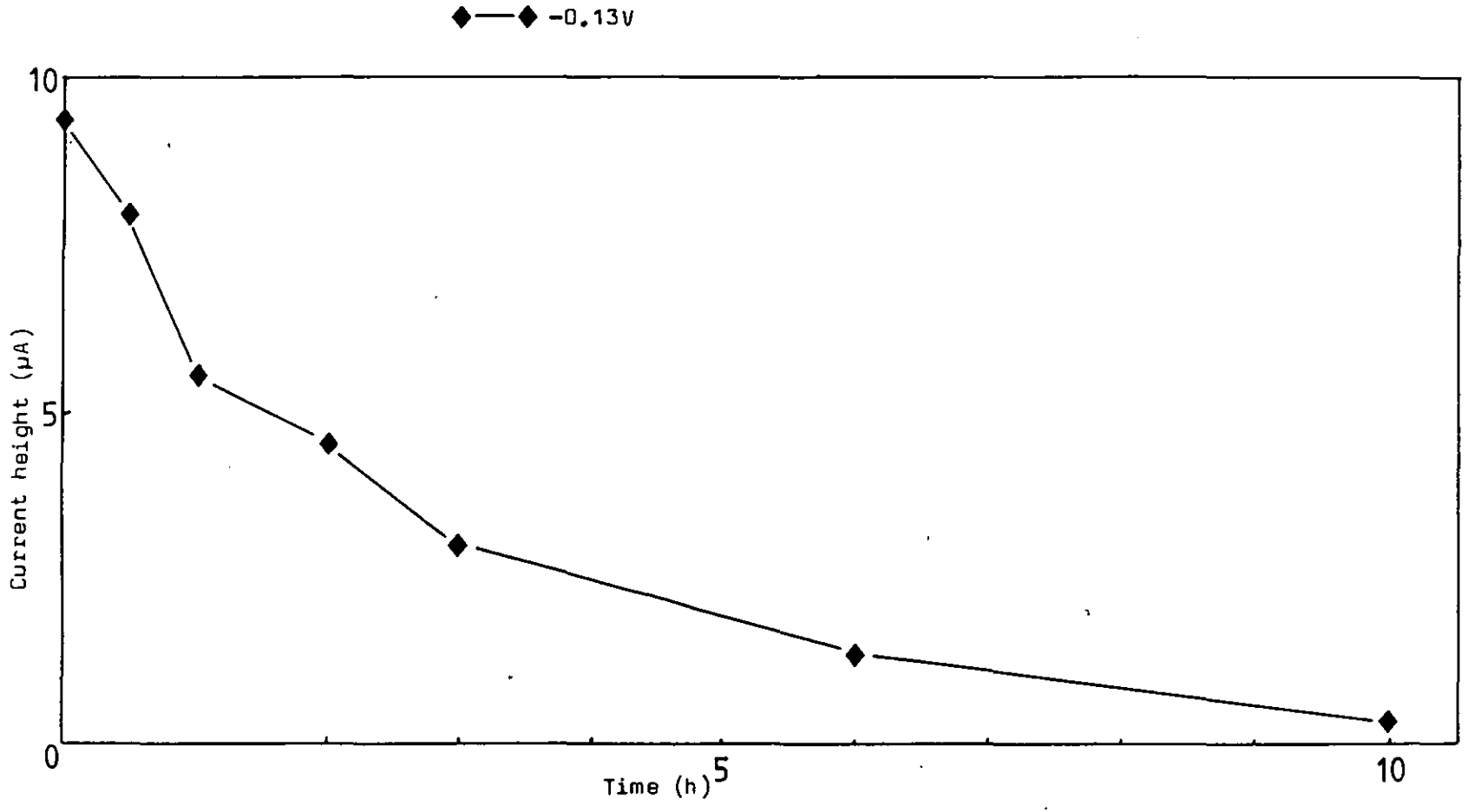


Fig. 21 Plot of the peak height of a wave (-0.13V) obtained from the alkaline hydrolysis of penicillamine, NaOH (0.025M), 25°C, polarography at pH 2.

Key as Fig. 9

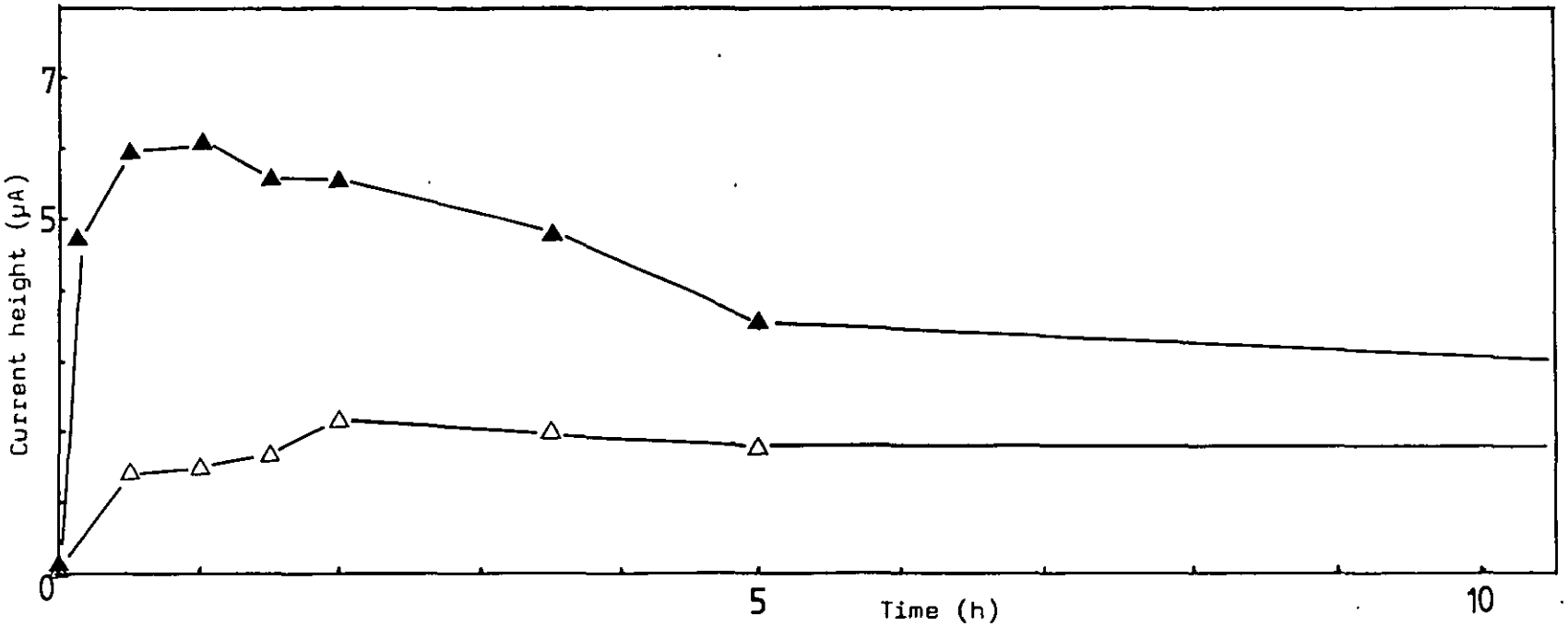


Fig. 22 Plot of peak heights of the degradation products of cephaloglycin after alkaline hydrolysis in the absence of molecular oxygen, NaOH (0.025M), 25°C, polarography at pH 2.

pH versus potential for the waves obtained from each degraded solution and hence from the graphs calculating the dependence on pH of the half-wave potential, $E_{\frac{1}{2}}$, for each peak.

Ampicillin (96 ppm) (2.38×10^{-4} M) was degraded for 1 hour in sodium hydroxide (0.1 M) at 100°C. After this time the solution was cooled and polarographed at various pH values. The pH was adjusted by the addition of HCl (2.0 M) and NaOH (2.0 M).

Two waves were recorded, both around the 3 μA range in height. The $E_{\frac{1}{2}}$ values are given below.

wave A; $E_{\frac{1}{2}} = (-0.56 - 0.08\ pH)$ V

wave B; $E_{\frac{1}{2}} = (-0.36 - 0.071\ pH)$ V

(see fig. 23).

Penicillin G (196 ppm) (5.26×10^{-4} M) was degraded and polarographed as ampicillin (above).

One small wave (2 μA range) was recorded, linear in the range pH 3-6.5.

wave C; $E_{\frac{1}{2}} = (-0.83 - 0.06\ pH)$ V

(see fig. 24).

Penicillin V (138 ppm) (4.08×10^{-4} M) gave one wave in the 2 μA range in height. The linear range was pH 3-11.

wave D; $E_{\frac{1}{2}} = (0.01 - 0.028\ pH)$ V

(see fig. 25).

Penicillamine (161 ppm) (1.09×10^{-3} M) showed two waves. The first with a linear range of pH 2.5-7.5, wave E, gave its maximum wave height in the range pH 9-13 and is due to penicillamine.

wave E; $E_{\frac{1}{2}} = (-0.05 - 0.065\ pH)$ V

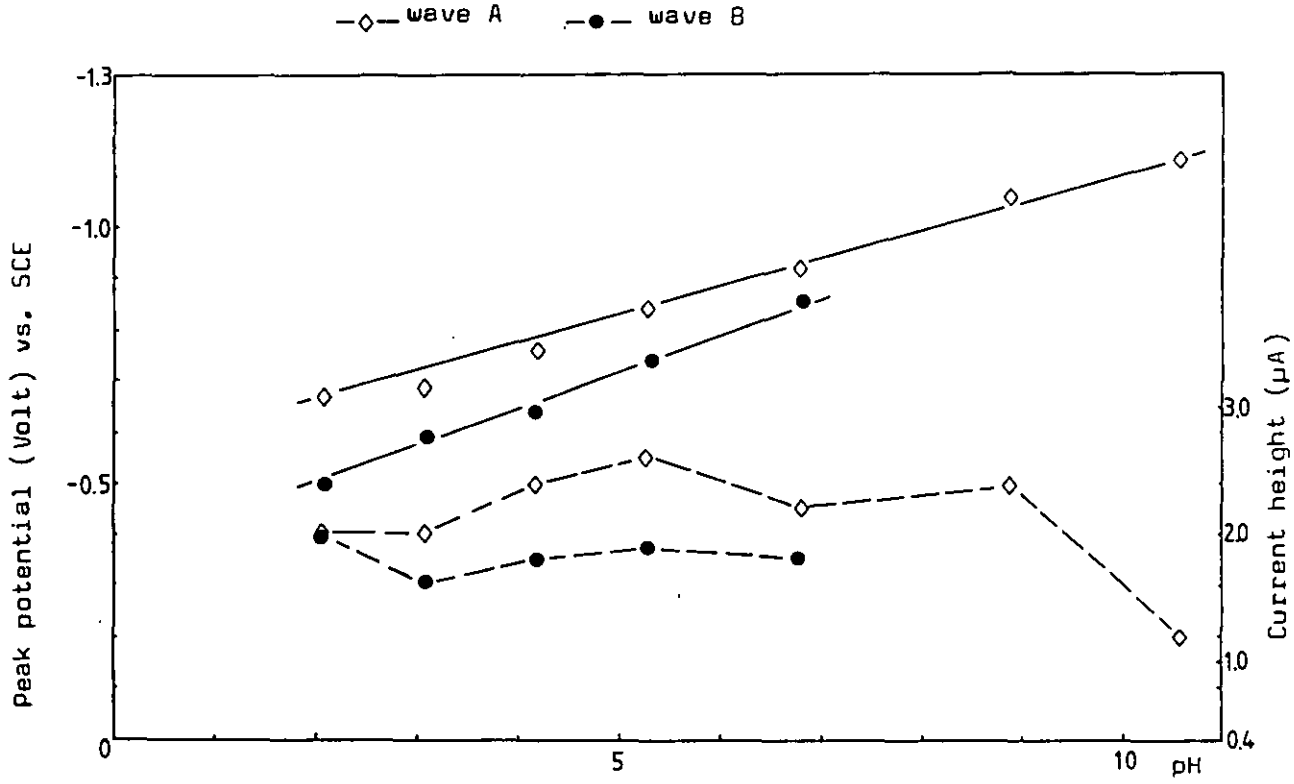


Fig. 23 Plot of changes in reduction potential and current height (dashed line) with pH for ampicillin after alkaline hydrolysis, NaOH (0.1M), 100°C, for 1 hour.

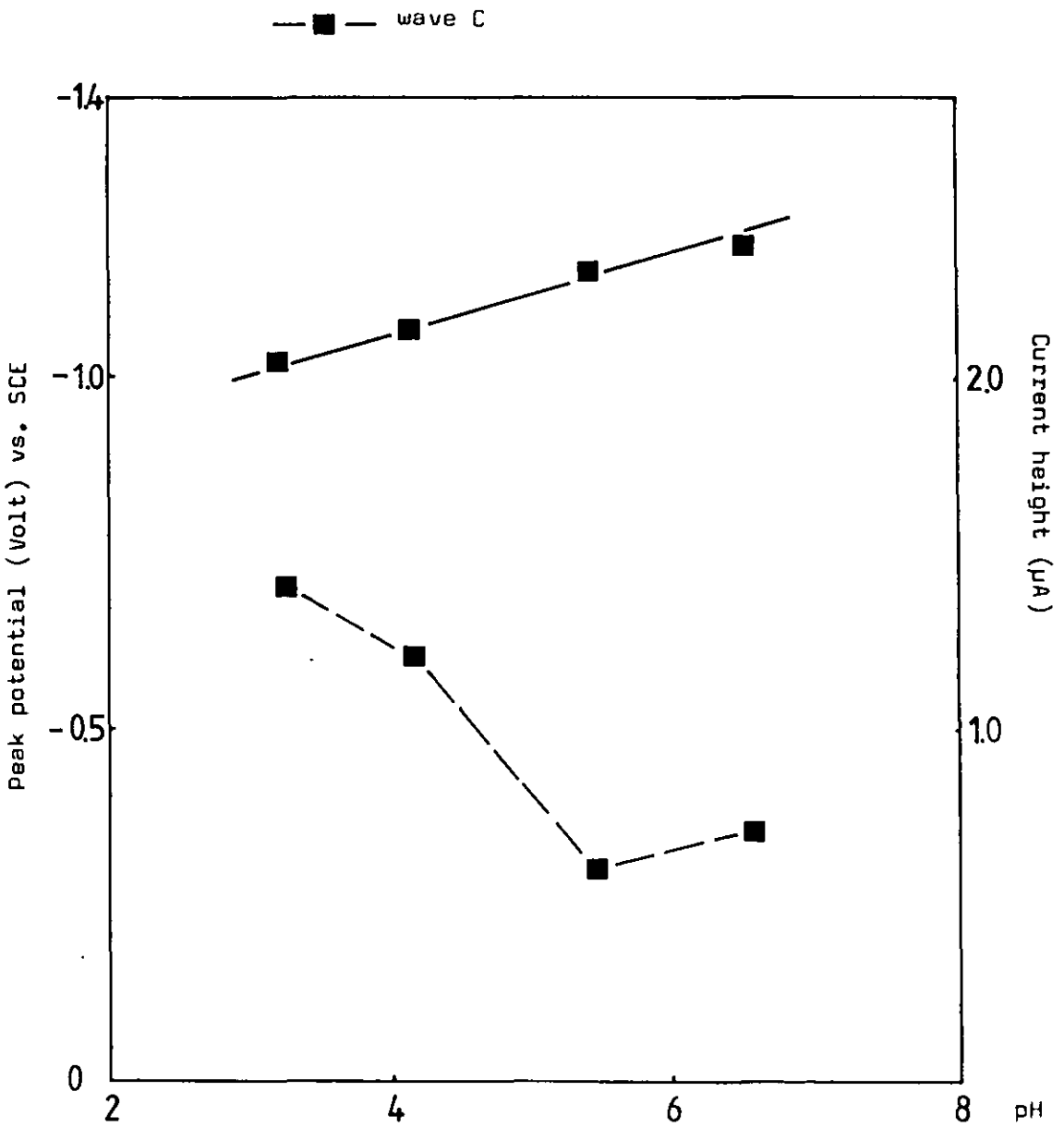


Fig. 24 Plot of changes in reduction potential and current height (dashed line) with pH for penicillin G after alkaline hydrolysis, NaOH (0.1M), 100°C for 1 hour.

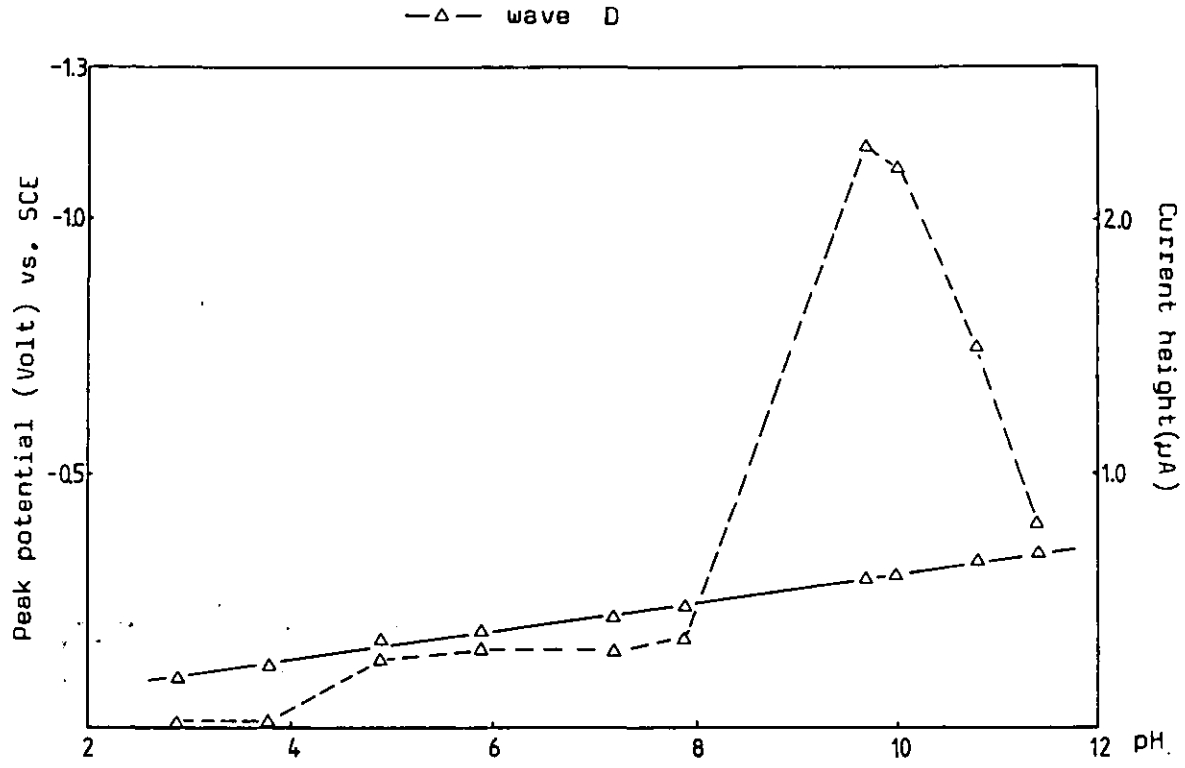


Fig. 25 Plot of the changes in the reduction potential and current height (dashed line) with pH for penicillin V after alkaline hydrolysis, NaOH (0.1M), 100°C for 1 hour.

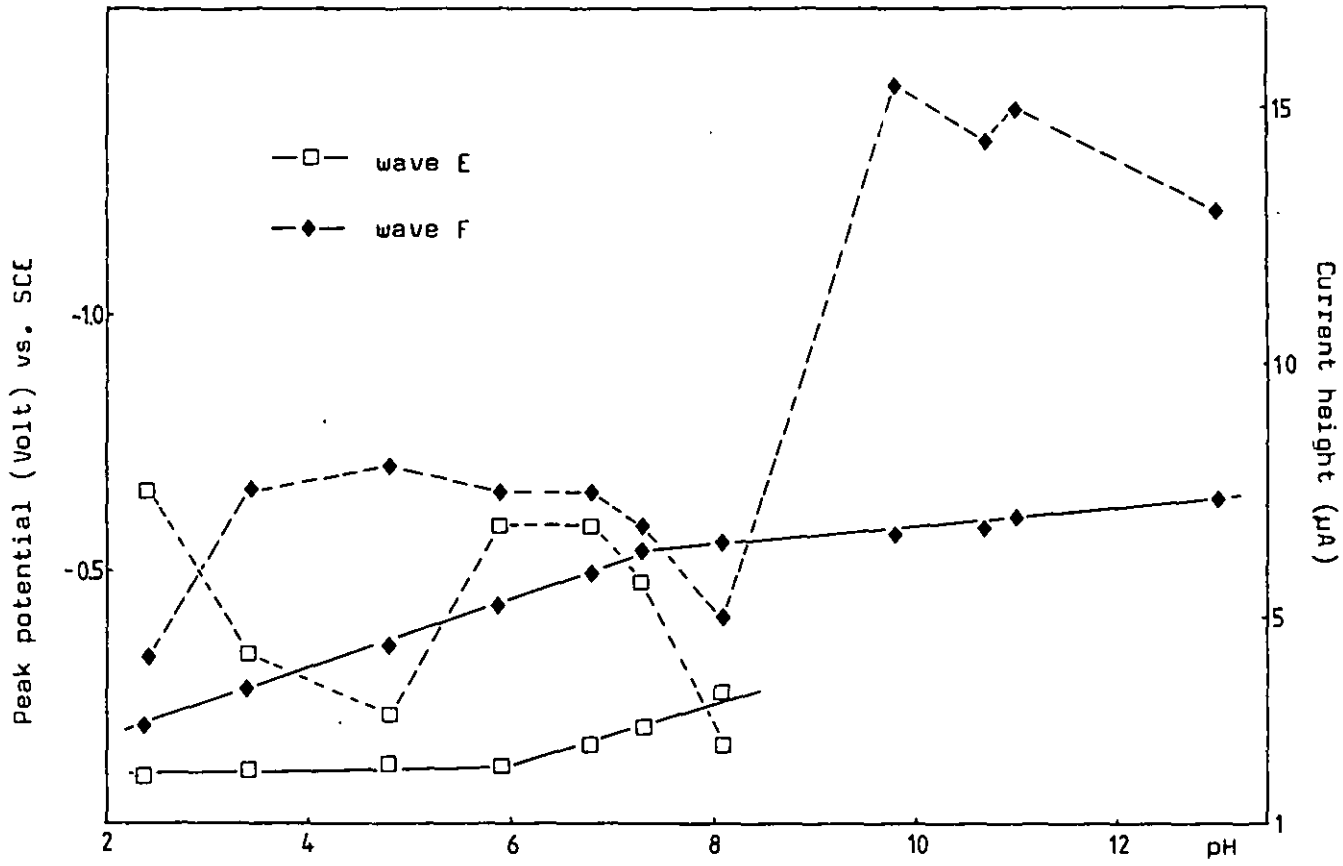


Fig. 26 Plot of the changes in the reduction potential and current height (dashed line) with pH for penicillamine after alkaline hydrolysis, NaOH (0.1M), 100°C for 1 hour.

The second wave, wave F, has a small linear range pH 6-8 (see fig. 26).

7.

Alkaline hydrolysis of cephalosporin followed by pH plots

A number of cephalosporins were degraded in sodium hydroxide (0.1 M) at 25°C for 1 hour. At the end of this time period pH plots were obtained and the dependence of the half-wave potential ($E\frac{1}{2}$) on pH for each polarographically active degradation product was calculated. The $E\frac{1}{2}$ equation for these waves so produced was compared in order to observe similarities in hydrolysis products.

Cephalexin (100 ppm) (2.88×10^{-4} M) was degraded as above and a pH plot was carried out.

Three waves were recorded. As well as the change of potential with pH the change in wave height with pH was also recorded (see Table 3).

Table 3.

pH	wave A		wave B		wave J	
	V (-)	i	V (-)	i	V (-)	i
1.90	-	0.0	0.37	5.4	-	0.0
2.80	-	0.0	0.45	6.1	-	0.0
4.00	-	0.0	0.55	5.4	-	0.0
4.90	-	0.0	0.63	5.4	-	0.0
5.80	0.48	0.5	0.71	5.3	1.31	1.8
6.90	0.54	0.9	0.80	2.3	1.41	5.6
7.70	0.58	1.6	0.85	1.0	1.47	4.9
8.40	0.62	2.2	-	0.0	1.54	3.3
9.60	0.65	2.8	-	0.0	1.64	2.1
10.25	0.68	3.1	-	0.0	1.64	0.5
11.50	0.72	3.0	-	0.0	-	0.0
13.00	0.77	3.8	-	0.0	-	0.0

i = measurement of peak current in units of $\mu A \times 10^{3}$/mole for this and all subsequent tables unless otherwise stated.

Cephaloglycin (125 ppm) (3.08×10^{-4} M) was treated as cephalexin (above). Three waves were observed (see Table 4).

Table 4.

pH	wave A		wave B		wave D	
	V (-)	i	V (-)	i	V (-)	i
2.0	-	0.0	0.39	3.0	-	0.0
2.6	-	0.0	0.49	3.6	1.05	1.5
3.4	-	0.0	0.52	3.9	1.02	2.3
4.8	-	0.0	0.68	6.5	1.02	2.9
5.8	-	0.0	0.77	4.4	1.02	3.2
6.7	-	0.0	0.85	4.2	1.04	3.2
7.5	0.59	0.6	0.96	6.5	1.03	3.9
9.0	0.63	0.8	-	0.0	1.04	3.9
10.1	0.67	0.7	-	0.0	1.04	0.9
11.9	0.75	4.7	-	0.0	-	0.0
13.0	0.79	8.9	-	0.0	-	0.0

Cephalonium (154 ppm) (3.35×10^{-4} M) was treated as cephalexin (above). Two major waves and one very minor wave were recorded (see Table 5).

Table 5.

pH	wave B		wave E	
	V (-)	i	V (-)	i
1.9	0.38	6.0	0.78	35.6
2.6	0.42	7.4	0.81	43.0
3.8	0.53	6.3	0.89	54.0
4.9	0.60	5.5	0.99	57.1
5.9	0.67	12.2	1.09	58.2
7.0	0.76	13.5	1.17	58.8
7.8	0.83	13.0	1.23	36.4
8.9	0.91	11.8	1.28	32.0
9.6	0.96	3.7	1.30	28.6
10.9	1.00	1.4	1.35	29.8
12.0	-	0.0	1.38	37.0
13.0	-	0.0	1.40	44.0

Although not recorded in Table 5 a small wave (0.1 μA) was observed in NaOH (0.1 M) (pH 13) at -0.77 V, and is denoted as wave A.

Cefuroxime (104 ppm) (2.61×10^{-4} M) was treated as cephalexin (above). Two major waves and one minor wave were recorded (see Table 6). Although not recorded in Table 6 a small wave (0.1 μA), denoted as wave A, was observed in NaOH (0.1 M) (pH 13) at -0.77 V.

Table 6.

pH	wave B		wave D	
	V (-)	i	V (-)	ii
2.0	0.39	12.0	-	0.0
2.7	0.46	10.4	1.02	0.6
4.0	0.53	8.8	1.00	1.1
4.6	0.57	7.6	1.00	2.4
6.0	0.66	7.4	1.08	4.4
6.6	0.70	7.4	1.04	3.3
7.7	0.79	5.8	1.04	0.6
8.6	0.84	2.0	1.05	0.2
9.6	0.87	0.3	1.09	0.1
11.0	-	0.0	-	0.0
12.0	-	0.0	-	0.0
13.0	-	0.0	-	0.0

Cephaloridine (100 ppm) (2.41×10^{-4} M) was treated as cephalexin (above). Four waves were recorded (see Table 7).

Cephalothin (181 ppm) (4.33×10^{-4} M) was treated as cephalexin (above). Three waves were recorded (see Table 8).

The peak denoted as A has two components to its pH plot ($E_{\frac{1}{2}}$ equation) A_1 and A_2.

Table 7.

pH	wave A		wave B		wave D		wave F	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i
2.0	-	0.0	0.39	8.3	-	0.0	0.50	5.8
2.9	-	0.0	0.46	9.9	1.02	5.4	0.57	6.6
3.5	-	0.0	0.51	9.9	1.02	5.4	0.61	7.1
4.7	-	0.0	0.59	12.4	1.02	8.3	0.73	7.5
5.6	-	0.0	0.66	13.3	1.02	8.3	-	0.0
6.4	-	0.0	0.74	22.0	1.02	7.5	-	0.0
7.5	-	0.0	0.82	22.0	-	0.0	-	0.0
8.6	0.58	0.1	0.88	2.16	-	0.0	-	0.0
9.8	0.63	0.1	0.98	12.5	-	0.0	-	0.0
10.8	0.66	0.2	1.02	10.4	-	0.0	-	0.0
11.7	0.70	1.7	1.02	0.4	-	0.0	-	0.0
13.0	0.75	1.7	1.02	0.8	-	0.0	-	0.0

Table 8.

pH	wave A		wave B		wave G	
	V (-)	i	V (-)	i	V (-)	i
2.0	-	0.0	0.39	4.2	0.93	0.2
2.9	-	0.0	0.46	4.9	0.99	1.2
3.9	-	0.0	0.57	5.1	1.03	2.8
4.7	-	0.0	0.62	5.3	1.05	4.4
5.5	-	0.0	0.65	5.8	1.11	3.5
6.7	0.50	0.1	0.76	13.6	1.18	6.5
7.4	0.56	0.5	0.81	6.5	-	0.0
8.1	0.60	1.2	0.86	6.9	-	0.0
8.4	0.64	3.9	0.89	6.9	-	0.0
9.7	0.69	12.5	0.97	4.3	-	0.0
10.8	0.72	12.7	1.00	0.2	-	0.0
11.8	0.76	9.7	-	0.0	-	0.0
13.0	0.78	7.4	-	0.0	-	0.0

Cephoxazole (149 ppm) (3.03×10^{-4} M) was treated as cephalexin (above). Three waves were recorded (see Table 9).

Table 9.

pH	wave A		wave B		wave G	
	V (-)	i	V (-)	i	V (-)	i
1.8	-	0.0	0.38	7.9	-	0.0
2.7	-	0.0	0.45	9.2	-	0.0
3.8	-	0.0	0.52	9.5	1.04	2.0
4.8	0.32	0.1	0.56	13.9	1.07	7.3
5.7	0.40	0.1	0.65	17.2	1.12	13.2
6.7	0.50	0.3	0.72	19.5	1.16	14.5
7.6	0.59	1.0	0.81	19.1	-	0.0
8.4	0.64	2.8	0.86	20.8	-	0.0
9.7	0.69	5.6	0.92	6.6	-	0.0
10.8	0.73	5.6	1.02	1.3	-	0.0
11.8	0.76	11.9	-	0.0	-	0.0
13.0	0.78	8.6	-	0.0	-	0.0

The wave denoted as A has two components to its pH plot ($E_{\frac{1}{2}}$ equation), A_1 and A_2.

Cephalosporin C (44 ppm) (1.09×10^{-4} M) was treated as cephalexin (above). Four waves were recorded (see Table 10).

The wave denoted as A has two components to its pH plot ($E_{\frac{1}{2}}$ equation), A_1 and A_2.

Desacetylcephalosporin C (100 ppm) (2.93×10^{-4} M) was treated as was cephalexin (above). Two major waves and one minor wave were recorded (see Table 11).

Although not recorded in Table 11 a very small wave (< 0.05 μA) at -0.39 V (pH 2) was observed. This has been denoted as wave B.

Table 10.

pH	wave A		wave B		wave C		wave G	
	V(-)	i	V(-)	i	V(-)	i	V(-)	i
2.0	-	0.0	0.39	0.9	0.50	0.6	0.92	0.05
2.7	-	0.0	0.44	0.9	0.55	0.4	1.00	0.1
3.3	-	0.0	0.49	1.2	0.61	0.3	1.00	0.4
4.6	0.38	< 0.05	0.60	1.1	0.71	0.2	1.06	0.4
5.7	0.45	< 0.05	0.66	0.9	-	0.0	1.15	0.4
6.5	0.52	< 0.05	0.77	0.8	-	0.0	1.22	0.2
7.4	0.55	0.05	0.84	0.7	-	0.0	1.24	0.1
8.6	0.60	0.1	0.94	0.1	-	0.0	1.37	0.1
9.6	0.63	0.1	-	0.0	-	0.0	-	0.0
10.8	0.67	0.2	-	0.0	-	0.0	-	0.0
11.8	0.71	0.6	-	0.0	-	0.0	-	0.0
13.0	0.74	0.6	-	0.0	-	0.0	-	0.0

Table 11.

pH	wave A		wave G	
	V(-)	i	V(-)	i
2.0	-	0.0	1.02	0.50
2.8	-	0.0	1.01	0.55
3.8	-	0.0	1.00	1.15
4.9	-	0.0	1.05	0.85
5.9	-	0.0	1.11	0.70
6.9	-	0.0	1.20	0.45
7.8	-	0.0	1.27	0.40
9.1	0.62	0.35	1.37	0.45
9.4	0.63	0.6	1.39	0.45
10.9	0.68	0.6	-	0.0
11.9	0.73	1.2	-	0.0
13.0	0.76	2.1	-	0.0

7-ACA (125 ppm) (4.60×10^{-4} M) was treated as cephalexin (above). Three waves were recorded (see Table 12).

Table 12.

pH	wave A		wave B		wave H	
	V (-)	i	V (-)	i	V (-)	i
1.9	-	0.0	0.39	14.8	0.77	2.0
2.5	-	0.0	0.45	15.5	0.80	3.9
3.8	-	0.0	0.56	11.3	0.88	5.0
4.9	0.48	1.5	0.60	4.4	0.97	5.7
6.0	0.50	2.6	0.67	3.7	1.05	3.1
6.8	0.55	4.0	0.72	2.7	1.11	0.8
7.8	0.60	5.9	0.85	1.3	-	0.0
8.7	0.62	8.3	0.92	1.0	-	0.0
10.0	0.66	8.7	-	0.0	-	0.0
10.7	0.69	9.8	-	0.0	-	0.0
12.0	0.74	10.2	-	0.0	-	0.0
13.0	0.76	9.4	-	0.0	-	0.0

7-ADCA (164 ppm) (7.65×10^{-4} M) was treated as cephalexin (above). Three waves were recorded (see Table 13)

A wave denoted by A has two components to its pH plot ($E_{\frac{1}{2}}$ equation), A_1 and A_2.

The DKP derivative of cephalexin (100 ppm) (2.90×10^{-4} M) was treated as cephalexin (above). Three waves were recorded (see Table 14).

The wave denoted by A has two components to its pH plot ($E_{\frac{1}{2}}$ equation), A_1 and A_2.

By comparing the $E_{\frac{1}{2}}$ equations obtained for alkaline hydrolysis products of the cephalosporins similarities can be observed.

(i) A wave polarographing at approximately -0.77 V at pH 13 (0.1 M, OH^-) is common to all cephalosporins examined and this has been tabulated as wave A or A_1 (see Table 15). The compound responsible for this wave gives

Table 13.

pH	wave A		wave B		wave D	
	V (-)	i	V (-)	i	V (-)	i
2.0	0.25	1.0	0.41	16.5	-	0.0
2.8	0.33	1.3	0.51	12.8	-	0.0
4.0	0.39	1.6	0.65	11.0	1.16	1.6
4.9	0.45	2.4	0.76	5.7	1.16	2.1
6.1	0.52	2.2	0.92	5.1	1.16	1.3
6.7	0.56	2.5	1.00	1.4	1.16	2.1
7.9	0.60	2.9	-	0.0	1.18	1.2
8.9	0.63	3.1	-	0.0	1.23	0.3
9.7	0.65	3.4	-	0.0	-	0.0
10.8	0.69	4.1	-	0.0	-	0.0
12.1	0.74	4.5	-	0.0	-	0.0
13.0	0.77	3.3	-	0.0	-	0.0

Table 14.

pH	wave A		wave B		wave D	
	V (-)	i	V (-)	i	V (-)	i
1.7	0.28	3.1	0.35	3.4	-	0.0
3.0	0.32	2.4	0.44	2.7	-	0.0
4.0	0.40	2.7	0.48	1.2	1.00	1.2
5.1	0.46	3.4	0.54	1.4	1.02	3.7
6.3	0.52	3.7	0.60	2.1	1.04	2.4
7.3	0.57	4.2	0.66	2.7	1.06	2.4
8.4	0.62	7.5	0.70	3.4	1.08	2.0
9.8	0.66	7.1	0.76	3.1	-	0.0
11.0	0.68	6.8	0.83	2.4	-	0.0
11.8	0.72	7.6	-	0.0	-	0.0
13.0	0.76	8.5	-	0.0	-	0.0

a higher signal in alkaline solutions and at pH values below 6-7 the signal obtained is negligible.

This signal has a linear range of approximately pH 7 - 13. It was later found to be due to hydrogen sulphide.

Table 15. Comparison of the $E_{\frac{1}{2}}$ equations obtained for wave A or A_1 from the cephalosporins examined

Compound	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephalexin	-0.29 - 0.039 pH	6 - 13
Cephaloglycin	-0.29 - 0.036 pH	7.5 - 13
Cephalonium	unknown	unknown
Cefuroxime	unknown	unknown
Cephaloridine	-0.26 - 0.039 pH	9 - 13
Cephalothin	-0.38 - 0.038 pH	8 - 13
Cephoxazole	-0.39 - 0.029 pH	8.5 - 13
Cephalosporin C	-0.28 - 0.035 pH	6.5 - 13
Desacetylcephalosporin C	-0.31 - 0.035 pH	9 - 13
7-ACA	-0.36 - 0.036 pH	5 - 13
7-ADCA	-0.33 - 0.034 pH	7 - 13
DKP (cephalexin)	-0.33 - 0.031 pH	7.5 - 13

(ii) The peak, previously observed at -0.39 V (pH 2) after alkaline hydrolysis, is common to most cephalosporins studied,(the exception is desacetylcephalosporin C) (see Table 16). This wave has been tabulated as B. It has a linear range of approximately pH 2 - 9. This wave gives its greatest signal at pH values below 7.

Table 16. Comparison of the $E_{\frac{1}{2}}$ equations obtained for wave B from the cephalosporins examined

Compound	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephalexin	-0.21 - 0.084 pH	2 - 6
Cephaloglycin	-0.18 - 0.100 pH	2 - 7
Cephalonium	-0.33 - 0.075 pH	2 - 9
Cefuroxime	-0.26 - 0.066 pH	2 - 9
Cephaloridine	-0.24 - 0.074 pH	2 - 10
Cephalothin	-0.23 - 0.076 pH	2 - 10
Cephoxazole	-0.26 - 0.071 pH	2 - 11
Cephalosporin C	-0.22 - 0.087 pH	2 - 8
Desacetylcephalosporin C	-	-
7-ACA	-0.29 - 0.064 pH	2 - 8.5
7-ADCA	-0.17 - 0.123 pH	2 - 7
DKP (cephalexin)	-0.28 - 0.067 pH	2 - 11

(iii) The wave that had been previously recorded at -0.50 V (pH 2) after alkaline hydrolysis of cephalosporins was only observed in this study in one case, that of cephalosporin C. This wave has been tabulated as C. This could be due to (i) the higher concentration of sodium hydroxide (0.1 M) (previously 0.025M used) causing this derivative to degrade within the 1 hour hydrolysis; (ii) not all cephalosporins may give this compound, previously (0.025 M study) cefuroxime and cephalonium did not give this wave C. The $E_{\frac{1}{2}}$ equation obtained for cephalosporin C was

$$E_{\frac{1}{2}} = (-0.34 - 0.082\ pH)\ V.$$

(iv) A wave in the middle pH ranges, approximately 4 - 8, was observed to be independent of pH, although with the DKP derivative of cephalexin this wave is not quite independent of pH. This wave has been tabulated as D (see Table 17). In the case of the DKP the addition of the carbonyl inhibitor, sodium metabisulphite, did not affect the wave. If cephalexin

is dissolved in sodium hydroxide (0.1 M) at 25°C a wave at -1.05 V, independent of pH in the range 5 - 9, is observed.

Table 17. Comparison of the $E_{\frac{1}{2}}$ equations obtained for wave D from the cephalosporins examined

Compound	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephaloglycin	-1.03	2 - 10
Cefuroxime	-1.03	3 - 9
Cephaloridine	-1.02	2 - 5
7-ADCA	-1.16	4 - 9
DKP (cephalexin)	-0.92 - 0.02 pH	4 - 8

8.

Degradation of some cephalosporins in alkaline solutions to form the -0.77 V wave(H_2S)

A number of cephalosporins were degraded in sodium hydroxide (0.1 M) at 25°C. Periodically samples were removed and polarographed.

Cephalexin (155 ppm) (4.46×10^{-4} M) was degraded and polarographed as above (see fig. 27).

The DKP derivative of cephalexin(VIII) (102 ppm) (2.93×10^{-4} M) was degraded and polarographed as cephalexin (above) (see fig. 28).

7-ACA (154 ppm) (5.66×10^{-4} M) was degraded and polarographed as cephalexin (above) (see fig. 29).

7-ADCA (102 ppm) (4.76×10^{-4} M) was degraded and polarographed as cephalexin (above) (see fig. 30).

With polarography in NaOH (0.1 M) (pH 13) two waves common to all four compounds examined are observed. The wave at -0.77 V forms steadily in the case of cephalexin, but with the DKP derivative the compound responsible for this wave is formed very rapidly (or is present already in a high concentration), reaching its maximum value quickly then degrading.

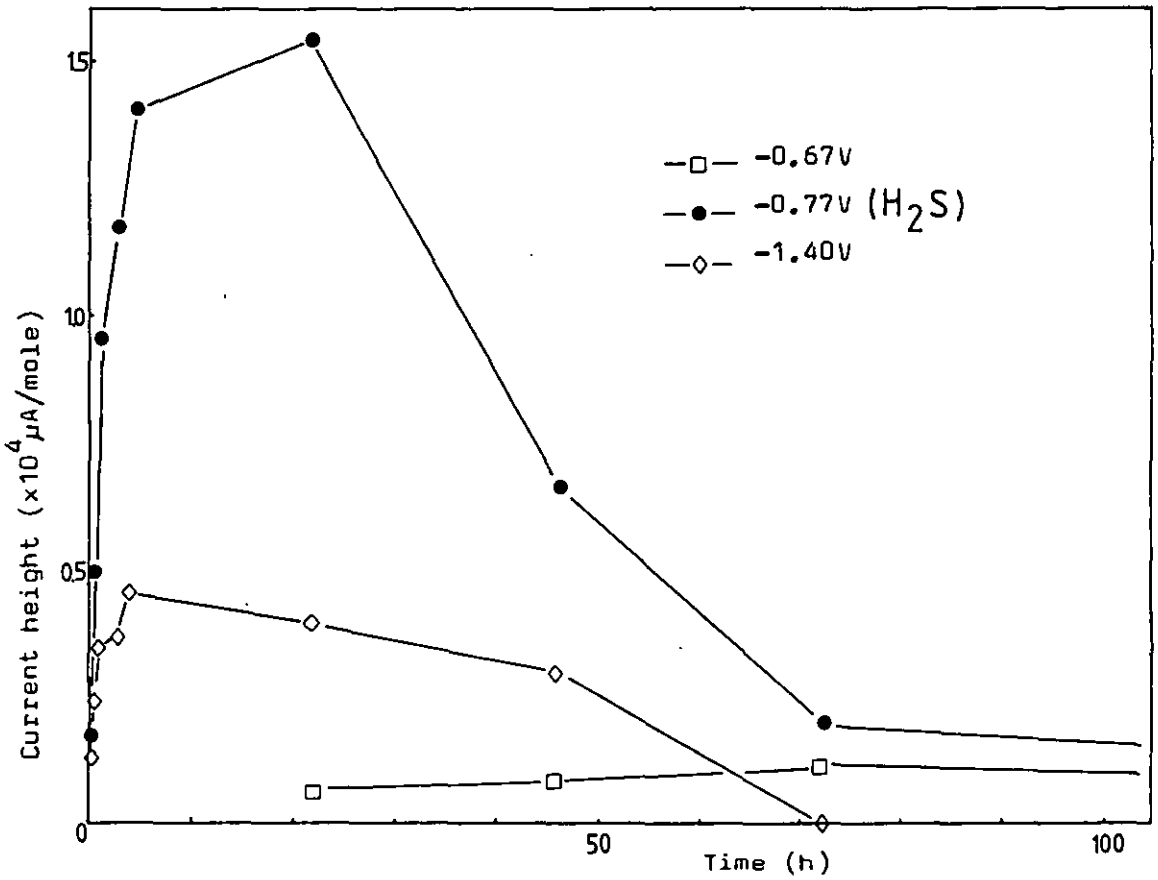


Fig. 27 Plot of peak heights for the alkaline hydrolysis products of cephalexin, NaOH (0.1M), 25°C. Polarography at pH 13.

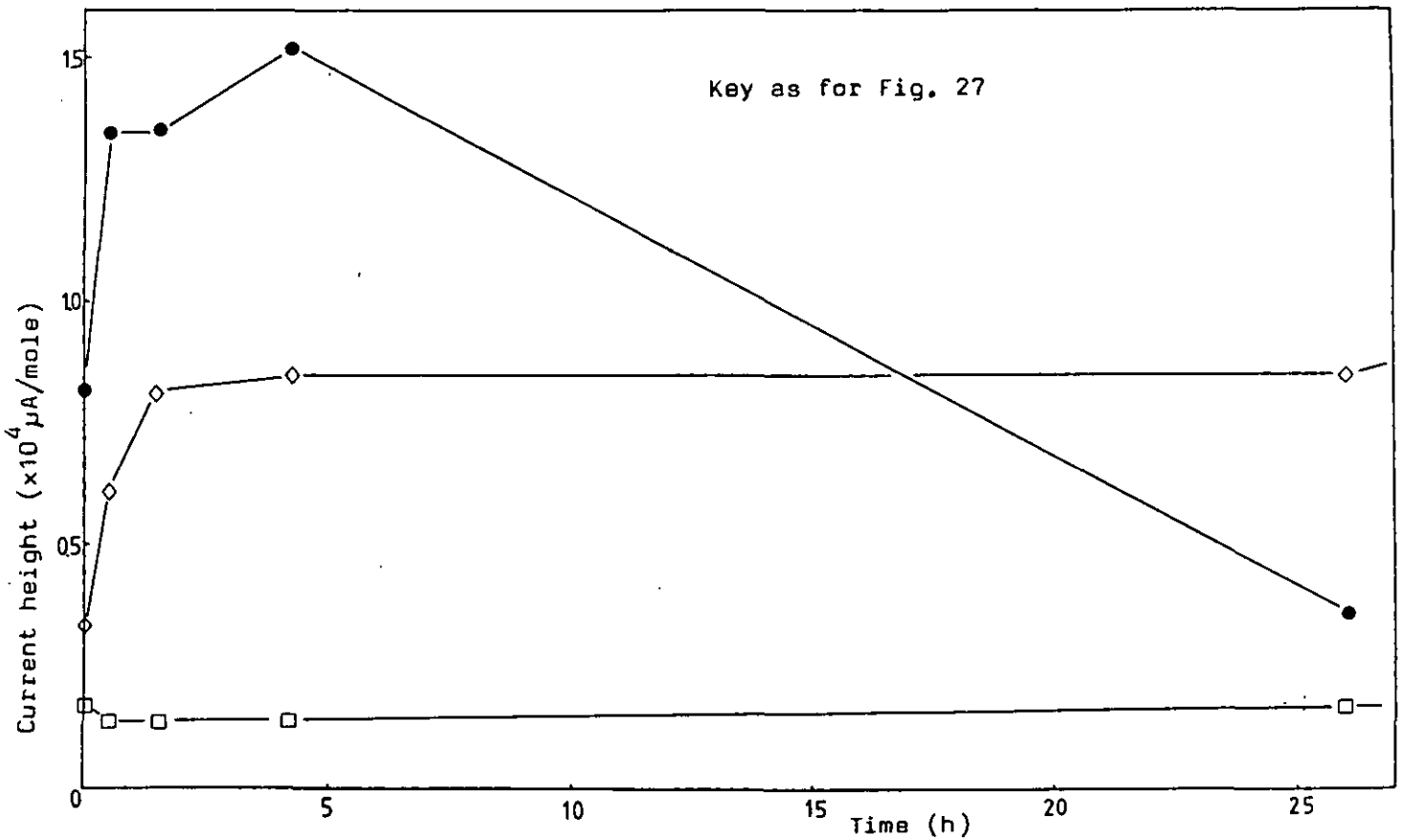


Fig. 28 Plot of peak heights for the alkaline hydrolysis products of the DKP dervative of cephalexin, NaOH(0.1M), 25°C. Polarography at pH 13.

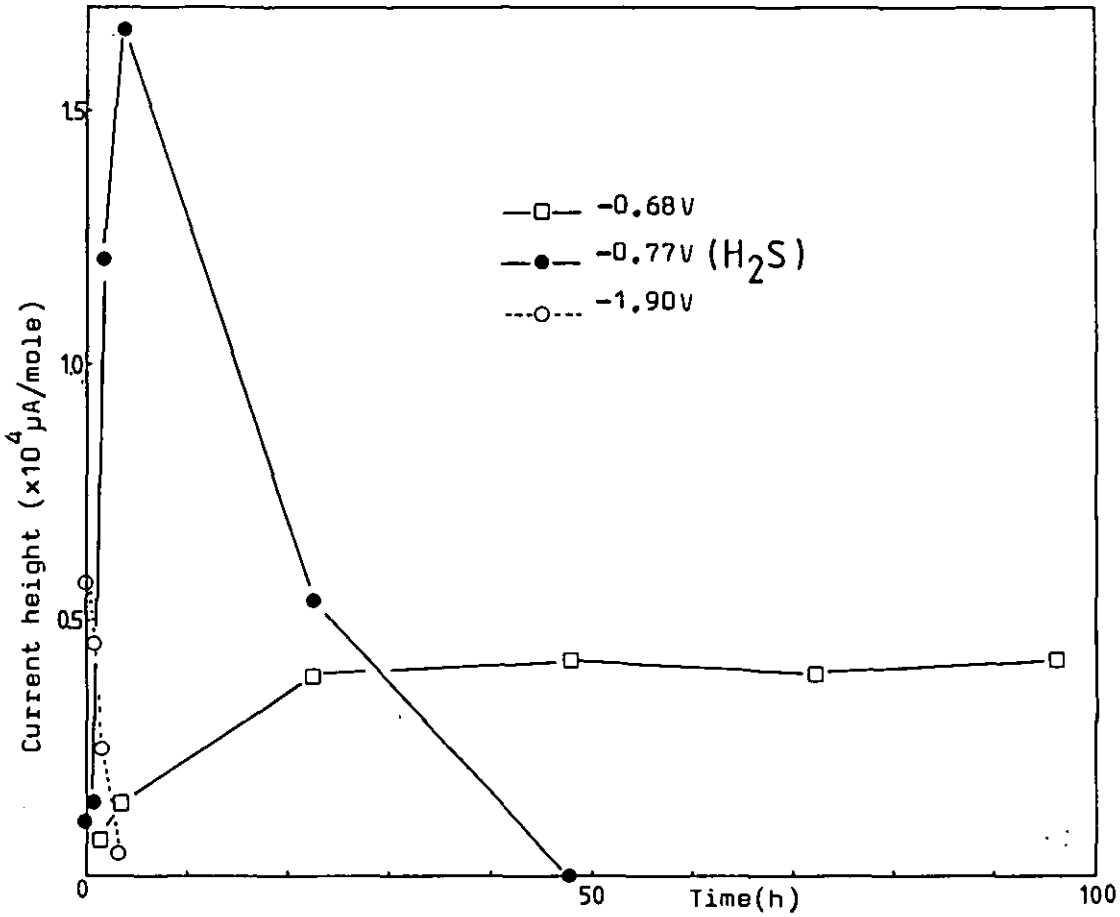


Fig. 29 Plot of peak heights for the alkaline hydrolysis products of 7-ACA, NaOH (0.1M), 25°C. Polarography at pH 13.

Key as for Fig. 27

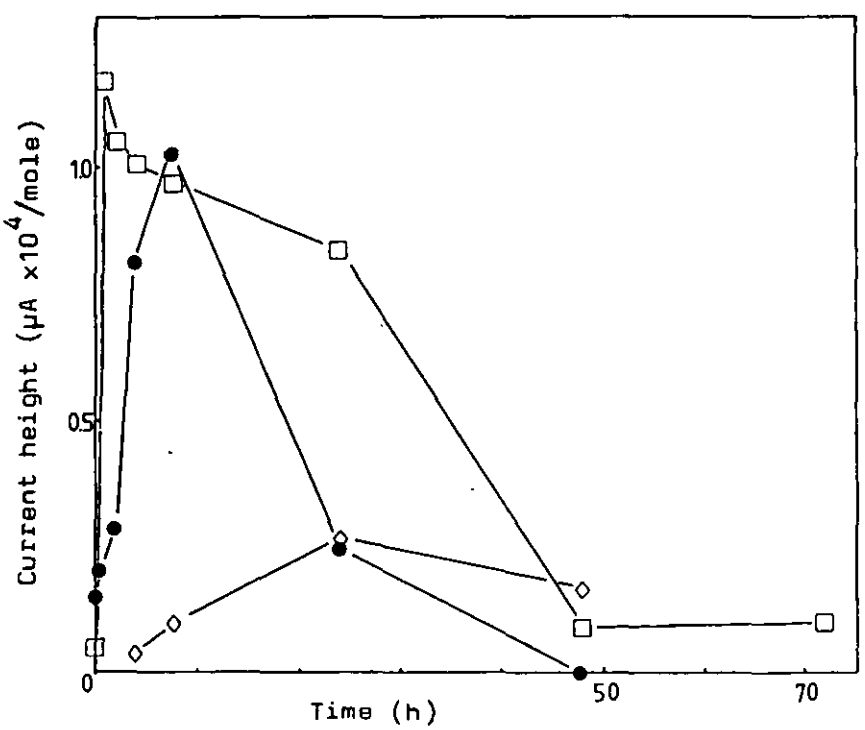


Fig. 30 Plot of peak heights for the alkaline hydrolysis products of 7-ADCA, NaOH (0.1M), 25°C . Polarography at pH 13.

Both cephalexin and the DKP reach the same maximum values for this -0.77 V wave. Formation of this peak from 7-ADCA and 7-ACA is similar. The wave reaches its maximum value (similar to the DKP and cephalexin studies) at between 5-10 hours, then declining over the next 40 hours. The other wave common to the four compounds is at -0.67 V.

9.

Alkaline degradation of penicillins at 100°C

A number of penicillins were degraded at 100°C in sodium hydroxide (0.1 M). Periodically samples were removed, cooled and polarographed.

Ampicillin (150 ppm) (3.72×10^{-4} M) was degraded and polarographed as above. Four peaks were observed (see figs. 31A and 31B).

Penicillin V (156 ppm) (4.60×10^{-4} M) was treated as ampicillin (above). Two peaks were recorded (see fig. 32).

Two peaks are common to both studies, -0.90 V and -1.34 V. No waves analogous to the waves found in the alkaline hydrolysis of cephalosporins were observed.

10.

Strong alkaline hydrolysis of cephalosporins at 100°C

The alkaline hydrolysis of cephalosporins has been reported to give large yields of hydrogen sulphide by Abdalla et al.[198] and the amount of hydrogen sulphide produced in sodium hydroxide (0.5 M) and 100°C has been quantified. Using the results from this study a number of cephalosporins were degraded to produce a maximum yield of hydrogen sulphide (each cephalosporin requiring a different hydrolysis time at 100°C in sodium hydroxide (0.5 M)). These solutions were then examined polarographically by DPP.

Cephalexin (100 ppm) (2.88×10^{-4} M) was hydrolysed in sodium hydroxide (0.5 M) at 100°C for 30 minutes. At the end of this time period the solution was cooled and a pH plot of wave potential and corresponding wave height against pH was carried out. The pH adjustments were made with HCl

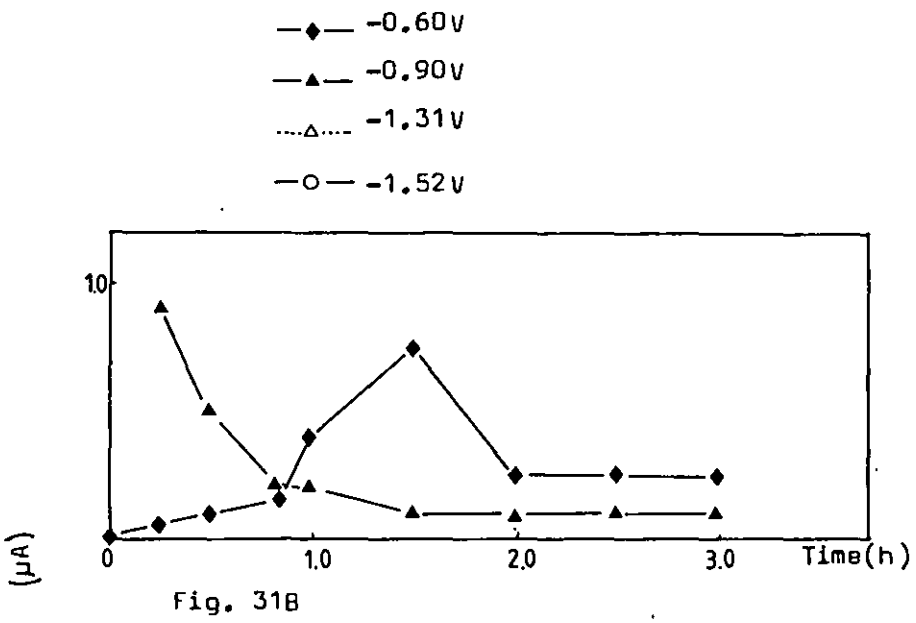


Fig. 31B

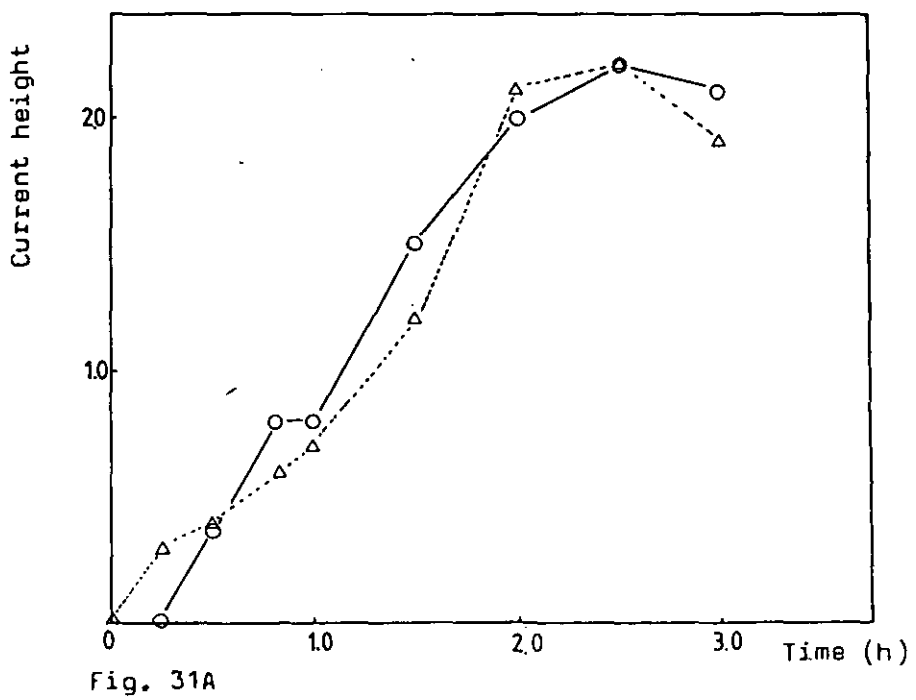


Fig. 31A

Figs. 31A and 31B Plot of the peak heights for the alkaline hydrolysis products of ampicillin, NaOH (0.1M), 100^{o}C, polarography at pH 13.

Key as for Fig. 31.

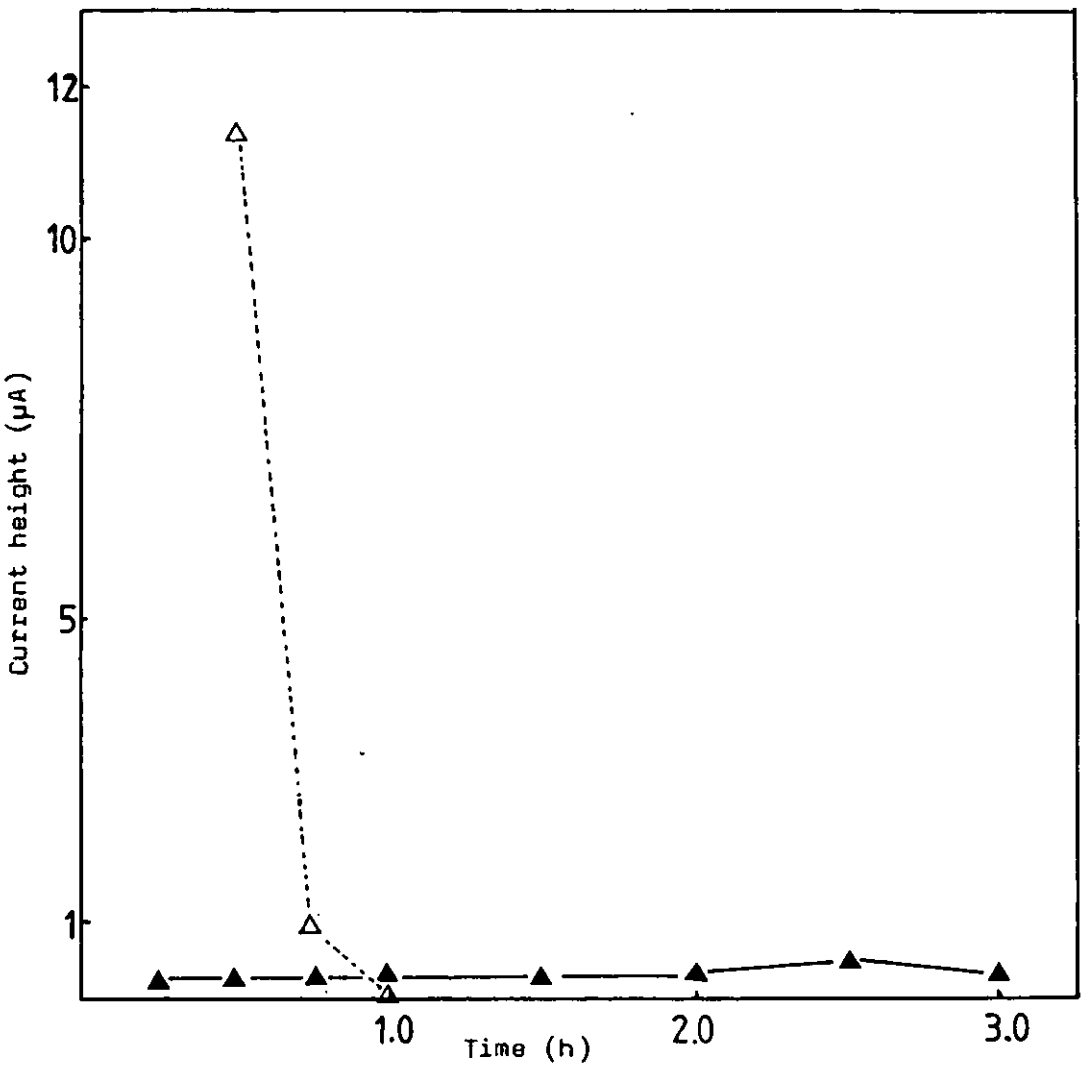


Fig.32 Plot of the peak heights for the alkaline hydrolysis products of penicillin V, NaOH (0.1M),100^{o}C, polarography at pH 13.

(2 M) and NaOH (2 M). From this data the dependence of the half-wave potential ($E_{\frac{1}{2}}$) on pH was calculated graphically.

The other cephalosporins were similarly examined. Table 18 relates the cephalosporin examined with the data obtained from its study.

Table 18.

Cephalosporin	concentration used ($\times 10^{-4}$ M)	hydrolysis time (m)	Table data on
Cephalexin	2.88	30	19
Cephaloglycin	4.49	40	20
Cephalonium	4.68	60	21
Cephaloridine	2.70	60	22
Cephalothin	3.42	45	23
Cephoxazole	2.20	70	24
7-ACA	6.79	40	25
7-ADCA	9.48	35	26
Cephradine	2.96	30	27
Cefazolin	2.04	30	28
Cefaclor	6.31	40	29

Table 19. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline degradation products of cephalexin (NaOH, (0.5 M), 100°C, 30 mins)

pH	wave A		wave B		wave C		wave D		wave E	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i
2.0	0.38	7.3	0.49	7.8	-	0.0	-	0.0	0.18	1.8
3.1	0.45	7.3	0.57	6.0	-	0.0	-	0.0	0.23	1.8
5.1	0.56	7.7	0.72	6.2	-	0.0	-	0.0	0.31	1.6
7.2	0.68	4.4	-	0.0	1.02	1.6	1.26	0.6	0.39	1.0
7.8	0.70	6.0	-	0.0	1.02	1.6	1.26	0.6	0.44	1.2
8.9	0.80	7.8	-	0.0	1.02	1.8	1.24	0.8	0.48	0.8
10.2	0.87	5.8	-	0.0	1.03	2.6	1.27	1.0	0.52	0.8
11.1	-	0.0	-	0.0	1.05	3.6	1.27	0.7	0.56	0.8
12.0	-	0.0	-	0.0	1.08	3.6	-	0.0	0.60	2.3
12.7	-	0.0	-	0.0	1.11	3.6	-	0.0	0.64	1.6
13.7	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0

Table 20. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline hydrolysis products of cephaloglycin (NaOH, (0.5 M), 100°C, 40 mins.)

pH	wave A		wave B		wave C		wave E		wave G	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i
2.1	0.39	6.7	0.50	3.9	0.68	0.6	0.19	3.6	0.13	3.7
3.3	0.46	6.3	0.55	4.3	0.76	1.4	0.24	3.0	0.17	2.2
5.8	0.62	6.2	-	0.0	0.79	2.1	0.34	2.8	0.30	2.2
7.7	0.75	4.2	-	0.0	1.00	1.6	-	0.0	0.41	2.1
8.8	0.80	5.4	-	0.0	1.01	2.3	-	0.0	0.48	2.5
10.0	0.90	3.7	-	0.0	1.02	2.8	-	0.0	0.51	2.4
11.0	-	0.0	-	0.0	1.03	3.3	-	0.0	0.56	2.4
12.3	-	0.0	-	0.0	1.01	3.6	-	0.0	-	0.0
13.7	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0

Table 21. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline hydrolysis products of cephalonium (NaOH, (0.5 M), 100°C, 60 mins.)

pH	wave B		wave C		wave E		wave H	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i
2.1	0.51	1.2	-	0.0	0.24	0.8	0.83	46.0
2.9	0.60	1.5	-	0.0	0.29	0.9	0.89	43.0
3.9	-	0.0	-	0.0	0.34	0.9	0.98	44.0
4.8	-	0.0	-	0.0	0.40	0.9	1.07	40.0
5.7	-	0.0	1.01	2.0	0.45	1.0	1.15	38.0
6.8	-	0.0	1.04	1.8	0.49	0.7	1.24	9.0
8.0	-	0.0	1.04	3.2	0.50	0.6	1.33	2.7
8.7	-	0.0	1.04	1.9	0.50	0.3	-	0.0
10.3	-	0.0	1.08	0.6	-	0.0	-	0.0
10.9	-	0.0	-	0.0	-	0.0	-	0.0
12.0	-	0.0	-	0.0	-	0.0	-	0.0
13.7	-	0.0	-	0.0	-	0.0	-	0.0

Table 22. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline hydrolysis products of cephaloridine (NaOH, (0.5 M), 100°C, 60 minutes)

pH	wave A		wave E		wave G		wave J	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i
1.7	0.40	0.8	0.20	2.4	0.13	2.6	0.64	0.6
2.5	0.46	1.5	0.24	1.5	0.17	1.6	0.70	0.7
3.5	0.53	1.3	0.28	1.4	0.21	1.2	0.81	2.4
4.5	0.60	1.2	0.33	1.7	0.26	1.4	0.88	1.9
5.6	0.68	1.2	0.37	1.9	0.29	1.4	0.94	0.5
7.3	0.80	0.8	0.44	1.9	0.38	1.9	1.03	0.4
8.9	0.90	0.5	-	0.0	0.45	1.3	-	0.0
10.1	1.10	0.3	-	0.0	0.48	1.1	-	0.0
11.1	-	0.0	-	0.0	0.50	1.1	-	0.0
12.3	-	0.0	-	0.0	0.50	0.7	-	0.0
13.7	-	0.0	-	0.0	-	0.0	-	0.0

Table 23. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline degradation products of cephalothin (NaOH, (0.5 M), 100°C, 45 minutes)

pH	wave A		wave B		wave D		wave E		wave G	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i
2.0	0.38	0.8	0.47	0.7	-	0.0	0.20	1.4	0.12	2.1
3.5	0.45	1.0	0.58	0.9	-	0.0	0.27	1.5	0.19	1.0
4.1	0.48	1.0	0.60	0.8	-	0.0	0.32	1.4	0.21	1.0
5.0	0.53	0.8	0.69	0.6	1.20	0.4	0.35	2.9	0.25	1.0
7.0	-	0.0	0.78	0.7	1.20	0.5	0.38	3.0	0.33	1.0
8.3	-	0.0	0.90	0.4	1.21	0.5	0.47	2.6	0.40	0.8
9.0	-	0.0	-	0.0	1.21	0.7	0.50	2.3	0.43	0.7
10.0	-	0.0	-	0.0	1.23	0.6	0.54	2.1	0.48	0.8
11.3	-	0.0	-	0.0	1.23	0.3	0.59	2.3	-	0.0
12.0	-	0.0	-	0.0	-	0.0	0.62	2.6	-	0.0
13.7	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0

Table 24. Changes in the reduction potential (V) and current height (i) (in μ A) for the alkaline degradation products of cephoxazole (NaOH, (0.5 M), 100°C, 70 minutes

pH	wave C		wave E		wave G		wave J		wave K		wave L	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i
2.7	-	0.0	0.24	1.2	0.71	1.1	0.13	1.0	-	0.0	-	0.0
3.5	-	0.0	0.28	2.0	0.81	3.2	0.17	1.0	-	0.0	-	0.0
4.7	-	0.0	0.34	2.4	0.90	2.2	0.22	0.6	-	0.0	-	0.0
6.1	1.05	1.0	0.40	1.5	-	0.0	0.28	0.3	-	0.0	-	0.0
7.5	1.05	0.5	0.46	2.1	-	0.0	-	0.0	-	0.0	-	0.0
8.9	1.05	0.4	0.53	2.4	-	0.0	-	0.0	0.85	0.7	1.21	0.7
10.1	1.06	0.3	0.56	1.5	-	0.0	-	0.0	0.89	0.7	1.26	0.7
11.2	1.06	0.4	-	0.0	-	0.0	-	0.0	0.92	0.1	1.30	0.6
12.2	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	1.34	0.8
13.7	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0

Table 25. Changes in the reduction potential (V) and current height (i) (in μ A) for the alkaline degradation products of 7-ACA (NaOH, (0.5 M), 100°C, 40 minutes

pH	wave A		wave D		wave E		wave G	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i
1.4	0.36	2.2	-	0.0	0.17	7.6	0.12	6.0
2.0	0.39	2.8	-	0.0	0.20	7.8	0.15	6.0
3.3	0.47	3.4	1.10	7.5	0.25	8.9	0.20	5.5
5.6	0.63	1.6	1.20	2.4	0.35	7.6	0.28	4.1
6.9	-	0.0	1.23	3.8	0.43	5.2	0.34	2.4
8.2	-	0.0	1.21	5.2	0.46	7.2	0.38	3.0
9.2	-	0.0	1.23	6.0	0.51	7.0	0.42	3.1
10.0	-	0.0	1.23	6.4	0.52	6.4	0.45	3.2
11.2	-	0.0	1.25	6.8	0.52	4.6	-	0.0
12.3	-	0.0	1.25	4.8	0.53	4.5	-	0.0
13.7	-	0.0	-	0.0	-	0.0	-	0.0

Table 26. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline hydrolysis products of 7-ADCA (NaOH, (0.5 M), 100°C, 35 minutes

pH	wave A		wave D		wave E		wave M		wave N	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i
1.8	0.38	2.0	1.25	1.8	0.18	6.6	0.32	0.9	0.38	2.8
3.2	0.47	1.7	1.23	2.2	0.23	6.8	0.40	0.9	0.42	3.6
4.5	0.56	1.5	1.23	8.2	0.30	6.6	0.49	1.2	0.45	3.0
7.3	-	0.0	1.24	8.7	0.40	5.8	0.64	0.5	0.48	3.0
8.6	-	0.0	1.24	9.2	0.48	4.4	-	0.0	0.52	2.5
9.5	-	0.0	1.25	9.5	0.51	3.8	-	0.0	-	0.0
10.7	-	0.0	1.29	7.5	0.54	3.8	-	0.0	-	0.0
12.0	-	0.0	1.28	6.5	0.60	3.0	-	0.0	-	0.0
13.7	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0

Table 27. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline hydrolysis products of cephradine (NaOH, (0.5 M), 100°C, 30 minutes)

pH	wave A		wave B		wave C		wave E		wave J	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i
1.6	0.35	5.2	0.49	4.8	-	0.0	0.16	1.8	-	0.0
2.5	0.40	4.1	0.53	2.8	-	0.0	0.21	1.2	0.73	1.0
3.8	0.49	4.0	0.60	2.7	-	0.0	0.27	1.0	0.84	2.3
4.6	0.54	3.1	0.66	2.9	-	0.0	0.31	1.0	0.90	1.5
5.6	0.60	2.2	0.71	3.0	-	0.0	0.36	1.0	0.97	2.0
6.7	0.66	1.8	0.77	1.7	-	0.0	0.40	1.0	1.09	2.4
8.0	0.74	1.3	0.81	2.1	1.03	0.7	0.45	0.8	1.17	4.4
9.2	0.82	2.2	-	0.0	1.02	1.4	-	0.0	1.26	3.4
10.0	0.86	2.0	-	0.0	1.02	1.2	-	0.0	1.30	1.4
11.1	-	0.0	-	0.0	1.05	1.5	-	0.0	-	0.0
12.1	-	0.0	-	0.0	1.08	1.0	-	0.0	-	0.0
13.7	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0

Table 28. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline hydrolysis products of cefazolin (NaOH, (0.5 M), 100°C, 30 minutes)

pH	wave A		wave C		wave G		wave P	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i
1.6	0.39	1.3	-	0.0	0.13	1.6	-	0.0
2.1	0.44	1.4	-	0.0	0.15	1.2	-	0.0
3.9	0.56	1.8	0.86	1.6	0.21	1.2	-	0.0
4.6	0.62	0.4	0.91	1.2	0.23	1.8	-	0.0
6.3	-	0.0	1.04	0.4	0.30	1.8	-	000
7.9	-	0.0	1.04	0.6	0.37	1.9	0.44	1.8
9.0	-	0.0	1.04	0.5	0.39	1.5	0.47	1.6
10.1	-	0.0	1.07	0.6	0.44	1.1	0.51	1.2
11.1	-	0.0	1.06	0.6	0.46	0.9	0.55	0.8
12.0	-	0.0	-	0.0	-	0.0	0.60	0.5
13.7	-	0.0	-	0.0	-	0.0	-	0.0

Table 29. Changes in reduction potential (V) and current height (i) (in μA) for the alkaline hydrolysis products of cefaclor (NaOH, (0.5 M), 100°C, 40 minutes)

pH	wave A		wave B		wave D		wave E		wave G		wave Q	
	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i	V (-)	i
2.0	0.39	5.3	0.50	4.8	-	0.0	0.18	3.4	0.11	2.9	-	0.0
3.1	0.46	5.4	0.58	3.7	-	0.0	0.24	2.3	0.15	1.8	0.71	2.9
4.0	0.52	5.6	0.63	3.3	-	0.0	0.29	2.0	0.19	1.3	0.83	5.7
5.1	0.61	5.5	0.68	4.0	-	0.0	0.33	2.0	0.24	1.4	0.89	4.1
6.2	0.70	4.8	0.75	4.2	-	0.0	0.38	1.8	0.29	1.5	1.00	1.0
7.2	0.74	4.0	0.80	3.0	1.23	3.2	0.42	1.9	0.34	1.4	1.00	1.3
8.2	0.80	3.6	0.87	2.0	1.24	5.0	0.48	1.8	0.39	1.2	1.02	1.4
9.1	0.85	3.4	0.94	1.6	1.25	5.3	0.52	1.8	0.41	1.0	1.04	1.6
10.2	0.91	2.4	0.99	1.7	1.26	6.0	0.56	1.7	0.48	0.6	1.06	2.6
11.1	-	0.0	-	0.0	1.27	6.2	0.58	1.7	-	0.0	1.08	3.1
12.1	-	0.0	-	0.0	1.27	5.2	-	0.0	-	0.0	1.11	3.0
13.7	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0

A major wave produced from all cephalosporins examined was present at -0.77 V, only in pH 13.7 and is denoted as wave F (see Table 30). A differential pulse polarogram of a solution of sodium sulphide (Na_2S) in NaOH (0.5 M) showed a wave at -0.77 V. The degraded solutions of cephalosporins produced by alkaline hydrolysis was brought down to pH 2. A purge gas (nitrogen) was bubbled through the solutions, which were then brought up to pH 13.7 again by the addition of NaOH. This purged solution was polarographed. No wave was seen at -0.77 V (see fig. 33). It was therefore concluded that the DP peak at -0.77 V produced from the cephalosporins was due to sulphide.

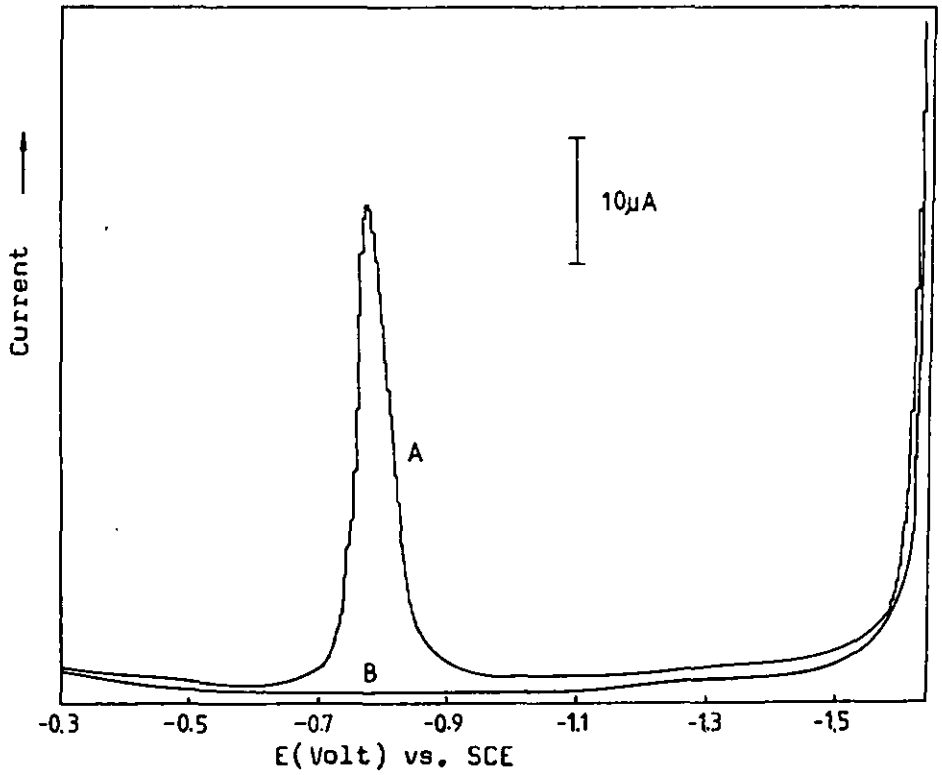
Table 30. Tabulation of the major wave (F) produced by the strong alkaline hydrolysis of cephalosporins (polarography at pH 13.7)

Cephalosporin	V (-)	peak height ($\times 10^4$ μA/mole)
Cephalexin	-0.77	12.85
Cephaloglycin	-0.77	5.44
Cephalonium	-0.77	3.93
Cephaloridine	-0.77	9.27
Cephalothin	-0.77	2.81
Cephoxazole	-0.77	11.38
7-ACA	-0.77	12.07
7-ADCA	-0.77	5.70
Cephradine	-0.77	13.50
Cefazolin	-0.77	17.70
Cefaclor	-0.77	2.62

Some relatively minor waves were observed in the solutions of more than one cephalosporin.

(i) The wave that was denoted as A in the Tables was also present in the NaOH, 0.025 M, 25°C study (denoted as wave B there) for most compounds investigated (see Table 31).

Fig. 33 DP polarograms of cephalexin (3.94×10^{-4}M) after alkaline hydrolysis, NaOH (0.5M), 100°C, 0.5 hours, polarography at pH 13.7.



B, polarogram after bringing solution down to pH 2 and purging with nitrogen gas for 10 mins.

A, polarogram of solution before bringing down to pH 2 and purging.

Table 31. Tabulation of the $E_{\frac{1}{2}}$ equation and linear range of wave A produced from the alkaline hydrolysis of cephalosporins

Cephalosporin	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephalexin	-0.27 - 0.056 pH	2 - 10
Cephaloglycin	-0.24 - 0.063 pH	2 - 10
Cephaloridine	-0.28 - 0.071 pH	2 - 10
Cephalothin	-0.29 - 0.046 pH	2 - 5
7-ACA	-0.26 - 0.065 pH	2 - 5.5
7-ADCA	-0.26 - 0.067 pH	2 - 4
Cephradine	-0.25 - 0.061 pH	2 - 10
Cefazolin	-0.28 - 0.057 pH	2 - 4
Cefaclor	-0.26 - 0.065 pH	2 - 10

(ii) The wave denoted as B was also present in the NaOH, 0.025 M, 25°C study to a much lesser extent (as wave C), and to a greater extent in the alkaline hydrolysis of cephalosporins, with polarography at pH 2, at -0.50 V (see Table 32).

Table 32. Tabulation of the $E_{\frac{1}{2}}$ equation and the linear range of wave B produced from the alkaline degradation of cephalosporins

Cephalosporin	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephalexin	-0.34 - 0.073 pH	2 - 5
Cephaloglycin	-0.42 - 0.04 pH	2 - 3
Cephalonium	-0.27 - 0.11 pH	2 - 3
Cephalothin	-0.32 - 0.07 pH	2 - 8
Cephradine	-0.40 - 0.055 pH	2 - 8
Cefaclor	-0.38 - 0.06 pH	3 - 10

(iii) The wave denoted as C is present in the linear range pH 6 - 10 (approximately) and is independent of pH (see Table 33). This wave is also present in the NaOH, 0.025 M, 25°C study (as wave D).

Table 33. Tabulation of the $E_{\frac{1}{2}}$ equation and the linear range of wave C produced from the alkaline degradation of cephalosporins

Cephalosporin	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephalexin	-1.02	7 - 11
Cephaloglycin	-1.01	7.5 - 12.5
Cephalonium	-1.04	6 - 10
Cephoxazole	-1.05	6 - 11
Cephradine	-1.03	8 - 12
Cefazolin	-1.04	6.5 - 11

(iv) Another wave that is independent of pH is that denoted as D. This wave occurs in the linear range pH 6 - 13 (approximately) with an $E_{\frac{1}{2}}$ of -1.23 V (approximately). This is suspected to be due to a carbonyl compound which gives high yields in the neutral degradation of cephalexin[61] and 7-ADCA in pH 7.4 buffer at 100°C. However testing of the degraded solutions with sodium metabisulphite show that it is due only to a carbonyl compound in the case of cephalexin and 7-ADCA (see Table 34).

Table 34. Tabulation of the $E_{\frac{1}{2}}$ equation and the linear range of wave D from the alkaline hydrolysis of cephalosporins

Cephalosporin	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephalexin	-1.26	7 - 11
Cephalothin	-1.21	5 - 11
7-ACA	-1.22	5.5 - 13
7-ADCA	-1.24	3 - 13
Cefaclor	-1.17 - 0.009 pH	7 - 12

(v) Two anodic waves denoted as E and G were also observed (see Tables 35 and 36). These waves were not observed in the NaOH, 0.025 M, 25°C study. Addition of the thiol inhibitor para-hydroxymercuribenzoate resulted in the disappearance of these waves. It is therefore probable that these waves are due to thiols and do not form to any significant

extent in the milder alkaline study.

Table 35. Tabulation of the $E_{\frac{1}{2}}$ equation and the linear range of wave E from the alkaline hydrolysis of cephalosporins

Cephalosporin	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephalexin	-0.09 - 0.044 pH	2 - 12
Cephaloglycin	-0.11 - 0.038 pH	2 - 5.5
Cephalonium	-0.12 - 0.058 pH	2 - 6
Cephaloridine	-0.13 - 0.044 pH	2 - 7
Cephalothin	-0.17 - 0.037 pH	2 - 12
Cephoxazole	-0.12 - 0.047 pH	3 - 10
7-ACA	-0.12 - 0.042 pH	2 - 10
7-ADCA	-0.12 - 0.045 pH	2 - 12
Cephradine	-0.10 - 0.045 pH	2 - 8
Cefaclor	-0.09 - 0.049 pH	2 - 10

Table 36. Tabulation of the $E_{\frac{1}{2}}$ equation and the linear range of wave G from the alkaline hydrolysis of cephalosporins

Cephalosporin	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Cephaloglycin	-0.02 - 0.049 pH	2 - 11
Cephaloridine	-0.05 - 0.046 pH	2 - 9
Cephalothin	-0.05 - 0.038 pH	2 - 11
Cephoxazole	-0.01 - 0.045 pH	3 - 6
7-ACA	-0.07 - 0.048 pH	2 - 10
Cefazolin	-0.07 - 0.037 pH	2 - 11
Cefaclor	-0.01 - 0.045 pH	2 - 10

11.

Study of the formation of H_2S from the alkaline hydrolysis of cephalexin

Cephalexin (146 ppm) (4.20×10^{-4} M) was degraded at 100°C in sodium hydroxide (0.5 M). Periodically samples were removed, cooled and polarographed (see fig. 34).

The hydrogen sulphide wave is seen to rise quickly to its maximum value (61 μA) after 30 minutes. The recommended hydrolysis quoted by

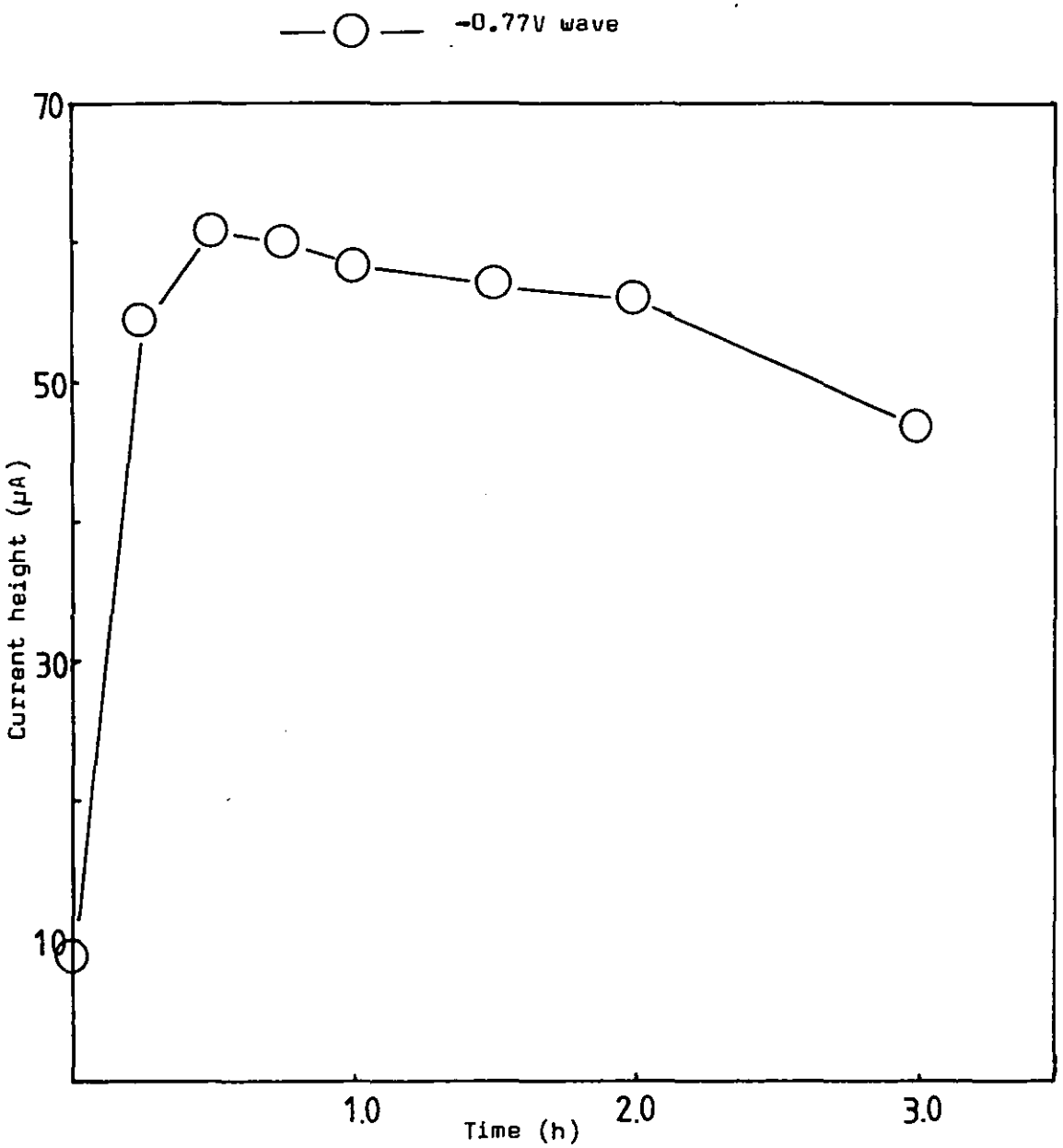


Fig. 34 Formation of the − 0.77V wave(sulphide) from the alkaline hydrolysis of cephalexin, NaOH (0.5M), 100°C , polarography at pH 13.7.

Abdalla et al.[198] for maximum production of H_2S from cephalexin, under the same conditions, measured by visible spectrophotometry was 30 minutes. After this maximum value the H_2S concentration is observed to drop (fig. 34) though not significantly for the first 30 minutes after the maximum value had been obtained. Hydrogen sulphide is also seen to be formed in the cold from the solution of cephalexin in sodium hydroxide.

12.

The effect of the sodium hydroxide concentration (and therefore pH) on the -0.77 V wave's potential

Cephalexin (133 ppm) (3.83×10^{-4} M) was degraded in NaOH (0.5 M) (100 ml) at 100°C for 30 minutes. The solution was then cooled and aliquots of NaOH (0.51 M) and HCl (1.0 M) were added to change the pH of the solution, and the resulting solution was polarographed. The change in the -0.77 V wave with pH can be seen in fig. 35.

Between pH 13.4 and pH 14.3 the hydrogen sulphide reduces at -0.77 V. As the pH decreases so does the reduction potential. Below pH 12.5 a small change in pH results in a large change in the sulphide reduction potential. Below pH 12.1 the H_2S wave almost disappeared.

13. The determination of hydrogen sulphide by normal pulse polarography has been carried out by Turner et al.[212]. NPP is used in preference to DPP. The electrode reaction is anodic, $S^{2-} + Hg \longrightarrow HgS + 2e^-$, and results in the formation of layers of insoluble HgS at the electrode surface. DPP uses a slowly increasing DC ramp; as this DC scan moves into the region where filming occurs the differential pulse is being applied to a filmed drop. In this region the current is determined by the electrode film characteristics rather than by the rate of diffusion of material to the electrode surface. Since the film characteristics may change with increasing (anodic) voltage and with concentration, the resulting differential current may not be reproducible.

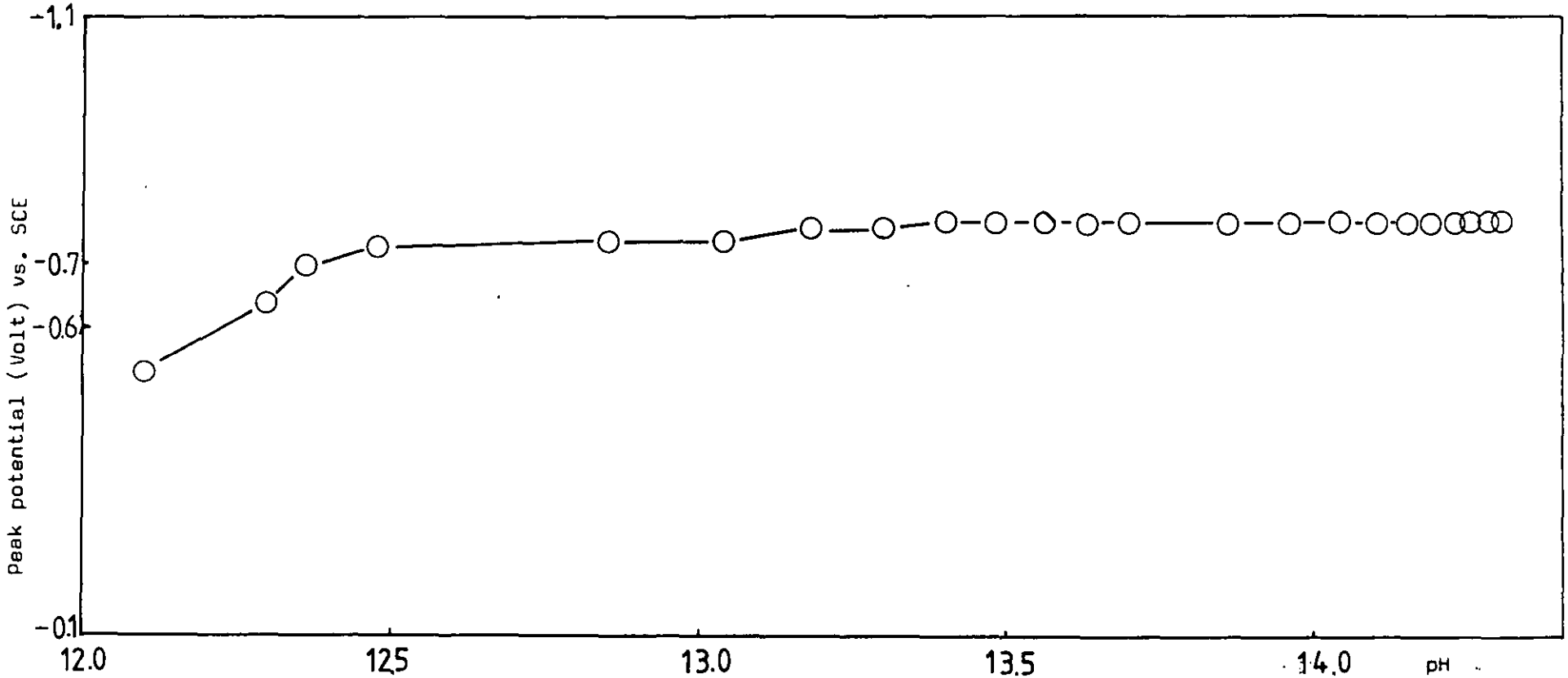


Fig. 35 Effect of the sodium hydroxide concentration (measured as pH for convenience) on the reduction potential of the -0.77V wave (sulphide) produced from the alkaline degradation of cephalexin, NaOH (0.5M), 100°C, 0.5 hours.

With NPP the electrode rests while the drop grows at a potential where no film formation is possible (in this study at -1.1 V; any HgS available would immediately be reduced to Hg and S^{2-}). At a specific time in the drop life a pulse is applied and the current is measured at the end of this pulse. Since the pulse is applied for only a very short time (typically less than 50 msec) there is less film formation and therefore the electrode is much better behaved.

14.

Construction of a calibration graph for determining H_2S by normal pulse polarography

A solution of Na_2S was made up from $Na_2S.xH_2O$ (where x = 9 approximately), of roughly 2 x 10^{-2} M in NaOH (0.5 M). This solution was standardised against $Pb(NO_3)_2$ (0.02 M) using a sulphide electrode as indicator electrode to detect the end point (potentiometric titration).

25 ml of S^{2-} (x molar) consumed 22.55 ml of Pb^{2+} (0.02 M)

$25.x \quad 22.55.(0.02)$

$x = (22.55)(0.02)/25$

The molarity of the standard solution is 1.8 x 10^{-2} M.

Dilutions were made of this standard and polarographed in the normal pulse mode using a drop-time of 0.5 seconds, scanning in the positive direction from an initial potential of -1.1 V, with a scan rate of 5 mV/second. The normal pulse wave is recorded at -0.79 V and is well defined (see Table 37 and figs. 36 and 37).

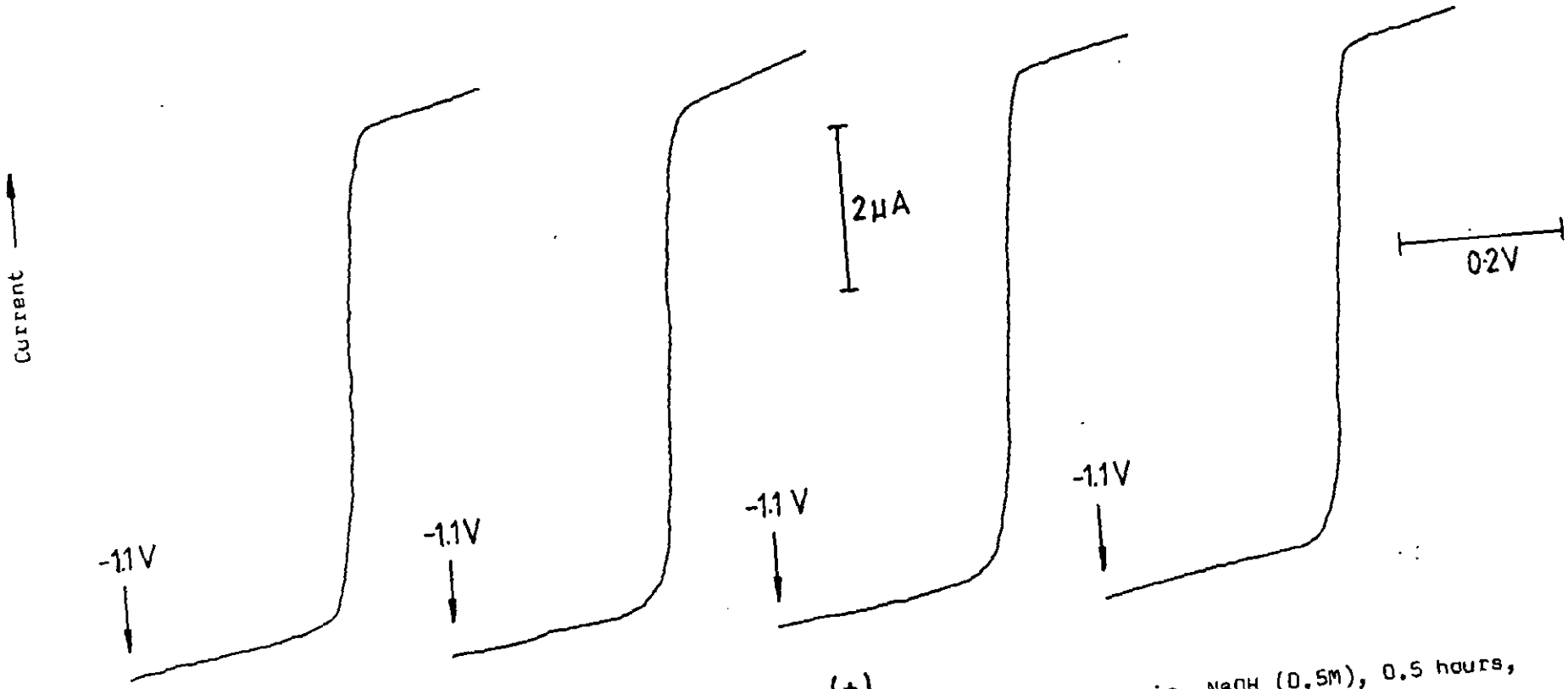


Fig. 36 NP polarograms obtained from cephalexin after alkaline hydrolysis, NaOH (0.5M), 0.5 hours, with a cephalexin molarity of 3.94×10^{-4}M.

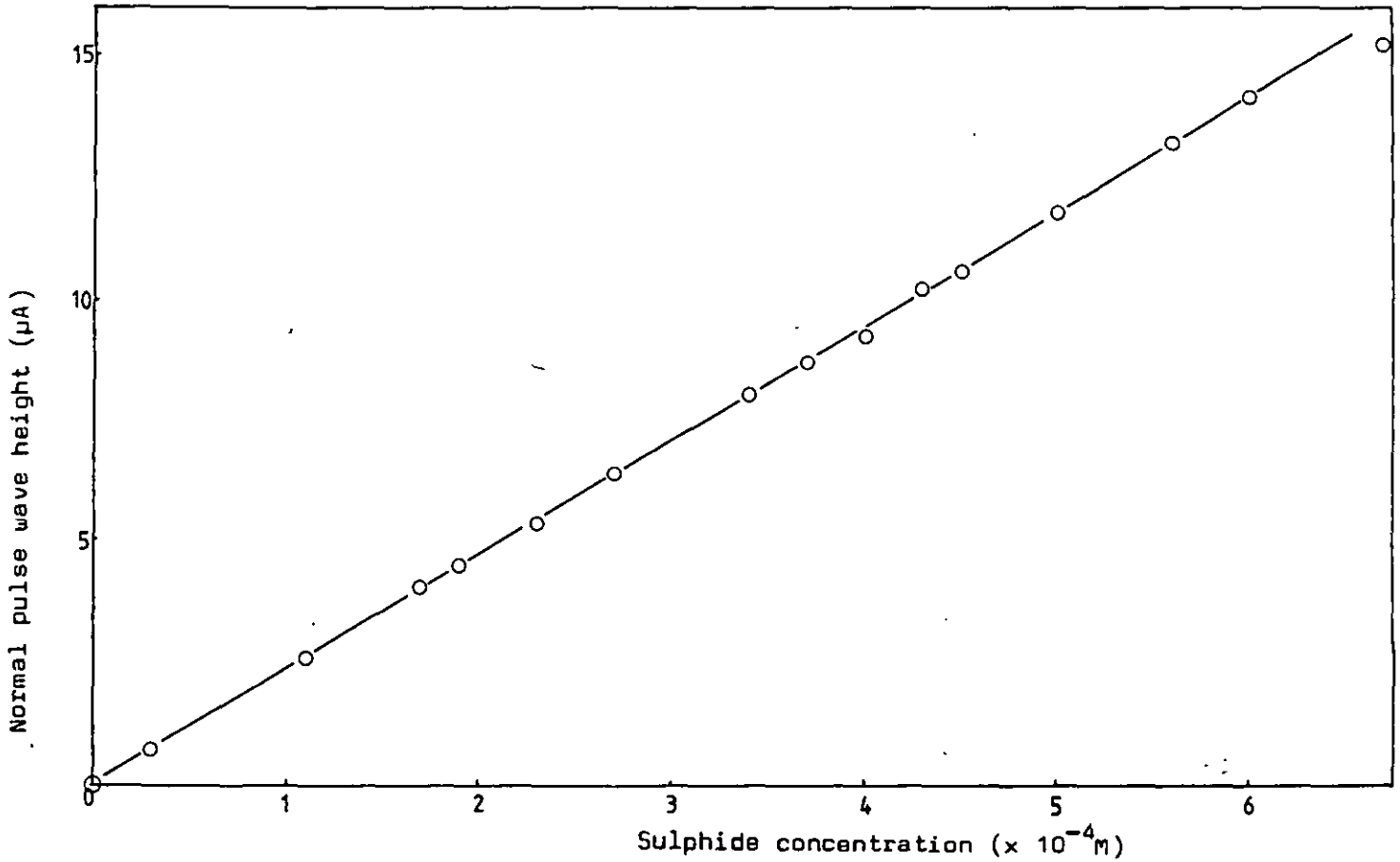


Fig. 37 Calibration graph for the Normal pulse method for sulphide determination.

Table 37. Tabulation of the NP signals obtained with changing concentration of H_2S for the calibration graph (fig. 37) for the determination of H_2S

molarity of S^{2-} (x 10^{-4} M)	peak height/μA				Average current obtained (μA)
	Trial				
	1	2	3	4	
Blank	0.0	0.0	0.0	0.0	0.0
0.3	0.65	0.7	0.75	0.7	0.70
0.7	1.5	1.6	1.6	1.6	1.58
1.1	2.6	2.6	2.6	2.6	2.60
1.7	4.05	4.0	4.1	4.0	4.04
1.9	4.5	4.5	4.5	4.5	4.50
2.3	5.4	5.4	5.4	5.2	5.35
2.7	6.4	6.4	6.4	6.4	6.40
3.4	8.05	8.0	8.1	8.0	8.04
3.7	8.7	8.75	8.7	8.7	8.71
4.0	9.2	9.3	9.25	9.3	9.26
4.3	10.2	10.2	10.2	10.3	10.23
4.5	10.6	10.6	10.6	10.6	10.60
5.0	11.85	11.9	11.8	11.85	11.85
5.6	13.0	13.5	13.5	13.0	13.25
6.0	14.2	14.2	14.15	14.25	14.20
6.7	15.2	15.2	15.2	15.2	15.20

A linear relationship was observed between 0.3 x 10^{-4} M and 6.0 x 10^{-4} M sulphide.

15.

Determination of the amount of H_2S produced from the alkaline hydrolysis of a number of cephalosporins

The cephalosporin was dissolved in NaOH (0.5 M) (100 ml). This solution was heated at 100°C to produce the maximum sulphide concentration (heating time for each cephalosporin obtained from Abdalla et al.[198]). The solution was cooled and polarographed in the normal pulse mode as described above. The data presented below shows the concentration of

cephalosporin used, the heating time required, and the percentage of sulphide produced as an average over four readings.

(i) Cephalexin (137 ppm) (3.94×10^{-4} M)

Trial	μ A
1	5.8
2	5.7
3	5.8
4	5.8
$\bar{x}$	5.78

Heating for 30 minutes.

5.78 μ A is equivalent to 2.45×10^{-4} M $\left[S^{2-}\right]$

$S^{2-}\% = 62.20$

(see fig. 36)

(ii) Cephaloglycin (120 ppm) (2.96×10^{-4} M)

Trial	μ A
1	1.55
2	1.50
3	1.60
4	1.55
$\bar{x}$	1.55

Heating for 40 minutes.

1.55 μ A $\quad$ 0.65×10^{-4} M $\left[S^{2-}\right]$

$S^{2-}\% = 22.00$

(iii) Cephalonium (302 ppm) (6.57×10^{-4} M)

Trial	μ A
1	3.9
2	4.0
3	4.1
4	3.9
$\bar{x}$	3.98

Heating for 60 minutes.

3.98 μ A $\quad$ 1.70×10^{-4} M $\left[S^{2-}\right]$

$S^{2-}\% = 25.90$

(iv) Cephaloridine (132 ppm) (3.18×10^{-4} M)

Trial	μ A
1	1.55
2	1.55
3	1.60
4	1.55
$\bar{x}$	1.56

Heating for 60 minutes.

1.56 μ A $\quad 0.65 \times 10^{-4}$ M $\left[S^{2-}\right]$

$S^{2-}\% = 20.00$

(v) Cephalothin (97 ppm) (2.32×10^{-4} M)

Trial	μ A
1	1.00
2	1.00
3	1.00
4	1.00
$\bar{x}$	1.00

Heating for 45 minutes.

1.00 μA $\quad 0.43 \times 10^{-4}$ M $\left[S^{2-}\right]$

$S^{2-}\% = 18.50$

(vi) Cephoxazole (548 ppm) (11.15×10^{-4} M)

Trial	μA
1	6.90
2	6.90
3	6.85
4	6.90
$\bar{x}$	6.89

Heating for 70 minutes.

6.89 μA $\quad 2.93 \times 10^{-4}$ M $\left[S^{2-}\right]$

$S^{2-}\% = 26.30$

(vii) 7-ACA (178 ppm) (6.54×10^{-4} M)

Trial	μ A
1	7.0
2	7.1
3	7.0
4	7.2
$\bar{x}$	7.08

Heating for 40 minutes.

7.08 μ A $\quad 3.00 \times 10^{-4}$ M $\left[S^{2-}\right]$

$S^{2-}\% = 45.90$

(viii) 7-ADCA (112 ppm) (5.23×10^{-4} M)

Trial	μA
1	7.00
2	7.05
3	7.05
4	7.05
$\bar{x}$	7.04

Heating for 35 minutes.

7.04 μA 2.98×10^{-4} M $\left[S^{2-}\right]$

$S^{2-}\% = 57.00$

(ix) Cephradine (153 ppm) (4.53×10^{-4} M)

Trial	μA
1	7.00
2	7.00
3	7.05
4	6.90
$\bar{x}$	6.99

Heating for 30 minutes.

6.99 μA 2.97×10^{-4} M $\left[S^{2-}\right]$

$S^{2-}\% = 65.60$

(x) Cefazolin (307 ppm) (6.44×10^{-4} M)

Trial	μA
1	6.10
2	6.10
3	6.00
4	6.10
$\bar{x}$	6.08

Heating for 30 minutes

6.08 μA 2.60×10^{-4} M $\left[S^{2-}\right]$

$S^{2-}\% = 40.40$

(xi) Cefaclor (103 ppm) (2.80×10^{-4} M)

Trial	μA
1	2.8
2	2.8
3	2.8
4	2.8
$\bar{x}$	2.8

Heating for 40 minutes.

2.8 μA 1.2×10^{-4} M $\left[S^{2-}\right]$

$S^{2-}\% = 42.90$

Table 38 (below) compares the percentage of hydrogen sulphide produced from the alkaline degradation of the cephalosporins using the normal pulse method (above) with that of (i) a spectrophotometric method to form methylene blue[198] and (ii) a potentiometric method using an Orion sulphide electrode[213], both of which produce H_2S under the same conditions as the NP method.

Table 38.

Compound	Percentage sulphide found		
	NP method	Potentiometric method	Spectrophotometric method
Cephalexin	62.2	65.8	64.4
Cephaloglycin	22.0	19.5	18.9
*Cephalonium	25.9	17.4	16.0
Cephaloridine	20.0	18.1	18.9
Cephalothin	18.5	19.7	19.8
*Cephoxazole	26.3	14.8	15.7
7-ACA	45.9	48.1	47.4
7-ADCA	57.0	57.3	57.9
Cephradine	65.6	61.9	63.7
*Cefazolin	40.4	25.1	26.9
Cefaclor	42.9	43.5	45.1

Good agreement is found between the methods for all the cephalosporins except those asterisked; cephalonium, cephoxazole and cefazolin all give significantly higher results in the normal pulse determination as compared with the spectrophotometric and the potentiometric methods. When the degraded solutions of these three compounds were brought down to pH 2, any H_2S present was blown off by purging with nitrogen gas. The solutions were then brought back up to pH 13.7. No wave at -0.79 V was observed.

These anomalies may be explained by assuming that the alkaline hydrolysis of these three compounds produces sulphur compounds and when brought down to low pH they break down into non-electroactive compounds. They

also did not react with the Pb^{2+} used in the potentiometric method.[213] The identity of these compounds is unknown.

B.
DEGRADATION OF α-AMINOBENZYL-β-LACTAMS TO FORM PYRAZINE DERIVATIVES.

α-Aminobenzylpenicillins and -cephalosporins, when subjected to strong alkaline hydrolysis at room temperature followed by mild acid hydrolysis at pH 5 in a boiling water bath, produce strongly fluorescent products which have been identified as pyrazine derivatives[58,154]. These pyrazines are polarographically active and are produced in low yield when α-aminobenzylcephalosporins are degraded at neutral pH[79] and when α-aminobenzylpenicillins are degraded in acidic pH[80]. The presence of formaldehyde as 1% v/v at the mild acid hydrolysis stage accelerates the rate of pyrazine production.

1.
Production of a pyrazine derivative from cephalexin.

A sample of 2-hydroxy-3-phenyl-6-methylpyrazine (V) was isolated according to the method of Barbhaiya *et al.*[58]. Cephalexin (0.5 g) in distilled water (50 ml) was treated (10 min., 20°C) with NaOH (25 ml, 1.0 M). HCl (25 ml, 1.0 M) was then added followed by Sorensen's citrate buffer (150 ml) containing formaldehyde (1% v/v). The pH of the buffer was adjusted to 5.0. The mixture was then heated at 100°C for 30 min., cooled and extracted repeatedly with ethyl acetate. The combined organic layers were then evaporated and the crude product thus obtained was crystallized from ethyl acetate. The yellow amorphous product had a m.p. of 206°C, compared with a m.p. in the literature of 206-208°C.

A pH plot was carried out on this compound (25 ppm) (see Table 39).

Table 39. Data obtained for the change in pH for a solution of the 6-methylpyrazine derivative.

pH	Reduction potential (V -)	Current height (μA)
2.0	0.61	6.2
3.0	0.70	7.4
4.0	0.77	7.4
5.0	0.83	6.8
6.0	0.89	6.4
7.0	0.96	6.0
8.0	1.03	5.2
9.0	1.09	4.8
10.0	1.15	4.2

2.

Comparison of the relative peak currents obtained from the isolated pyrazine in different media.

The 6-methylpyrazine derivative (V) (50 ppm) (2.67×10^{-4} M) was dissolved in (i) HCl (5 M); (ii) HCl (0.3 M); (iii) Sorensen's buffer pH 5 and (iv) H_2SO_4 (12.5%). The sample solutions were then polarographed at 25°C (see Table 40).

Table 40.

Solvent	Molarity of pyrazine ($\times 10^{-4}$ M)	Reduction potential V (-)	Current obtained (μA)	Current/mole ($\times 10^{4}$ μA/mole)
HCl (5 M)	2.67	0.42	12.6	4.72
HCl (0.3 M)	2.67	0.53	14.3	5.03
pH 5 buffer	2.67	0.83	17.75	6.65
H_2SO_4 (12.5%)	2.67	0.47	19.5	7.30

The pyrazine compound gives its greatest signal in H_2SO_4 (12.5%) with HCl (5 M) giving the smallest of the signals.

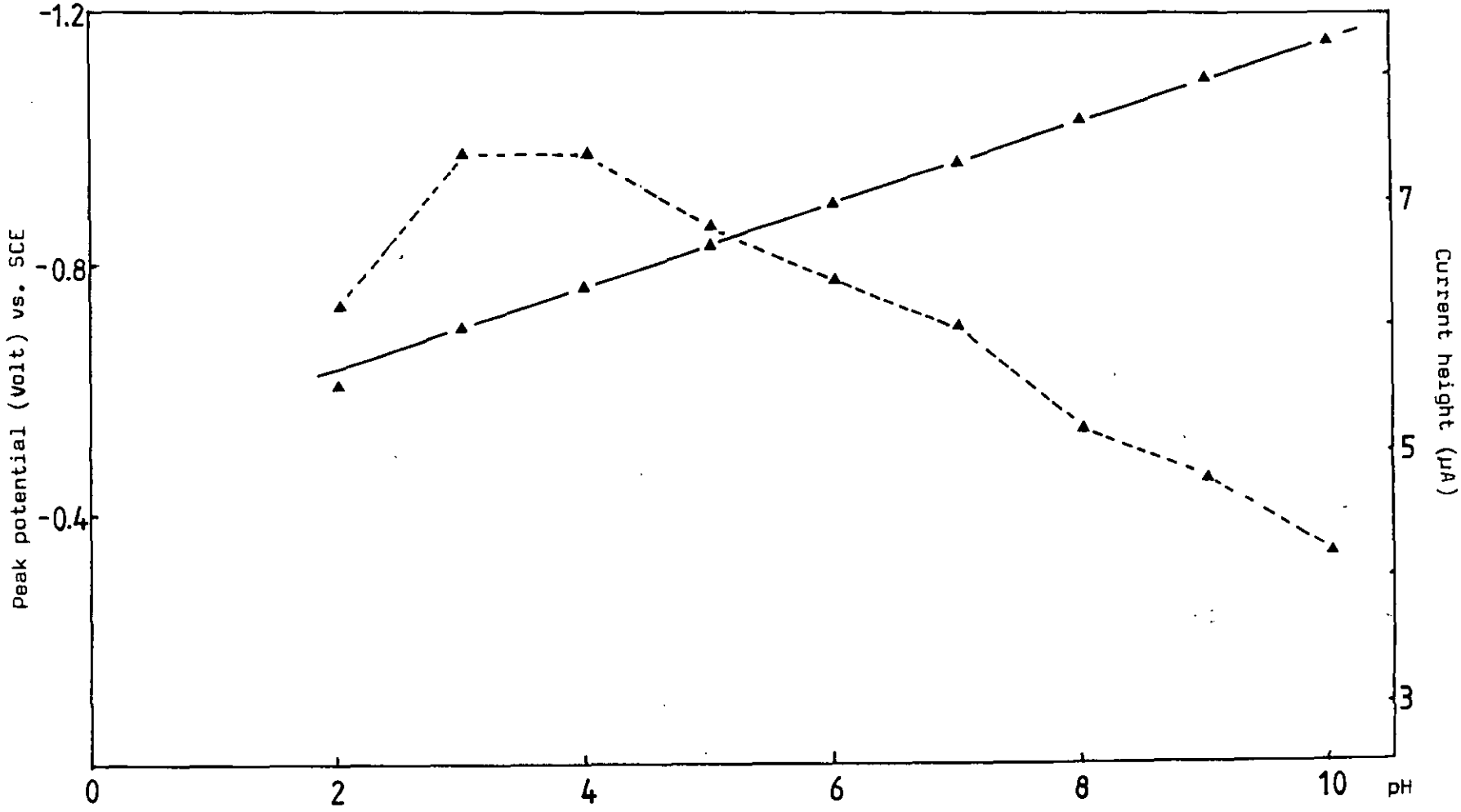


Fig. 38 Plot of the changes in the reduction potential and current height (dashed line) with pH for the 6-methylpyrazine (V) derivative of cephalexin.

A graph was plotted of these results (fig. 38). A linear slope is obtained in the pH range 2 - 10. The $E_{\frac{1}{2}}$ equation obtained was

$$E_{\frac{1}{2}} = (-0.50 - 0.065\ pH)\ V.$$

3.

An investigation of the alkaline hydrolysis step.

The recommended time period for the alkaline hydrolysis step is one hour in NaOH (0.1 M) at 25°C. The mild acid hydrolysis step is carried out in Sorensen buffer (pH 5) at 100°C for 30 minutes. The length of time undergoing alkaline hydrolysis was examined for a number of cephalosporins and ampicillin.

The drug (100 ppm) was degraded in NaOH (0.1 M) at 25°C. Periodically aliquots of 1 ml were removed and neutralized with HCl (1 ml). This solution was made up to 10 ml with either (a) Sorensen buffer pH 5 (8 ml) or (b) Sorensen buffer pH 5 (1% v/v formaldehyde) (8 ml). This solution was then boiled for 30 minutes, cooled and polarographed at 25°C.

Cephalexin, cephaloglycin and ampicillin were examined (see Tables 41 to 47 and figs. 39 and 40).

Table 41. Cephalexin (2.88×10^{-4} M) using pure buffer in the acid hydrolysis step.

Time/hrs (in 0.1 M, OH^-, 25°C)	Peak heights/(μA)			
	peak at -0.30 V	peak at -0.83 V	peak at -0.92 V	peak at -1.07 V
1 min	6.00	4.00	3.00	5.00
0.33	6.40	13.60	7.80	5.00
0.66	6.40	15.00	7.80	5.00
1.00	6.66	15.00	8.60	5.00
1.50	6.6	14.40	9.00	4.80
2.00	5.8	11.00	9.80	5.40
3.00	4.70	11.00	9.40	5.00
6.00	3.60	10.90	8.00	3.60

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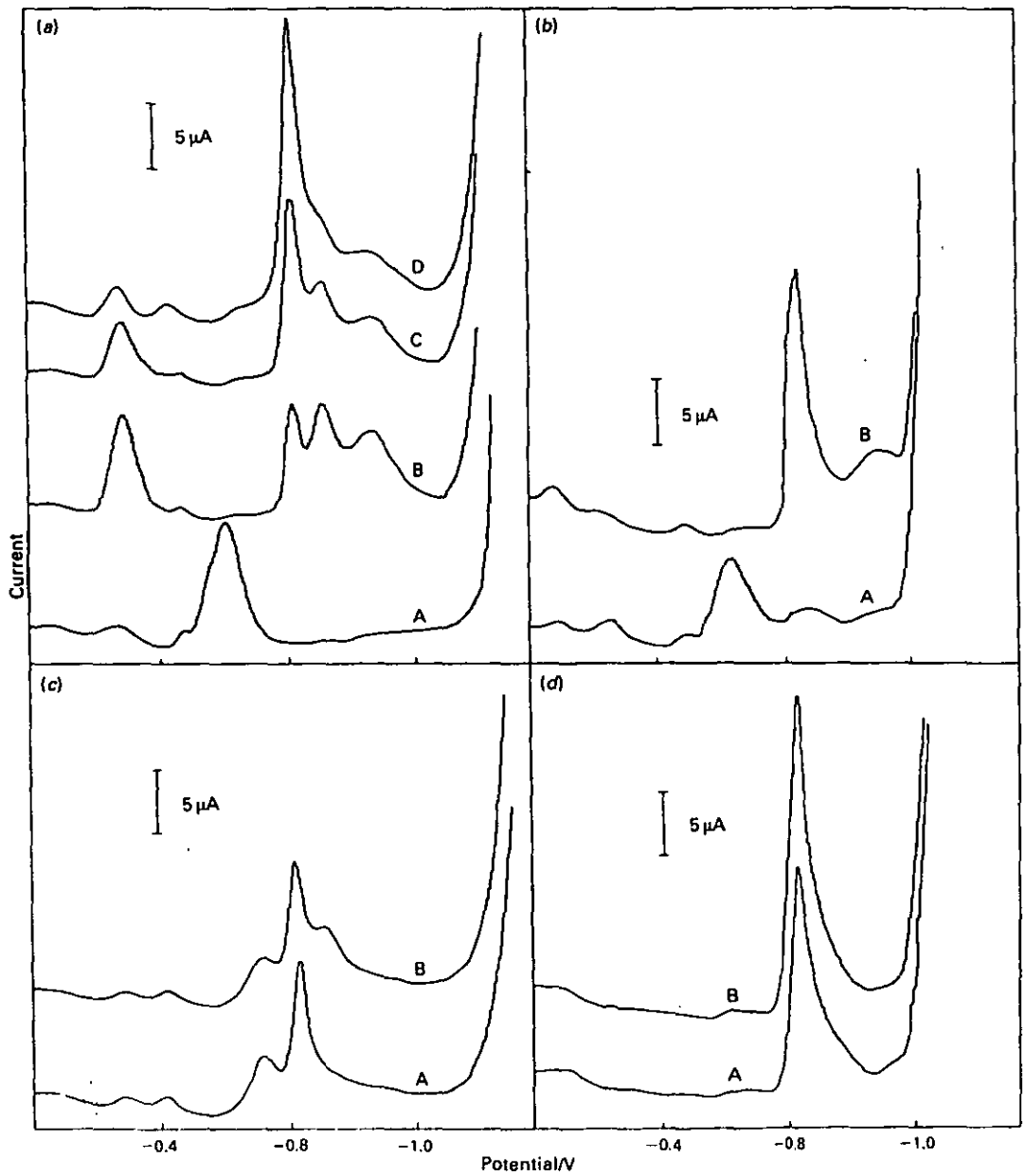


Above: pyrazine derivative formation from cephalexin (100 µg ml^{-1}): (*a*) without formaldehyde, A 1, B 10, C 20 and D 60 min; (*b*) with 1% formaldehyde; A 1 and B 30 min. Below: pyrazine derivative formation from ampicillin (100 µg ml^{-1}): (*c*) without formaldehyde; A 1 and B 30 min; (*d*) with 1% formaldehyde; A 1 and B 30 min.

Fig. 39

Table 42. Cephalexin (2.88×10^{-4} M) using buffer with 1% v/v HCHO in the acid hydrolysis step.

Time/hrs (in 0.1 M, OH^-, 25°C)	Peak heights/μA	
	peak at -0.83 V	peak at -1.05 V
1 min	18.50	0.0
0.5	26.50	2.5
1.0	27.00	3.0
1.5	27.00	2.5
2.0	27.00	2.5
4.0	27.00	2.0
6.0	26.00	1.5

Table 43. Cephaloglycin (2.47×10^{-4} M) using pure buffer in the acid hydrolysis step.

Time/hrs (in 0.1 M, OH^-, 25°C	Peak heights/μA		
	peak at -0.72 V	peak at -0.83 V	peak at -0.91 V
1 min	4.0	9.5	0.0
0.50	3.0	10.50	5.0
1.00	3.0	11.5	5.0
1.50	3.0	10.5	5.0
3.25	2.0	9.5	4.5
6.00	1.75	8.0	4.1
9.00	1.50	7.0	4.0

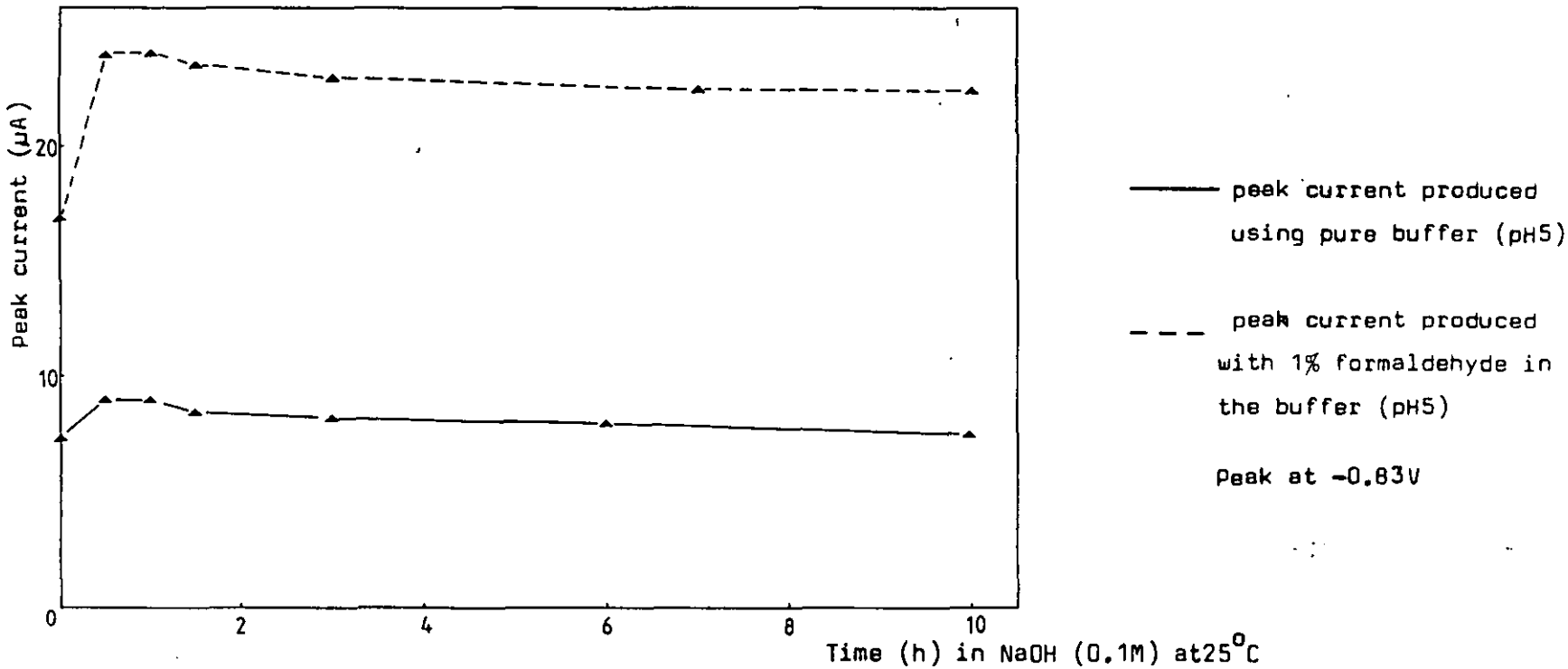


Fig. 40. Pyrazine derivative formation from ampicillin after treatment with NaOH and heating at 100°C in Sorensen's buffer (pH5).
Polarography at pH5.

Table 44. Cephaloglycin (2.47×10^{-4} M) using buffer with 1% v/v HCHO in the acid hydrolysis step.

Time/hrs (in 0.1 M, OH^-, 25°C	Peak heights/μA	
	peak at -0.72 V	peak at -0.83 V
1 min	1.5	9.5
0.5	3.0	18.0
1.0	2.5	17.0
1.5	2.2	15.0
3.0	1.5	15.0
6.25	1.0	12.0
9.0	0.5	10.5

Table 45. Ampicillin (2.48×10^{-4} M) using pure buffer in the acid hydrolysis step.

Time/hrs (in 0.1 M, OH^-, 25°C)	Peak heights/μA		
	peak at -0.30 V	peak at -0.83 V	peak at -0.91 V
1 min	0.0	7.3	6.0
0.5	0.5	9.0	7.3
1.0	0.6	9.0	7.3
1.5	1.0	8.5	7.8
3.0	1.0	8.3	7.0
6.0	1.0	8.0	6.7
10.0	1.0	7.5	6.5

Table 46. Ampicillin (2.48×10^{-4} M) using buffer with 1% v/v HCHO in the acid hydrolysis step.

Time/hrs (in 0.1 M, OH^-, 25°C)	Peak heights/μA
	peak at -0.83 V
1 min	17.0
0.5	24.0
1.0	24.0
1.5	23.5
3.0	23.0
7.0	22.5

Table 47. Relative amount of pyrazine produced from each compound measured as peak current.

Compound	Peak height/($\times 10^4$ μA/mole)	
	pure buffer	buffer (1% HCHO)
Cephalexin	5.20	9.37
Cephaloglycin	4.65	7.28
Ampicillin	3.63	9.68

The pyrazine derivatives from all the compounds reach their maximum concentration when 1 hour of alkaline hydrolysis is used. Cephalexin gives the largest signal when pure buffer is used, but ampicillin gives the highest value with the use of the formaldehyde. After reaching its maximum value the -0.83 V peak stays constant. The effect of formaldehyde is to increase the yield of pyrazine by between 1.5 and 2.5 times its value in pure buffer for the compounds.

The theory for ampicillin is that production of the corresponding penicilloic acid in alkaline solution leads to formation of the penicilloaldehyde (VI)[10] mild acid which in turn undergoes ring closure to pyrazine (see fig. 8).[154] This theory in turn might apply to the formation of the analogous cephalosporoic acid from the cephalosporins.

From the tables it may be seen that most of the α-aminobenzyl-β-lactam alkaline degradation product that is used in the acid hydrolysis step to form the pyrazine derivative is formed within 1 minute of alkaline hydrolysis although the maximum of this precursor and subsequently pyrazine occurs when the drug is subjected to 1 hour of alkaline hydrolysis. Therefore the time of 1 hour of alkaline hydrolysis (NaOH, (0.1 M)) at 25°C was chosen to maximise the production of pyrazine.

The -0.92 V wave observed in the study was also found in similar yields when cephaloridine, a compound that does not undergo pyrazine formation, was subjected to the above regime using pure buffer, suggesting that this wave is due to a breakdown mechanism common to all these compounds.

The addition of formaldehyde to the reaction scheme results in formation of few waves other than the pyrazine at -0.83 V.

The incorporation of HCHO into the reaction scheme produces a 6-methylpyrazine derivative (V)[58]. The 2-hydroxy-3-phenylpyrazine (VII) and the 6-methylpyrazine derivative (V) cannot be distinguished polarographically, as they both reduce at the same potential (-0.83 V), in pH 5 buffer solution.

4.

<u>The effect of varying the heating time at 100°C of the acid hydrolysis step.</u>

Cephalexin (2.88×10^{-4} M) was degraded in NaOH (0.1 M) at 25°C for 1 hour. A 10 ml aliquot was extracted and neutralized with HCl (0.1 M). Sorensen buffer (pH 5) (80 ml) or Sorensen buffer (pH 5, 1% v/v HCHO) (80 ml) was added to the solution. This solution was then heated at 100°C and sampled periodically. The samples were cooled and polarographed at pH5, 25°C (see Tables 48 and 49).

Table 48. Using pure buffer (pH 5).

Time/hrs (at 100°C)	Peak height/μA	
	Peak at -0.61 V	Peak at -0.83 V
0	6.3	0.0
0.16	0.0	5.7
0.33	0.0	9.3
0.50	0.0	11.3
1.00	0.0	15.0
2.00	0.0	16.3
3.00	0.0	17.3
4.00	0.0	16.7
7.00	0.0	15.3

Table 49. Using buffer with 1% v/v HCHO.

Time/hrs (at 100°C)	Peak height/μA	
	Peak at -0.61 V	Peak at -0.83 V
0	6.2	1.9
0.16	0.0	23.1
0.33	0.0	25.1
0.50	0.0	25.7
1.00	0.0	27.0
3.16	0.0	27.0
6.0	0.0	27.0
9.0	0.0	27.0

Cefaclor (2.72×10^{-4} M) was similarly treated as cephalexin (above), (see Tables 50 and 51).

Table 50. Using pure buffer (pH 5).

Time/hrs (at 100°C)	Peak height/μA	
	Peak at -0.61 V	Peak at -0.83 V
0	5.5	0.0
0.25	0.0	6.9
0.50	0.0	10.0
0.75	0.0	12.5
1.00	0.0	14.0
1.50	0.0	14.5
2.00	0.0	14.6
3.00	0.0	14.6

Table 51. Using buffer with 1% v/v HCHO.

Time/hrs (at 100°C)	Peak height/μA	
	Peak at -0.61 V	Peak at -0.83 V
0.0	5.6	2.1
0.25	0.0	24.0
0.50	0.0	24.5
0.75	0.0	25.0
1.00	0.0	24.9
1.50	0.0	24.7
2.00	0.0	24.0
3.00	0.0	24.0

Using pure buffer maximum pyrazine production was reached after 3 hours of boiling with both cephalexin and cefaclor. Using buffer with 1% v/v formaldehyde, maximum production of pyrazine is achieved after only 1 hour of boiling. Not only does the addition of HCHO increase the rate of formation of the pyrazine derivative but it increases the yield by approximately 1½ times the value without HCHO in both cefaclor and cephalexin (see Table 52).

Table 52. Comparison of the maximum production of pyrazine as the time of the acid hydrolysis step is varied.

Compound	Peak height/(x 10^4 μA/mole)	
	pure buffer	buffer (1% HCHO)
Cephalexin	6.01	8.93
Cefaclor	5.37	9.19

The wave at -0.61 V is an alkaline hydrolysis product common to both cephalexin and cefaclor which degrades on heating.

5.

The effect of lowering the temperature of the mild acid hydrolysis step from 100°C to 50°C.

Cephalexin (2.88 x 10^{-4} M) was degraded in NaOH (0.1 M) at 25°C for 1 hour. A 10 ml aliquot was extracted, neutralized with HCl, made up to 100 ml with (a) Sorensen buffer (pH 5) and (b) Sorensen buffer (pH 5, 1% v/v HCHO). These solutions were heated at 50°C, sampled at various intervals, cooled and polarographed at 25°C (see Tables 53 and 54).

Table 53. Using pure buffer (pH 5).

Time/hrs (at 50°C)	Peak height/μA	
	Peak at -0.61 V	Peak at -0.83 V
1 min	6.25	0.00
0.167	5.20	0.00
0.33	4.15	0.35
0.50	3.10	1.05
1.00	1.40	3.10
3.00	0.35	9.00
6.00	0.00	11.45
17.00	0.00	12.50
24.00	0.00	13.20
104.00	0.00	12.10

Table 54. Using buffer with 1% HCHO.

Time/hrs (at 50°C)	Peak height/μA	
	Peak at -0.61 V	Peak at -0.83 V
1 min	6.35	1.8
0.167	5.80	6.6
0.33	1.75	10.5
0.50	0.80	13.5
1.00	0.60	16.5
3.00	0.00	18.0
6.16	0.00	20.25
80.00	0.00	24.00

Lowering the temperature of the acid hydrolysis step to 50°C from 100°C results in a dramatic decrease in the rate of pyrazine formation, both in the pure buffer study and when using HCHO, although the relative yields in the two studies remains the same as using 100°C.

6.

Diketopiperazine derivatives and pyrazine formation.

Diketopiperazine derivatives are degradation products from the alkaline hydrolysis of α-aminobenzylcephalosporins[204,208]. In order to assign the role of these derivatives in the formation of pyrazine compounds an experiment involving the diketopiperazine derivative of cephalexin (VIII) was carried out.

The DKP derivative of cephalexin (100 ppm) (2.9×10^{-4} M) was degraded at 100°C in Sorensen buffer (pH 5) (i) with and (ii) without 1% HCHO incorporated in the buffer. Aliquots were removed periodically, cooled and polarographed at 25°C.

In both buffer cases no pyrazine wave at -0.83 V was observed. Both studies were carried out for periods of 6 hours. The DKP peak at 0.64 V decayed quickly in both cases. In pure buffer it disappeared after 1 hour,

and in the buffer containing 1% HCHO no DKP was observed after 20 minutes boiling. Pyrazine derivatives do not form directly from the mild acid hydrolysis of the DKP derivative of cephalexin. This DKP degrades rapidly at 100°C, pH 5, the presence of 1% HCHO accelerating this degradation.

7.

Alkaline hydrolysis of the DKP of cephalexin.

It was now thought that perhaps an alkaline degradation product of the DKP could be an intermediate in pyrazine formation from cephalexin.

DKP (2.90×10^{-4} M) was degraded at 25°C in NaOH (0.1 M). Periodically aliquots of 1 ml were removed, neutralized with HCl, made up to 10 ml with Sorensen buffer pH 5 (i) with and (ii) without 1% HCHO. This solution was then heated at 100°C for 30 minutes, cooled and polarographed at 25°C.

(a) Using pure Sorensen buffer pH 5 the study was carried out for 148 hours and at no time was a wave corresponding to a pyrazine derivative, at -0.83 V, observed. A wave at -0.64 V was found after 1 minute in the alkaline solution. This corresponds to the DKP wave at pH 5. On boiling for 30 minutes this wave had disappeared.

(b) Using Sorensen buffer pH 5 (1% v/v HCHO) the study was carried out for 120 hours. No wave corresponding to pyrazine (-0.83 V) was observed. The DKP wave at -0.64 V disappeared on boiling for 30 minutes.

Under these conditions the DKP of cephalexin does not give pyrazine derivatives, making it likely that the other major degradation product of α-aminobenzylcephalosporins in alkaline solution, the cephalosporoic acid, is the first stage in formation of pyrazine derivatives from these compounds.

8.

Investigating the role of the aldehyde in the formation of pyrazine.

Speculation exists as to whether the aldehyde (formaldehyde) added at the mild acid hydrolysis stage acts as a catalyst in the reaction or actually is incorporated into the pyrazine molecule to give the 6-methylpyrazine derivative (V). If the latter statement is correct then replacement of formaldehyde by the larger acetaldehyde would perhaps cause steric hindrance in the formation of the 6-alkylpyrazine derivative. The 6-ethylpyrazine derivative therefore would form at a slower rate than the 6-methylpyrazine derivative. In a previous experiment with 1% HCHO in the Sorensen pH 5 (Table 47) the pyrazine production from ampicillin was greatest. Ampicillin was therefore chosen to compare the rate of production of pyrazine using formaldehyde and acetaldehyde.

Ampicillin (2.48×10^{-4} M) was degraded in NaOH (0.1 M), 25°C. Periodically 10 ml aliquots were removed, neutralized and made up to 100 ml with Sorensen's buffer pH 5 containing 1% v/v acetaldehyde. This solution was then treated at 100°C for 30 minutes, cooled and polarographed at 25°C (see Table 55).

Table 55.

Time/hrs (in OH^- (0.1 M))	Peak height/μA
	Peak at -0.83 V
1 min.	10.7
0.5	17.6
1.0	17.9
1.5	17.9
3.0	17.9
6.0	18.2
9.0	17.0
25.0	17.0
48.0	17.0
72.0	17.0

maximum production of pyrazine = 7.34×10^4 μA/mole.

Comparison of Tables 45, 46 and 55 shows that the addition of $CH_3.CHO$ instead of HCHO to the buffer does not affect the t_{i_p} maximum of the pyrazine derivative. If the 6-ethylpyrazine is formed in the presence of acetaldehyde then it reduces at the same potential as 2-hydroxy-3-phenyl-pyrazine and the 6-methylpyrazine derivative at pH 5. Addition of $CH_3.CHO$ instead of HCHO decreases the maximum yield of pyrazine by 25% from 9.68×10^4 μA/mole to 7.34×10^4 μA/mole. This value implies that the addition of acetaldehyde does cause steric hindrance and suggests that the aldehyde is incorporated into the pyrazine molecule.

9.

The effect of the absence of dissolved oxygen on the production of pyrazine from ampicillin.

Ampicillin (2.48×10^{-4} M) was degraded in NaOH (0.1 M) at 25°C for 1 hour. Ten ml aliquots were neutralized and made up to 100 ml with (i) Sorensen buffer pH 5, (ii) Sorensen buffer pH 5 (1% v/v HCHO) and (iii) Sorensen buffer pH 5 (1% v/v $CH_3.CHO$). Each of the three 100 ml solutions was divided into two 50 ml portions, one of which was deoxygenated. The six 50 ml solutions were then heated for 30 minutes at 100°C, cooled and then polarographed at 25°C (see Table 56).

Table 56.

State of Sorensen buffer pH 5	Peak height/μA	
	Peak at -0.83 V	
	Hydrolysis conditions	
	with dissolved oxygen	without dissolved oxygen
pure buffer	9.1	9.2
buffer with 1% HCHO	24.0	23.9
buffer with 1% $CH_3.CHO$	17.8	17.6

The absence of dissolved oxygen does not have an effect on the production of the pyrazine derivative from ampicillin. It has been suggested by Indelicato et al.[159] that a route to pyrazine was from oxidation of the dihydropyrazine skeleton (IX) (formed by cyclization of the penicilloaldehyde (VII)). If this route does occur then the oxidation step does not require dissolved oxygen.

10.

The acidic degradation of ampicillin.

Ampicillin (2.48×10^{-4} M) was heated at 100°C in (i) Britton-Robinson buffer solution pH 2 and (ii) buffer with 1% v/v HCHO. Periodically aliquots were removed, cooled and polarographed at 25°C (see Tables 57 and 58) at pH 5.

(i)

Table 57. Using Britton-Robinson buffer pH 2.

Time/hrs	Peak height/uA		
	peak at -0.30 V	peak at -0.83 V	peak at -0.92 V
0.0	0.0	0.0	0.0
0.5	6.5	11.0	2.0
1.0	5.5	12.0	2.0
3.0	4.0	12.0	2.0
6.0	3.0	12.0	2.5
9.0	1.0	12.0	2.5

maximum molar yield of pyrazine as current

$= 4.9 \times 10^{4}$ µA/mole.

(ii)

Table 58. Using Britton-Robinson buffer pH 2 (1% v/v HCHO).

Time/hrs	Peak height/μA
	Peak at -0.83 V
0.0	0.0
1.0	10.5
3.0	12.0
6.0	9.0

maximum molar yield of pyrazine as current

$$\doteq 4.9 \times 10^{4}\ \mu A/mole.$$

Comparison of the maximum molar yields as current with hydrolysis at pH 2 with those obtained from the combined alkaline/acid hydrolysis steps in Table 47 shows that hydrolysis at pH 2 produces smaller yields of pyrazine. The penicillin degradation product penicillamine, -0.30 V (pH 5), is not seen to form when formaldehyde is present.

11.

The effect of the pH of the acid hydrolysis step on the production of pyrazine from ampicillin.

Ampicillin (2.48×10^{-4} M) was degraded in NaOH (0.1 M) at 25°C for 1 hour. Two 10 ml aliquots were removed, which were made up, after neutralization with HCl, to 100 ml with (i) Sorensen buffer pH 2 and (ii) Sorensen buffer pH 2 (1% v/v HCHO). Both these solutions were heated at 100°C for 30 minutes, cooled and polarographed at 25°C.

A comparison can be made between hydrolysis at pH 5 and at pH 2 of hydroxide degraded ampicillin to form pyrazines (see Table 59).

Table 59.

State of Sorensen buffer	Peak height/μA	
	Peak at -0.83 V	
	Hydrolysis conditions	
	pH 2	pH 5
pure buffer	9.25	9.00
buffer with 1% HCHO	17.80	23.80

Ampicillin has been shown by Fogg et al.[80] to degrade readily in acid solution (pH 2.5) to a pyrazine derivative. Alkaline hydrolysis produces the α-aminobenzylpenicilloic acid. Pyrazines are found in larger yields when this acid undergoes acid hydrolysis at pH 5 rather than at pH 2. The hydrolysis without 1% HCHO is not affected by the pH.

12.

Hydrolysis of ampicillin to form a pyrazine derivative without first forming the α-aminobenzylpenicilloic acid.

(a) Hydrolysis at 25°C.

Ampicillin (2.48×10^{-4} M) was hydrolysed at 25°C in Sorensen buffer pH 5 (i) with 1% v/v HCHO and (ii) without the aldehyde. Periodically samples were removed and polarographed.

(i) Using pure Sorensen buffer a pyrazine peak (-0.83 V) is not seen until 50 hours have elapsed. After 75 hours the peak had only reached 0.75 μA compared with a maximum of 9.0 μA using the combined alkaline/acid step (Table 45).

(ii) Using Sorensen buffer with 1% HCHO a pyrazine peak began to appear after 1.5 hours. The maximum signal was obtained after 28.5 h and was stable at 1.85 μA (see Table 60).

Table 60.

Time/hrs	Peak height/μA	
	Peak at -0.83 V	Peak at -1.06 V
0.0	0.0	0.0
0.25	0.0	0.0
0.5	0.0	0.0
1.5	1.05	0.8
3.0	1.15	1.35
6.5	1.40	2.1
28.5	1.85	2.8
50.0	1.85	2.8

This reading is 7.7% of maximum reading obtained using the combined alkaline/acid steps, 24 μA (see Table 46). The peak at -1.06 V is unknown.

(ii) Hydrolysis at 100°C.

Ampicillin (2.48×10^{-4} M) was degraded as in the last experiment, only the hydrolysis temperature was 100°C (see Tables 61 and 62).

Table 61. Using pure Sorensen buffer pH 5.

Time/hrs	Peak height/μA		
	Peak at -0.30 V	Peak at -0.83 V	Peak at -0.92 V
0.0	0.40	0.0	0.0
0.25	0.90	0.85	0.95
0.50	1.90	2.95	1.65
1.00	2.40	8.25	2.10
1.50	2.05	8.85	1.75
3.00	1.45	10.80	1.75
6.00	1.15	11.25	1.75

Table 62. Using Sorensen buffer (1% v/v HCHO).

Time/hrs	Peak height/μA			
	Peak at -0.30 V	Peak at -0.70 V	Peak at -0.83 V	Peak at -1.06 V
0.0	0.2	0.0	0.0	0.0
0.25	0.0	0.1	1.4	2.1
0.50	0.0	0.2	0.1	1.8
1.00	0.0	0.3	1.5	0.9
1.50	0.0	0.5	1.6	0.6
3.00	0.0	0.9	1.6	0.3
6.00	0.0	1.65	1.7	0.0

The wave at -0.30 V is due to penicillamine. This wave was absent from the 25°C study. It is either not formed or degraded very quickly in the presence of HCHO. The pyrazine compound at -0.83 V forms steadily in the buffer without HCHO (Table 61) at a slow rate. The maximum height at 6 h (11.25 μA) is higher than that obtained using the combined alkaline/acid hydrolysis steps (9.0 μA) (Table 45). In the presence of HCHO at 100°C ampicillin does not form high yields of pyrazine, even after 6 hours. The formaldehyde may be reacting with a compound which is a precursor to formation of penicilloaldehyde (VI) and therefore blocking formation of pyrazine.

13.

Degradation of ampicillin in the presence of Cu(II) ions.

The presence of Cu(II) ions in solutions of penicillins promotes the degradation of these penicillins to their penicilloic acids[130]. The presence of levels of $CuCl_2$ at 2.5×10^{-6} M resulted in comparable results to sodium hydroxide (0.1 M) hydrolysis of penicillins to their penicilloic acids. The effect of Cu(II) ions on the mild acid hydrolysis of ampicillin to produce pyrazines was examined.

Ampicillin (2.48×10^{-4} M) was hydrolysed at (i) 25°C and (ii) 100°C in Sorensen buffer pH 5 containing $CuCl_2$ (2.51×10^{-6} M). Periodically the

solutions were sampled and polarographed at 25°C (see Tables 63 and 64).

Table 63. Hydrolysis at 25°C.

Time/hrs	Peak height/μA
	Peak at -0.83 V
0.0	0.0
0.25	0.5
0.50	0.6
1.00	0.55
1.50	0.35
3.00	0.25
6.00	0.0
26.00	0.0
48.00	0.0

Table 64. Hydrolysis at 100°C.

Time/hrs	Peak height/μA		
	Peak at -0.30 V	Peak at -0.83 V	Peak at -0.92 V
0.0	0.35	0.0	0.0
0.25	0.60	0.25	0.75
0.50	1.65	2.80	1.20
1.00	1.75	5.80	1.60
1.50	1.80	7.60	1.80
3.00	1.50	8.60	1.80
6.00	1.20	8.90	1.80

The presence of Cu(II) ions in the 25°C study does not produce significant amounts of pyrazine. In the 100°C study the presence of Cu(II) ions, unlike that of formaldehyde, does not prevent the production of pyrazine but it does not produce larger amounts than the study using pure buffer at 100°C where the maximum amount produced was 11.25 μA (Table 61).

14.

A pH plot of the pyrazine produced from ampicillin using 1% HCHO was carried out. Ampicillin (2.48×10^{-4} M) was degraded to produce the pyrazine (see fig. 41). The $E_{\frac{1}{2}}$ equation obtained is

$$E_{\frac{1}{2}} = -0.45 - 0.070 \text{ pH}$$

The result shows that the pyrazine derivative from ampicillin (using 1% HCHO) has an $E_{\frac{1}{2}}$ equation experimentally identical to the prepared pyrazine derivative from cephalexin, (V),

$$E_{\frac{1}{2}} = (-0.50 - 0.065 \text{ pH}) \text{ V} .$$

15.

Determination of the amount of H_2S produced during the production of pyrazine from cephalexin.

Cephalexin (500 ppm) (1.44×10^{-3} M) was degraded in NaOH (0.1 M) at 25°C for 1 hour. At the end of this time period the amount of H_2S present was determined by the normal pulse polarographic method.

A reading of 5 μA was obtained, this is equivalent to 2.1×10^{-4} M of H_2S. Therefore the percentage of H_2S produced under these conditions in 1 h = 14.6%.

Cephalexin (1.44×10^{-3} M) was degraded as above. Two 10 ml aliquots were removed, and neutralized. One was made up to 100 ml with (i) Sorensen buffer pH 5, the other made up to 100 ml with Sorensen buffer pH 5 (1% v/v HCHO). These solutions were then heated at 100°C. Nitrogen purge gas swept any H_2S produced into NaOH (0.5 M) (15 ml). Periodically the 15 ml solution was replaced by 15 ml of fresh NaOH (0.5 M). The sample solution was made up to 100 ml with NaOH (0.5 M) and the H_2S determined (see Tables 65 and 66).

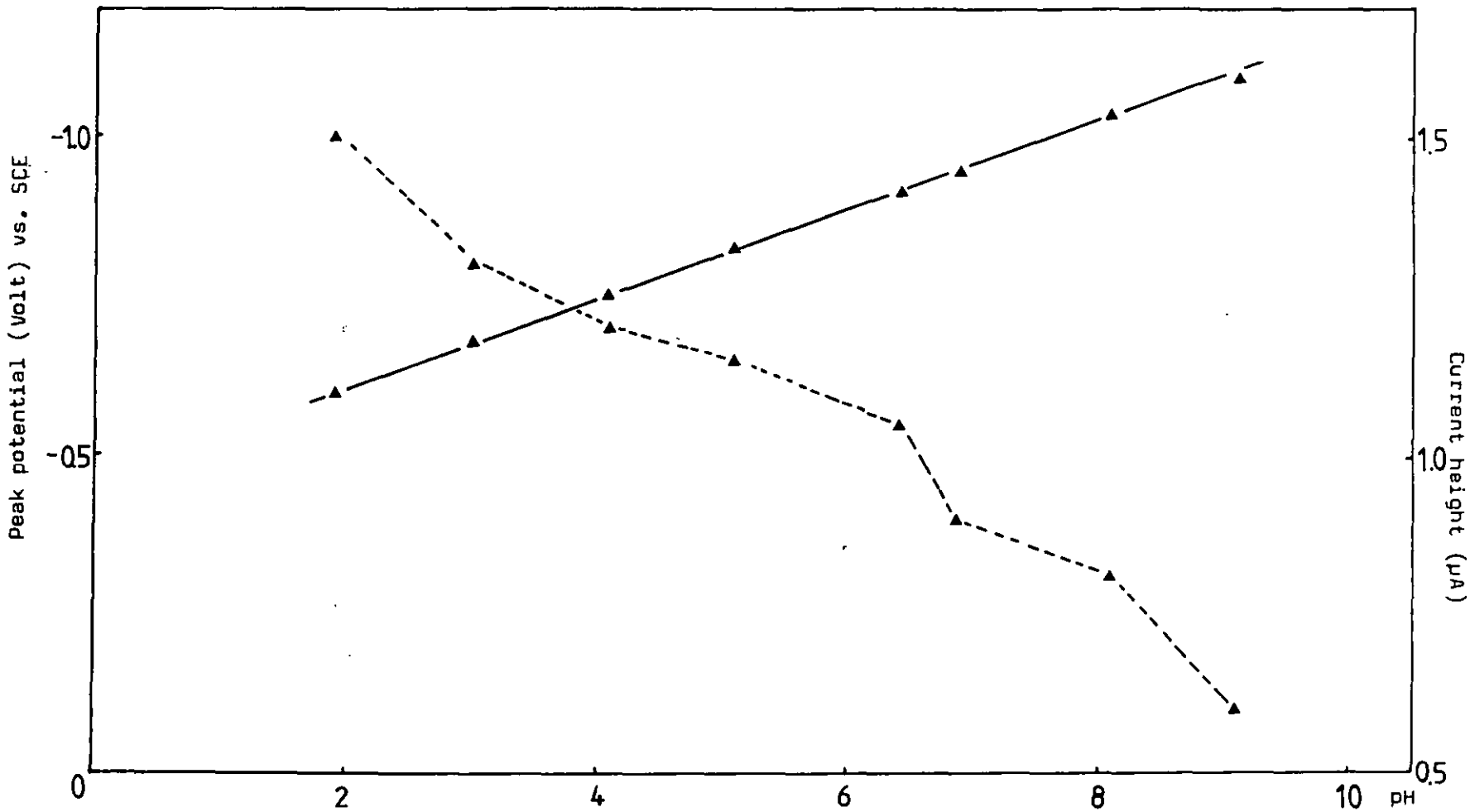


Fig. 41 Plot of the changes in the reduction potential and current height (dashed line) with pH for the pyrazine derivative of ampicillin.

Table 65. Using Sorensen buffer pH 5.

Time/hrs (in 100°C)	pulse signal (μA)	S^{2-} (x 10^{-4} M)	% S^{2-} produced
0.5	5.8	2.45	17.01*
1.0	1.6	0.70	4.86
1.5	0.5	0.40	2.77
2.0	0.2	0.20	1.39

* As the value for 0.5 h contains the S^{2-} produced in NaOH (0.1 M), 25°C, 1 h (14.6%), then the correct value for the 0.5 h mild acid hydrolysis step is 2.41%.

Percentage of sulphide produced by mild acid step = 11.43. Total percentage produced = 11.43 + 14.6

= 26.03%.

Table 66. Using Sorensen buffer pH 5 (1% v/v HCHO).

Time/hrs (in 100°C)	pulse signal (μA)	S^{2-} (x 10^{-4} M)	% S^{2-} produced
0.5	6.0	2.55	17.70*
1.0	1.8	0.75	5.21
1.5	1.4	0.60	4.17
2.0	0.7	0.30	2.08

* True value for 0.5 h, 100°C is (17.7 - 14.56)% = 3.1%. Percentage of sulphide produced by mild acid hydrolysis step = 14.56. Total percentage produced = (14.56 + 14.6

= 29.16%.

The difference in the total amount of sulphide produced by each method (3.13%) is not significant. Slightly higher concentrations of sulphide are being produced with formaldehyde. Therefore the sulphur part of the degraded cephalexin does not have a significant role to

play in pyrazine formation.

16.

Degradations of glycine anhydride.

Glycine anhydride (X) is similar structurally to the DKP derivatives of α-aminobenzylcephalosporins (VIII, XI). Glycine anhydride was subjected to a number of experiments in order to observe if it mimics the diketopiperazine ring part of the DKP derivatives in any way.

Glycine anhydride (100 ppm) was dissolved in phosphate buffer (pH 7.4) and polarographed. No polarographic waves were observed.

Glycine anhydride (100 ppm) as prepared as above was heated at 100°C. Aliquots were removed periodically and polarographed at 25°C and pH 7.4.

No polarographic waves were observed in the time study which was carried out for 3 hours.

Glycine anhydride (100 ppm) was degraded in NaOH (0.1 M), 25°C for 1 hour. Polarography at pH 7.4 showed no peaks.

Polarography of a 100 ppm solution of glycine anhydride that had been hydrolysed for 1 h in NaOH (0.1 M), 25°C then degraded at 100°C in Sorensen buffer pH 5 for 30 minutes revealed no waves. Replacement of the buffer solution with buffer containing 1% HCHO also showed no waves.

Glycine anhydride does not oxidise under the above conditions to pyrazine derivatives. The possible analogue of glycine anhydride that is present in cephalexin degradations is the diketopiperazine aldehyde[207] (XII).

17.

Degradations of an aminomethylene derivative of cephalexin.

An aminomethylene derivative of cephalexin has been isolated from the neutral (pH 7.4) degradation of cephalexin and characterized as 3-aminomethyl-6-phenylpiperazine-2,5 dione (XIII). This compound has been suggested by Bundgaard[208] to convert readily to the diketopiperazine aldehyde (XII) which may then go on to pyrazine derivatives.

The aminomethylene derivative (XIII) was isolated from a solution of degraded cephalexin as from Bundgaard[208]. It was found to be insoluble in distilled water, however addition of ethanol to 5% v/v resulted in its dissolution. A UV spectra was obtained for a 4.88×10^{-5} M solution. A single broad absorption signal at 265 nm was recorded. The absorbance was 0.54 (265 nm). The molar absorbtivity was determined as 11 065.6 at 265 nm. ($L\ mol^{-1}\ cm^{-1}$).

Polarography of (100 ppm) solution of the aminomethylene derivative in phosphate buffer (pH 7.4) (5% v/v ethanol) and at three other pH values, 2, 5 and 12, gave no polarographic peaks, showing that this compound is polarographically inactive.

The aminomethylene derivative of cephalexin (AMDC) (XIII) (160 ppm) was treated at 25°C in NaOH (0.1 M) for 1 hour, after which a 10 ml aliquot was removed, neutralized and made up to 100 ml with Sorensen buffer pH 5. This solution was polarographed.

No polarographic peaks were observed showing that alkaline hydrolysis of this compound does not form pyrazine derivatives.

The above solution was divided into two 50 ml portions. To one of these portions 0.5 ml of HCHO was added. Both solutions were heated at 100°C. Periodically samples were removed and polarographed at 25°C.

(a) Using pure Sorensen buffer a small wave at -0.58 V was visible after 0.5 h. This did not increase in height when the boiling solution was sampled after 1 h.

(b) Using 1% HCHO in the buffer no waves were visible in the study either at 0.5 h or 1 h.

The AMDC (XIII) (130 ppm) was dissolved in Sorensen buffer pH 5 (5% v/v ethanol) (i) with and (ii) without 1% v/v HCHO. Both solutions were heated at 100°C. Periodically samples were removed, cooled and polarographed at 25°C.

(a) Using pure Sorensen buffer no polarographic waves of significant height were observed, although three small waves were recorded after one hour at -0.28 V (0.5 μA), -0.67 V (0.3 μA) and -1.10 V (0.3 μA).
(b) Using buffer with 1% HCHO a single wave at -0.91 V was observed, which remained stable at 30 minutes and 1 hour at 1.5 μA.

Thus the AMDC is not a precursor to the formation of pyrazine derivatives, however the wave at -0.91 V observed may be the same wave recorded when cephalexin undergoes the combined alkaline/acid steps to produce pyrazine (see Table 41); that wave reducing at -0.92 V (pH 5).

A pH plot of the -0.91 V wave from hydrolysis of the AMDC as described above was carried out. This was compared with a pH plot of the -0.92 V wave obtained from the degradation of cephalexin to form pyrazine (see figs. 42 and 43).

The $E_{\frac{1}{2}}$ equation obtained from the AMDC = (-0.64 - 0.05 pH) V. That obtained from cephalexin = (-0.63 - 0.055 pH) V.

Experimentally these equations are identical. The AMDC and cephalexin may then degrade to give the same product responsible for the -0.91 V (pH 5).

Dinner[207] has reported the formation of diketopiperazine aldehyde (XII) in high yield from the acidic degradation (pH 3.3) of cephalexin at 75°C. This possibility of this compound forming pyrazine derivatives was investigated.

Cephalexin (422 ppm) (1.21×10^{-3} M) was degraded at pH 3.3 (unbuffered) for 90 minutes at 75°C. After this time period two 25 ml aliquots of solution were made up to 100 ml with (i) Sorensen buffer pH 5 and (ii) Sorensen buffer pH 5 (1% v/v HCHO). Each solution was heated at 100°C. At intervals of 0.5 h and 1 h samples were removed and polarographed at 25°C.

(i) Using Sorensen buffer pH 5 a pyrazine peak was observed, although a large anodic wave at -0.13 V, a thiol, was observed, as well as a large wave at -0.90 V, possibly due to hydrolysis of the diketopiperazine aldehyde (XII) at pH 3.3. Previously the AMDC (XIII) which is possibly a hydrolysis precursor to the aldehyde (XII) gave a wave in this region (-0.92 V) on heating at pH 5 in the presence of HCHO.

(ii) Addition of 1% v/v HCHO to the buffer gave similar results, pyrazine and large waves at -0.13 V and -0.90 V.

Degradation of the diketo aldehyde (formed in pH 3.3 solution), with and without the presence of HCHO does not produce pyrazines. It is unlikely that the AMDC (XIII) and the diketopiperazine aldehyde (XII) are precursors to pyrazine formation.

The AMDC (125 ppm) was dissolved in phosphate buffer (pH 7.4) (5% v/v ethanol). This solution was heated at 100°C for 1 hour.

Cephalexin, when treated as above, gives a high yield of a carbonyl compound at -1.26 V and a small yield of pyrazine at -0.96 V. No waves were observed from the AMDC.

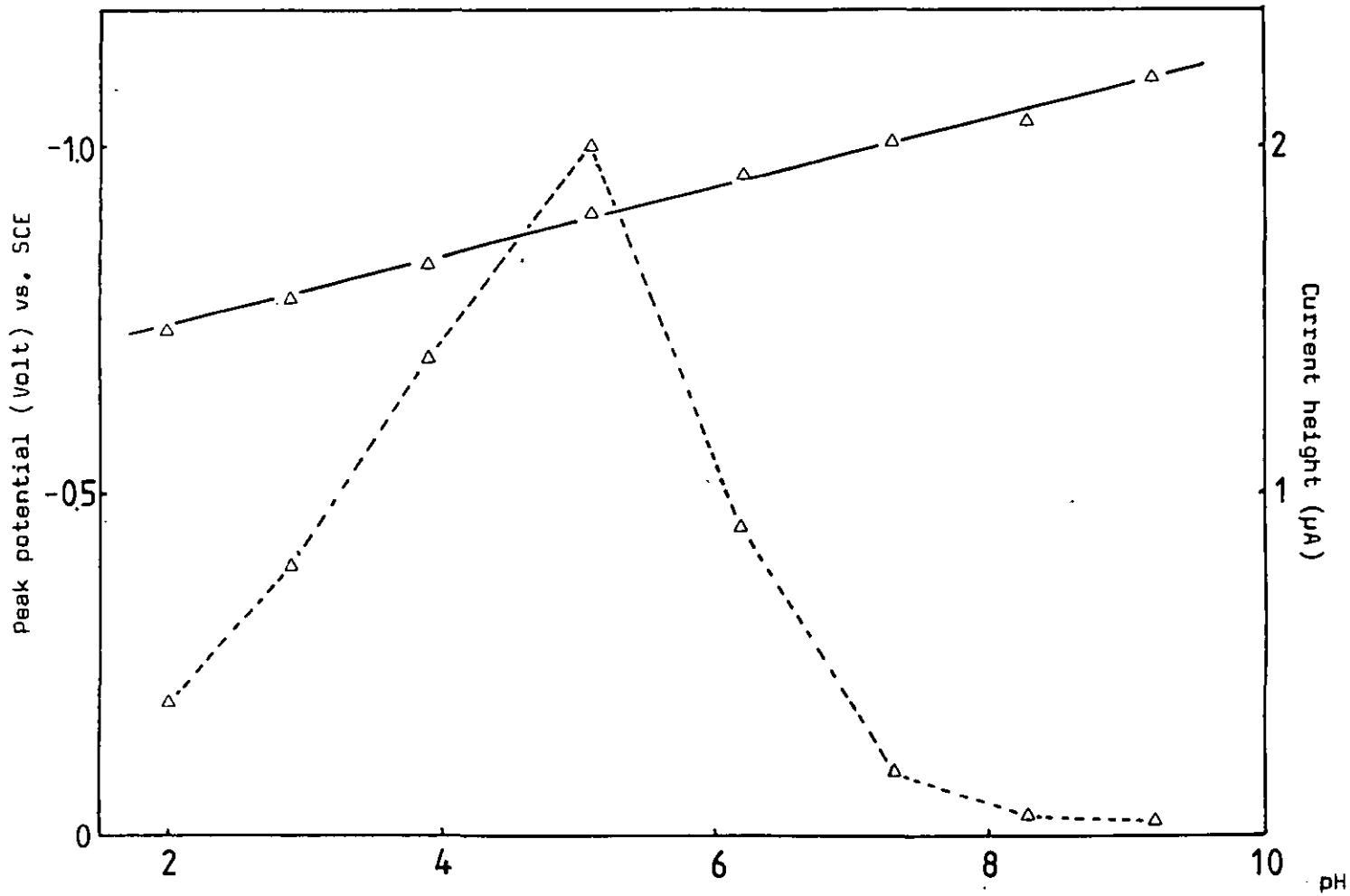


Fig. 42 Plot of the changes in the reduction potential and current height (dashed line) with pH for the aminomethylene derivative of cephalexin (XIII) after degradation at pH5.

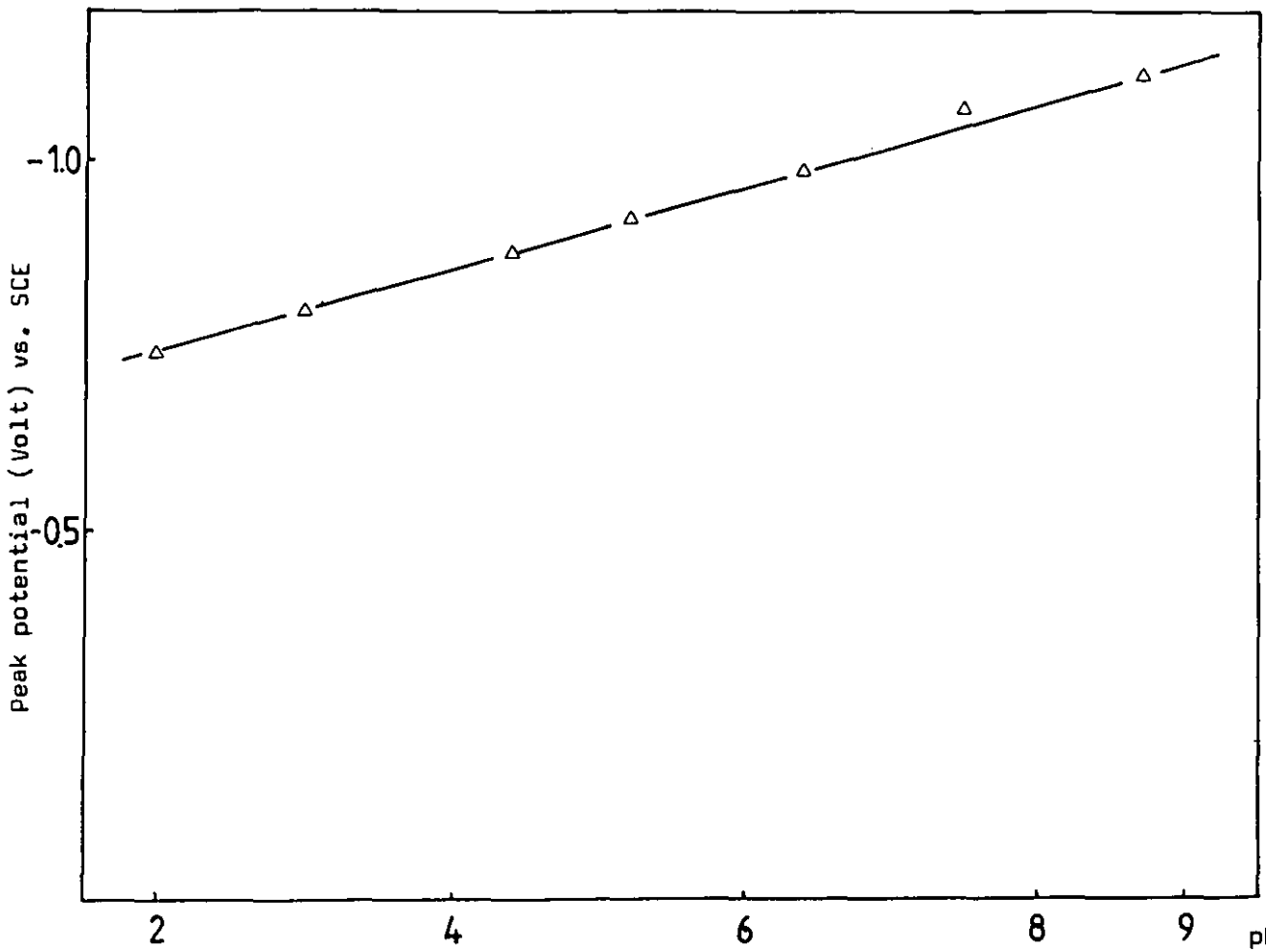


Fig. 43 Plot of the change in the reduction potential with pH for the -0.90V wave (pH5) produced from cephalexin after alkaline and acid hydrolysis.

18.

PRODUCTION OF PYRAZINES BY ACID HYDROLYSIS.

Squella et al.[86] have found a pyrazine derivative being formed by subjecting ampicillin to HCl (0.3 M) (1% v/v HCHO) at 80°C and running a DC scan.

Ampicillin (2.33×10^{-4} M) was hydrolysed at 80°C in HCl (0.3 M) (1% v/v HCHO) for 70 minutes after which time the solution was cooled and polarographed at 25°C.

A large wave (9.1 μA) is seen at -0.53 V which corresponds to the pyrazine derivative found by Squella et al.[86]. A smaller wave (1.0 μA) was seen at -0.72 V. The pyrazine signal in HCl (0.3 M) (9.1 μA) is equivalent when polarographed at pH 5 to 11.4 μA.

Hydrolysis of ampicillin (2.33×10^{-4} M) (as above) using pure HCl (0.3 M) gave a pyrazine signal at -0.53 V of 4.8 μA (equivalent to 6 μA at pH 5).

Cephalexin (5.59×10^{-4} M) and cephaloglycin (5.78×10^{-4} M) were treated as ampicillin (above) in HCl (0.3 M) with and without 1% v/v HCHO. The amount of pyrazine produced from each experiment was calculated as μA/mole (see Tables 67 and 68).

Table 67. Polarography in HCl (0.3 M).

Compound	Current height obtained for pyrazine peak (-0.53 V) ($\times 10^4$ μA/mole)	
	with HCHO (1%)	without HCHO
Ampicillin	3.91	2.06
Cephalexin	0.04	0.07
Cephaloglycin	2.76	2.12

Table 68. Equivalent signal at pH 5.

Compound	Current height for pyrazine ($\times 10^4$ μA/mole)	
	with HCHO (1%)	without HCHO
Ampicillin	4.89	2.58
Cephalexin	0.05	0.09
Cephaloglycin	3.45	2.66

Under this reaction regime, ampicillin, as in the combined alkaline/acid hydrolysis steps, produces the greatest level of pyrazine. The yield from cephalexin is very low, with the addition of HCHO having a detrimental effect.

The pyrazine compound produced from hydrolysis with HCl (0.3 M) (1% HCHO) gave an $E_{\frac{1}{2}}$ equation of $E_{\frac{1}{2}} = (-0.51 - 0.068\ pH)$ V, which is experimentally identical to that quoted previously for pyrazines produced from α-aminobenzyl-β-lactams.

19.

Hydrolysis of α-aminobenzyl-β-lactams in HCl (5 M).

Squella et al.[89] have reported the formation of a pyrazine compound from α-aminobenzyl-β-lactams in strong acid solutions (HCl, 5 M) at 80°C using 1% HCHO in the solution by DC polarography. A number of cephalosporins and ampicillin were treated as above.

Cephalexin (1.35×10^{-4} M) was heated at 80°C in HCl (5 M) (i) with 1% HCHO and (ii) without 1% HCHO. Periodically this solution was sampled and polarographed at 25°C (see Table 69).

Table 69.

Time/hrs	Peak height/μA			
	using 1% HCHO		with no HCHO	
	Peak at -0.42 V	Peak at -0.64 V	Peak at -0.42 V	Peak at -0.64 V
0	0.0	0.0	0.0	0.0
1	4.9	0.3	4.0	1.8
2	4.9	0.5	4.8	2.2
3	4.9	0.6	4.9	2.5
4	4.9	0.8	4.9	2.5

The peak at -0.42 V is due to pyrazine, that at -0.64 is unknown. The maximum pyrazine value (4.9 μA) is reached in both cases. The study with formaldehyde reaches the maximum value after only 1 hour's hydrolysis. This value is equivalent, at pH 5, to 5.11×10^4 μA/mole. This value is much greater than the value obtained from cephalexin in 0.3 M HCl (1% HCHO) (Table 68) which is only 0.05×10^4 μA/mole.

A pH plot on the pyrazine derivative of cephalexin, produced as above, using HCHO 1% gave an $E\frac{1}{2}$ equation of $E\frac{1}{2}$ = (-0.50 - 0.067 pH) V, which agrees well with the behaviour of other pyrazines.

Cefaclor (2.72×10^{-4} M) was degraded as cephalexin (above) in HCl (5 M) with and without HCHO 1% at 80°C (see Table 70).

Table 70.

Time/hrs	Peak height/μA	
	Peak at -0.42 V	
	using HCHO 1%	with no HCHO
0.0	0.80	0.80
0.25	23.20	5.50
0.50	23.00	6.20
1.00	22.70	6.10
1.50	22.50	5.70
2.00	23.00	5.80
3.00	21.50	5.70
4.00	20.50	5.75
6.00	20.50	5.80

The peak at -0.42 V (pyrazine) is the only wave observed. Unlike the cephalexin study the solution with HCHO gives a much greater maximum pyrazine value than that without HCHO. Both maximum values are attained within 0.5 h.

Cephaloglycin (2.47×10^{-4} M) was degraded at 80°C for 1 h in HCl (5 M) with HCHO (1% v/v) and without HCHO. After this time period the solutions were polarographed at 25°C.

The pyrazine peak at -0.42 V was the only peak observed. The formaldehyde case gave a value of 7.8 μA, whilst the study without the aldehyde gave a reading of 1.6 μA.

Ampicillin (4.26×10^{-4} M) was similarly treated as cephaloglycin (above).

Again the pyrazine peak at -0.42 V was the only peak recorded. With 1% HCHO a current height of 4.1 μA was observed and with no HCHO a current height of 1.05 μA was obtained.

The DKP derivative of cephalexin (VIII) (2.11×10^{-4} M) was degraded as cephalexin (above).

In both cases no pyrazine was observed and the only wave visible was a small peak (0.6 μA) at -0.38 V in the formaldehyde study.

The AMDC (8.42×10^{-4} M) was degraded as cephaloglycin (above).

An ill-defined wave at -0.42 V with a peak height of 2.1 μA was observed in the formaldehyde study. A dirty brown precipitate was found in the flask. A sharp peak at -0.42 V was found in the study without formaldehyde.

Table 71 compares the production of pyrazine (as μA/mole) from the above compounds converted to the pH 5 value.

Table 71.

Compound	Current height for pyrazine ($\times 10^4$ μA/mole)	
	with HCHO 1%	without HCHO
Cephalexin	5.11	5.11
Cefaclor	11.95	3.20
Cephaloglycin	4.41	0.91
Ampicillin	1.35	0.35
DKP (cephalexin)	-	-
AMDC	0.35	0.48

20.

The effect of the absence of dissolved oxygen on the production of the pyrazine compound at -0.42 V in HCl (5 M) at 80°C was investigated for cephaloglycin.

A solution of cephaloglycin (2.47×10^{-4} M) in HCl (5 M) (600 ml) was prepared.

(i) 100 ml was degraded at 80°C for 1 hour.

(ii) 100 ml was deoxygenated then degraded as (i).

(iii) Formaldehyde (2 ml) was added to 200 ml. 100 ml of this was degraded as (i), the other 100 ml was deoxygenated and then degraded as (ii).

(iv) Acetaldehyde (2 ml) was added to the remaining 200 ml. 100 ml of this solution was degraded as (i), the other 100 ml was deoxygenated and then degraded as (i). At the end of the degradation period, 1 hour, the solutions were cooled and polarographed (see Tables 72 and 73).

Table 72. Comparison of peak heights of pyrazine obtained in HCl (5 M).

State of acid	Peak height/μA	
	oxygenated	deoxygenated
no aldehyde	1.6	1.95
1% HCHO	7.6	7.6
1% CH_3.CHO	5.5	5.4

Table 73. Representation of figures from Table 72 as μA/mole at pH 5.

State of acid	Peak current/(x 10^4 μA/mole)	
	oxygenated	deoxygenated
no aldehyde	0.91	1.09
1% HCHO	4.31	4.31
1% CH_3.CHO	3.12	3.12

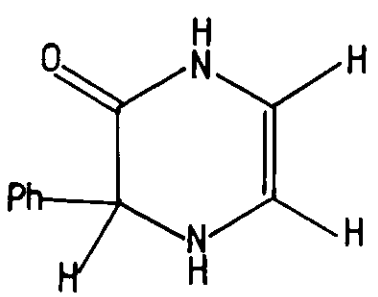
Comparing this 5 M study with the HCl (0.3 M) study (Tables 68 and 71) it can be seen that cephalexin increases its yield of pyrazine dramatically. With cephaloglycin the increase in the acid concentration in the formaldehyde case increases the yield of pyrazine but results in a smaller amount of pyrazine with no formaldehyde. With ampicillin increasing the acid concentration results in an overall decrease in the yield of pyrazine.

Cefaclor gave the highest production of pyrazine with HCHO present. The DKP derivative of cephalexin gave no pyrazine although the aminomethylene derivative of cephalexin gave small amounts of pyrazine. The absence of dissolved oxygen in the degradation of cephaloglycin did not affect the yield of pyrazine when aldehydes were included but doubled the pyrazine yield when pure acid was used.

21.

Degradation of α-aminobenzyl-β-lactams in sulphuric acid (12.5%) at 100°C.

Bontchev et al.[184] have reported that strong acid hydrolysis at 100°C (H_2SO_4, 12.5%) of cephalexin produces an aminoaldehyde (VI) which, it is suggested, cyclizes to a structure such as the compound (XV).



XV

This compound may undergo oxidation to pyrazine derivatives. A number of cephalosporins and ampicillin were examined under the above hydrolysis conditions.

Cephalexin (5.47×10^{-4} M) was degraded at 100°C in sulphuric acid (12.5%) with (i) HCHO 1% v/v added and (ii) without HCHO. Periodically samples were removed, cooled and polarographed at 25°C (see Tables 74 and 75 and fig. 44).

Cephaloglycin (3.01×10^{-4} M), cefaclor (2.83×10^{-4} M) and ampicillin (2.65×10^{-4} M) were treated as cephalexin above (see Tables 76 to 81).

Table 74. Hydrolysis of cephalexin (12.5%, H_2SO_4) at 100°C.

Time/hrs	Peak height/μA		
	Peak at -0.29 V	Peak at -0.47 V	Peak at -0.70 V
0	0.0	0.0	0.0
0.25	0.0	3.4	2.9
0.50	0.2	9.2	5.8
0.75	0.6	12.0	7.0
1.00	1.2	15.8	7.6
1.50	1.5	20.0	8.2
2.00	1.5	21.2	8.2
3.00	1.5	22.9	8.2
4.50	1.75	23.5	8.2
6.00	2.0	23.5	8.2

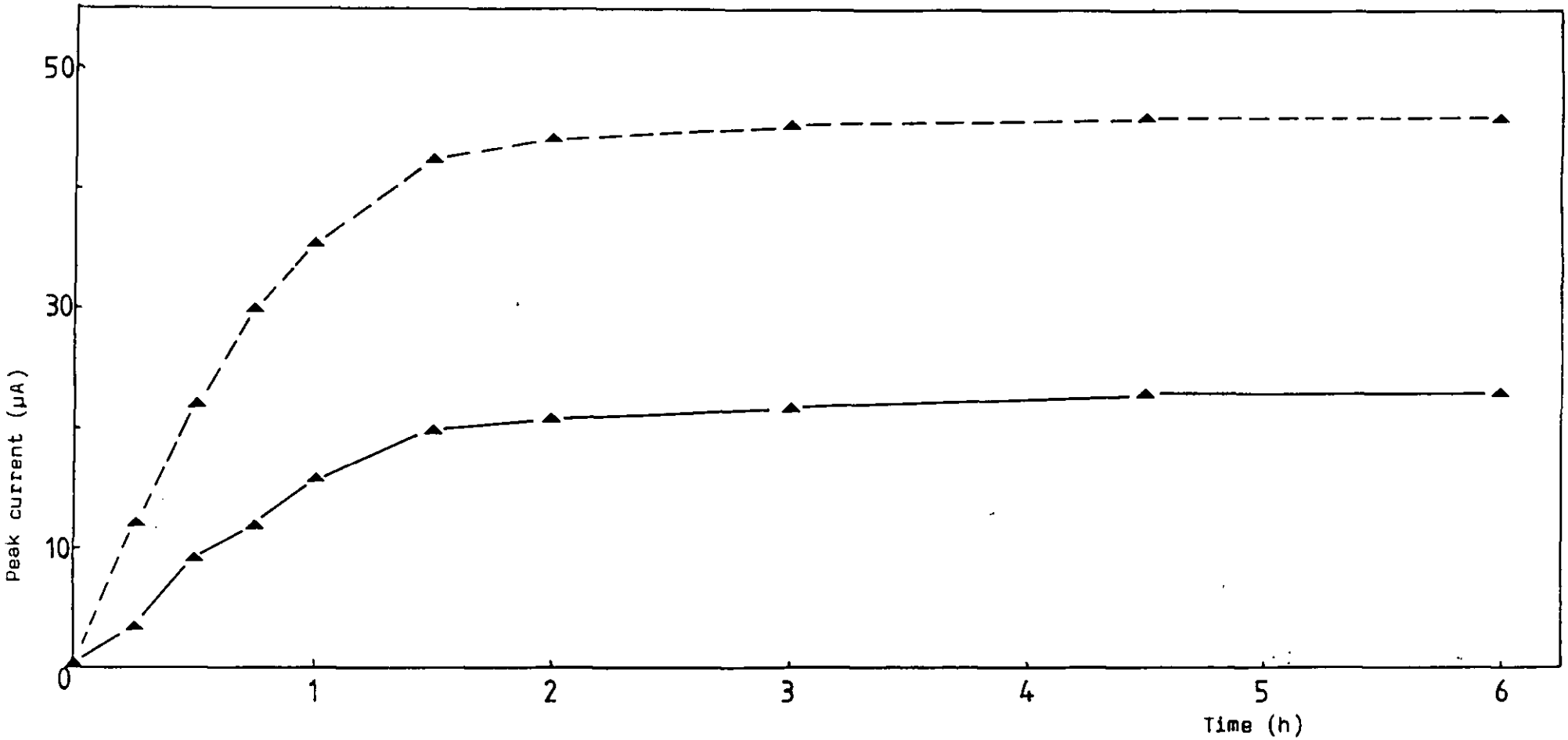


Fig. 44 Pyrazine derivative formation from cephalexin after treatment in H_2SO_4 (12.5%), 100°C, polarography in H_2SO_4 (12.5%). Peak at -0.47V.

——— peak current produced using pure H_2SO_4

– – – peak current produced with 1% formaldehyde in the sulphuric acid.

Table 75. Hydrolysis of cephalexin (12.5%, H_2SO_4, 1% v/v HCHO) at 100°C.

Time/hrs	Peak height/μA		
	Peak at -0.29 V	Peak at -0.47 V	Peak at -0.70 V
0	0.0	0.0	0.0
0.25	0.0	12.2	0.4
0.50	0.5	22.25	1.75
0.75	1.0	30.00	3.00
1.00	1.25	35.5	3.50
1.50	1.75	42.5	4.50
2.00	2.00	44.25	5.00
3.00	2.75	45.5	5.50
4.50	3.00	46.0	5.50
6.00	3.00	46.0	5.50

Table 76. Hydrolysis of cephaloglycin (12.5%, H_2SO_4) at 100°C.

Time/hrs	Peak height/μA			
	Peak at -0.08 V	Peak at -0.29 V	Peak at -0.47 V	Peak at -0.70 V
0.0	0.0	0.0	0.0	0.0
0.25	7.20	0.50	7.60	3.20
0.50	6.75	0.75	12.25	4.25
0.75	5.75	1.00	14.50	4.50
1.00	4.25	1.00	12.75	4.25
1.50	3.00	1.25	12.25	4.25
2.00	2.00	1.50	12.00	4.25
3.00	1.00	1.50	11.75	3.75
6.00	0.50	2.75	11.50	3.75

Table 77. Hydrolysis of cephaloglycin (12.5%, H_2SO_4, 1% v/v HCHO) at 100°C.

Time/hrs	Peak height/μA		
	Peak at -0.29 V	Peak at -0.47 V	Peak at -0.70 V
0.0	0.0	0.0	0.0
0.25	0.0	23.25	2.00
0.50	0.25	29.50	3.00
0.75	0.35	33.75	3.00
1.00	0.55	29.00	3.50
1.50	0.75	29.50	3.50
2.00	1.00	30.50	3.75
3.00	1.25	29.00	3.75
6.00	2.00	28.50	6.00

Table 78. Hydrolysis of cefaclor (12.5%, H_2SO_4) at 100°C.

Time/hrs	Peak height/μA			
	Peak at -0.17 V	Peak at -0.29 V	Peak at -0.47 V	Peak at -0.70 V
0.0	0.0	0.0	0.0	0.0
0.25	0.0	1.50	4.00	2.00
0.50	0.75	2.00	6.00	2.50
0.75	1.00	2.50	8.75	3.25
1.00	1.50	2.50	10.00	3.50
1.50	3.25	2.75	10.75	3.50
2.00	4.25	3.00	11.00	4.00
3.00	4.50	3.50	10.75	3.50
6.00	4.25	3.50	10.25	3.00

Table 79. Hydrolysis of cefaclor (12.5%, H_2SO_4, 1% v/v HCHO) at 100°C.

Time/hrs	Peak height/μA			
	Peak at -0.17 V	Peak at -0.29 V	Peak at -0.47 V	Peak at -0.70 V
0.0	0.0	0.0	0.0	0.0
0.25	0.50	0.25	9.00	0.0
0.50	0.50	0.50	17.50	1.25
0.75	1.25	0.50	21.75	1.75
1.00	1.50	0.75	23.00	2.25
1.50	1.75	0.75	24.50	2.50
2.00	2.00	0.75	24.75	2.75
3.00	2.25	1.00	24.25	3.00
6.00	2.00	1.50	23.50	3.75

Table 80. Hydrolysis of ampicillin (12.5%, H_2SO_4) at 100°C.

Time/hrs	Peak height/μA		
	Peak at -0.29 V	Peak at -0.47 V	Peak at -0.70 V
0.0	0.0	0.0	0.0
0.25	0.8	0.8	0.4
0.50	0.9	2.0	1.0
0.75	1.0	3.4	1.8
1.00	1.3	5.2	2.5
1.50	1.6	7.4	3.3
2.00	1.8	9.0	3.8
3.00	2.1	11.4	4.6
6.00	3.2	13.6	5.3

Table 81. Hydrolysis of ampicillin (12.5%, H_2SO_4, 1% v/v HCHO) at 100°C.

Time/hrs	Peak height/μA		
	Peak at -0.29 V	Peak at -0.47 V	Peak at -0.70 V
0.0	0.0	0.0	0.0
0.25	0.0	9.5	1.25
0.50	0.0	17.5	2.5
0.75	0.0	22.0	3.25
1.00	0.25	24.5	3.5
1.50	0.50	27.0	4.5
2.00	0.50	27.25	4.5
3.00	1.00	28.0	4.0
6.00	1.75	33.0	4.0

A Table of the maximum molar production of pyrazine, as the equivalent height of the -0.47 V wave at pH 5 is shown below (Table 82).

Table 82.

Compound	Peak current ($\times 10^4$ μA/mole)	
	with 1% HCHO	without HCHO
Cephalexin	7.66	3.90
Cephaloglycin	10.20	4.39
Cefaclor	8.75	3.89
Ampicillin	10.30	4.67

Heating α-aminobenzyl-β-lactams at 100°C in sulphuric acid (12.5%) produces pyrazine derivatives as can be seen by the reduction potential at -0.47 V. The addition of formaldehyde to the degrading solution approximately doubles the yield of pyrazine produced (see Table 82). All four compounds gave about the same yield of pyrazine under the two different sets of conditions. The rate of pyrazine formation was greater when formaldehyde was included in the acid although stable levels were not

reached until 1.5 to 2 hours of hydrolysis. The other waves in the study (-0.08 V, -0.17 V, -0.29 V and -0.70 V) are unknown and they are not affected as the pyrazine peak by the addition of formaldehyde.

A wave for cephaloglycin at -0.94 V and a wave for cefaclor at -0.89 V were observed in the early stages of the hydrolysis. In the case of cephaloglycin the -0.94 V wave was seen only in the study using pure sulphuric acid. After 30 minutes heating this wave had completely disappeared. This wave is thought to be due to the reductive elimination of the acetyl group of the C-3 substituent of cephaloglycin. If so, then this suggests cephaloglycin degrades to its lactone at a greater rate in the presence of formaldehyde. The wave observed from cefaclor (-0.89 V) may be due to the elimination of chlorine at the C-3 position. This wave was absent in the study using formaldehyde and when the aldehyde was absent it was present until 1 hour.

The DKP derivative of cephalexin previously gave no pyrazine in HCl (5 M) (see Table 71). This compound was subjected to sulphuric acid (12.5%) hydrolysis with and without formaldehyde.

The DKP (2.94×10^{-4} M) was degraded at 100°C for 1.5 hours in H_2SO_4 (12.5%) with (i) HCHO 1% v/v and (ii) without HCHO in the acid. At the end of this time period the solution was cooled and polarographed at 25°C.

In the formaldehyde study two waves were observed, at -0.29 V (2 μA) and at -0.47 V (1.5 μA). The study with pure buffer gave the same waves, the -0.29 V with a height of 2 μA and the pyrazine at -0.47 V with a peak height of 1 μA. As a measure of molar production (polarography at pH 5) these values are equivalent to

(i) using HCHO = 0.510×10^4 μA/mole

(ii) using no HCHO = 0.340×10^4 μA/mole.

The time of 1.5 h was chosen for the hydrolysis because, previously, most of the α-aminobenzyl-β-lactams gave their i_p maxima at this heating time. Unlike the HCl (5 M) study the DKP compound did produce pyrazines,

with or without 1% v/v HCHO. The yield of the -0.29 $V_{\wedge}^{WAVE}$ was comparable to that produced by the α-aminobenzyl-β-lactams under these conditions (see Tables 74 to 81).

22.

Degradation of α-aminobenzyl-β-lactams in sulphuric acid at 25°C.

Cephalexin (2.45×10^{-4} M) was degraded in H_2SO_4 (12.5%) at 25°C. Periodically the degrading solution was sampled and polarographed.

This study was carried out for 170 hours. The only wave observed was that at -0.60 V which had reached a steady height of 6.4 μA after 30 minutes. The wave declined slowly after this and was at a level of 4.2 μA after 170 hours. A very small wave at -0.47 V, corresponding to pyrazine was observed in the late stages of the hydrolysis. After 170 hours this wave had reached 0.25 μA.

Cephaloglycin (3.01×10^{-4} M) was degraded and polarographed as cephalexin (above).

On polarographing the made up solution two waves were recorded, one at -0.60 V and one at -0.94 V. The -0.60 V wave reached a steady level of 6.0 μA after 1 hour, this was maintained throughout the study which lasted 192 hours. The wave at -0.94 V corresponds to the reductive elimination of the C-3 acetyl group of cephaloglycin. Initially this wave was 6.7 μA in height. After 1.5 hours it had completely disappeared. Therefore all intact cephaloglycin had degraded by this time. As in the cephalexin study a very small peak at -0.47 V corresponding to pyrazine was recorded in the latter stages of degradation. This wave was 0.5 μA in height after 192 hours.

Ampicillin (3.40×10^{-4} M) was degraded and polarographed as cephalexin (above).

A wave at -0.60 V was observed and remained steady at 6.1 μA throughout the study which was carried out for 168 hours. No pyrazine peak was observed.

Elevated temperatures, as in the 100°C study, are required for large yields of pyrazine derivatives. The wave at -0.60 V is unknown.

The production of hydrogen sulphide from the sulphuric acid degradation of cephalexin was investigated by the normal pulse polarographic method.

23. Cephalexin (4.46×10^{-4} M) was degraded at 100°C in H_2SO_4 (12.5%) for 1 hour. Nitrogen purge gas was continuously passed through the solution and any hydrogen sulphide being produced was trapped as S^{2-} ion in NaOH (0.5 M) (15 ml). This trapping solution was polarographed in the normal pulse mode.

No hydrogen sulphide was detected by the NP method. Bontchev et al.[184] suggest that when cephalexin is hydrolysed as above the thiazine ring remains intact and forms a thiazine (XVI). Non-formation of H_2S may suggest that all of the cephalexin does form the compound, therefore locking the sulphur atom into the structure.

24.
ACID HYDROLYSIS OF A NUMBER OF PENICILLINS AND CEPHALOSPORINS AT pH 2 AND 25°C.

Penicillin G (2.63×10^{-4} M) was degraded in Britton-Robinson buffer (pH 2) at 25°C. Periodically samples were removed and polarographed at pH 2, pH 5 and pH 12.

No polarographic activity was recorded over the length of the study (70 hours).

Penicillin V (3.19×10^{-4} M) was degraded and polarographed as penicillin G (above).

During this study (carried out for 48 hours) no polarographic peaks were observed.

Benzylpenicilloic acid (2.52×10^{-4} M) was degraded and polarographed as penicillin G (above).

With pH 2 polarography no waves were recorded until 26 hours. A small wave (0.5 μA) at -0.07 V which remained stable until the end of the study (48 hours) was the only peak observed.

With pH 5 polarography an initial wave at -0.1 V declined quickly and disappeared after 3 hours. No other waves were recorded.

With polarography at pH 12 no polarographic peaks were observed.

Benzylpenillic acid (3.23×10^{-4} M) was degraded and polarographed as penicillin G (above).

At pH 2 a wave at -0.18 V (1 μA) was observed and this did not decline throughout the period of study (50 hours). At pH 12 there was little polarographic activity until 26 hours when a wave at -0.30 V (0.75 μA) was recorded. This was the same height at 50 hours. At pH 5 no polarographic

behaviour was observed.

Ampicillin (2.43×10^{-4} M) was degraded and polarographed as penicillin G (above).

With polarography at pH 2 no peaks were seen until 27 hours when small waves at -0.08 V -0.18 V and -0.58 V were observed. After 50 hours only a small increase in the waves at -0.58 V and -0.08 V was recorded. At pH 5 a small wave (0.5 µA) at -0.83 V (pyrazine) was observed towards the end of the study. At pH 12 no peaks were recorded.

Cephalexin (3.11×10^{-4} M) was degraded and polarographed as penicillin G (above).

At pH 2, at the beginning of the study, a wave of 1.5 µA was observed at -0.06 V. This wave increased throughout the study (48 hours in length) to 3 µA. At pH 5 a wave at -0.11 V which stayed at 3.5 µA was seen. A small wave (0.2 µA) at -0.81 V which may correspond to a pyrazine compound was observed after 48 hours. At pH 12,2 waves at -0.31 V (2 µA) and at -0.56 V (3.1 µA) were recorded. The peak at -0.56 V declined slowly.

Cephaloridine (2.45×10^{-4} M) was degraded and polarographed as penicillin G (above).

At all pH values little polarographic activity was observed.

25. Previously, it has been reported that penicillamine is a polarographically active degradation product from ampicillin at pH 2.5. Penicillamine is also found as a degradation product from other penicillins. The utilization of penicillamine as an indicator of penicillin quality was investigated.

Penicillamine (6.54×10^{-4} M) was degraded at 100°C in the absence of oxygen in citrate-phosphate buffer pH 2.5. Periodically, samples were removed and polarographed at 25°C.

Two waves were recorded, both were due to penicillamine although the first wave at -0.08 V is believed to be of non-faradaic origin, occurring only at high concentrations of penicillamine, therefore the change in the

height of the second wave, at -0.13 V was recorded to illustrate the effect of the above conditions on penicillamine (see Table 83).

Table 83. Effect of heating (at 100°C) on penicillamine at pH 2.5 in the absence of dissolved oxygen.

Time/hrs	Peak height/μA
	Peak at -0.13 V
0.0	13.9
0.25	12.5
0.50	11.9
0.75	11.5
1.00	11.25
1.50	11.0
2.00	9.8
3.00	8.3
6.00	5.5

Penicillamine is not stable at 100°C and degrades slowly in citrate-phosphate buffer pH 2.5.

Some penicillins were degraded at 100°C in citrate-phosphate buffer at pH 2.5 to observe the production of penicillamine.

Ampicillin (2.47×10^{-4} M) was degraded in citrate-phosphate buffer pH 2.5, with and without the absence of dissolved oxygen at 100°C. Periodically, samples were removed and polarographed at 25°C.

(i) In the presence of dissolved oxygen three major peaks were recorded (see Table 84). The peak at -0.13 V corresponds to penicillamine, that at -0.63 V is due to the pyrazine derivative and an unknown peak at -0.78 V, which corresponds to the -0.92 V wave (pH 5) formed in the alkaline/acid hydrolysis of α-aminobenzyl-β-lactams to produce pyrazines and in the degradation of the aminomethylene derivative of cephalexin (XIII). The penicillamine peak is only relatively stable from ampicillin degradation between 1 h and 2 h. Thereafter it degrades steadily until after 6 h it has completely gone. The pyrazine compound forms steadily under these

conditions and has not yet reached a plateau after 6 hours.

Table 84. Degradation of ampicillin in the presence of dissolved oxygen.

Time/hrs	Peak height/μA		
	Peak at -0.13 V	Peak at -0.63 V	Peak at -0.78 V
0.0	0.0	0.15	0.0
0.25	0.7	1.0	0.35
0.50	2.1	4.55	1.65
0.75	3.4	7.6	2.7
1.00	4.0	10.5	3.8
1.50	4.2	15.4	5.4
2.00	4.6	17.0	6.0
3.00	3.0	17.9	6.0
6.00	0.7	18.7	6.0

(ii) In the absence of dissolved oxygen the yield of penicillamine was increased (see Table 85), although the stability of penicillamine is not increased under these conditions. The rate of production of the pyrazine wave, at -0.63 V, is increased compared with the study with dissolved oxygen although the maximum peak height reached is in the same range (17-18 μA) for both cases.

Table 85. Degradation of ampicillin in the absence of dissolved oxygen.

Time/hrs	Peak height/μA		
	Peak at -0.13 V	Peak at -0.63 V	Peak at -0.78 V
0.0	0.0	0.1	0.0
0.25	3.3	6.3	2.7
0.50	8.0	10.4	5.0
0.75	10.25	11.5	5.4
1.00	12.05	13.4	5.4
1.50	10.35	17.0	5.4
2.00	8.35	17.2	5.6
3.00	6.1	15.6	5.4
6.00	6.1	13.8	5.4

Penicillin G (2.68×10^{-4} M) was degraded and polarographed as ampicillin (above), in the absence of dissolved oxygen.

The only wave recorded was that due to penicillamine, at -0.13 V (see Table 86). The production of penicillamine from penicillin G is swift but does not reach the same level as that formed from ampicillin. Penicillamine reaches a steady level after 2 hours' degradation.

Table 86. Degradation of penicillin G in the absence of dissolved oxygen.

Time/hrs	Peak height/μA
	Peak at -0.13 V
0.0	0.0
0.25	3.7
0.50	3.5
0.75	3.8
1.00	3.5
1.50	3.5
2.00	3.1
3.00	3.1
6.00	3.1

Penicillin V (2.95×10^{-4} M) was degraded as ampicillin (above) in the absence of dissolved oxygen.

As was the case with penicillin G the only wave recorded in this study was that at -0.13 V (penicillamine) (see Table 87). Penicillin V produces similar amounts of penicillamine as the structurally related penicillin G.

A comparison of the maximum molar production of penicillin from the three penicillins studied is given in Table 88.

Table 87. Degradation of penicillin V in the absence of dissolved oxygen.

Time/hrs	Peak height/μA
	Peak at -0.13 V
0.0	0.0
0.25	3.8
0.50	4.5
0.75	4.5
1.00	4.5
1.50	4.8
2.00	4.0
3.00	4.0
6.00	3.5

Table 88.

Compound	Peak current ($\times 10^4$ μA/mole)	
	with dissolved oxygen	without dissolved oxygen
Ampicillin	1.84	4.88
Penicillin G	not known	1.42
Penicillin V	not known	1.63

The α-aminobenzylpenicillin gives the largest amount of penicillamine, over three times that from penicillin G and penicillin V, whose values in the studies without dissolved oxygen are less than that of ampicillin with dissolved oxygen.

A plot of pH against measured potential was made for penicillamine.

Penicillamine (6.75×10^{-4} M) was dissolved in citrate-phosphate buffer (pH 2.5). pH adjustments were made with NaOH (0.2 M) and HCl (0.2 M). Two waves were recorded. The change in reduction potential (V) and peak current (μA) for each wave is recorded in Table 89 (see fig. 45).

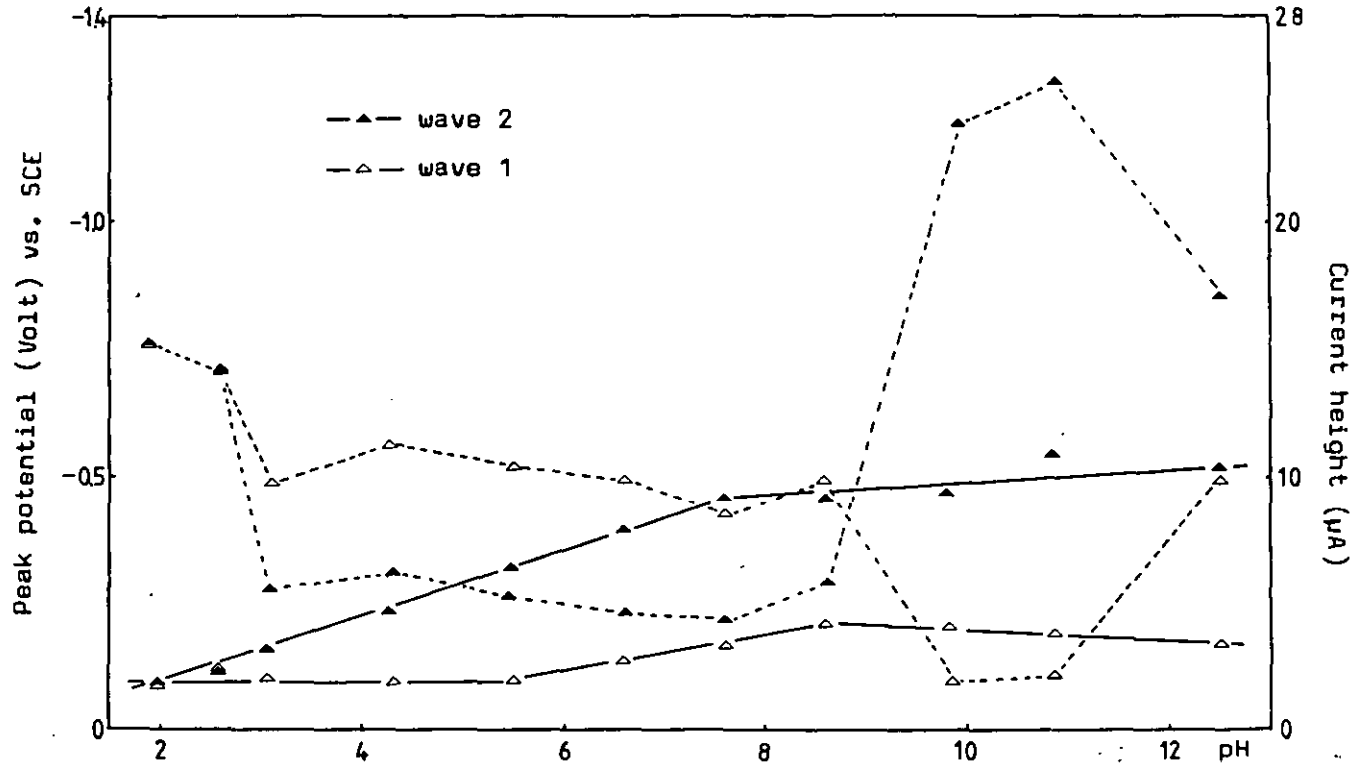


Fig. 45 Plot of the changes in the reduction potential and current height (dashed line) for penicillamine.

Table 89.

pH	wave 1		wave 2	
	V (-)	μA	V (-)	μA
1.9	0.10	15.2	0.10	15.2
2.6	0.13	14.0	0.13	14.0
3.1	0.11	9.7	0.17	5.5
4.3	0.10	11.3	0.24	6.1
5.5	0.10	10.4	0.34	5.2
6.6	0.14	9.7	0.43	4.6
7.6	0.17	8.5	0.46	4.3
8.6	0.21	9.7	0.46	5.8
9.8	0.20	1.8	0.47	23.7
10.85	0.19	2.1	0.54	25.5
12.5	0.17	10.1	0.52	17.0

Wave 2 has a linear slope in the pH range 2 - 7. The $E_{\frac{1}{2}}$ equation of this slope is

$$E_{\frac{1}{2}} = (+0.03 - 0.058\ pH)\ V.$$

This wave has two regions where the wave height is large, (a) pH 1.5 - 2.5 and (b) pH 10 - 11.5.

Ampicillin (3.32×10^{-4} M), penicillin G (4.62×10^{-4} M) and penicillin V (5.94×10^{-4} M) were degraded in citrate-phosphate buffer (pH 2.5) at 100°C in the absence of dissolved oxygen. pH plots were made of the suspected penicillamine waves produced from each compound (see Table 90).

Table 90. Comparison of $E_{\frac{1}{2}}$ equations from the acidic hydrolysis (pH 2.5) of some penicillins at 100°C

Compound	($E_{\frac{1}{2}}$ equation).V	linear range (pH)
Ampicillin	(-0.05 - 0.055 pH)	2.5 - 10
Penicillin G	(-0.02 - 0.058 pH)	2 - 8
Penicillin V	(-0.01 - 0.057 pH)	2.5 - 9.5

The $E_{\frac{1}{2}}$ equations compare well with that obtained from pure penicillamine

$$E_{\frac{1}{2}} = +0.03 - 0.058 \text{ pH},$$

showing that these waves are due to penicillamine.

Benzylpenicilloic acid (4.37×10^{-4} M) was dissolved in citrate-phosphate buffer (pH 2.5) and the $E_{\frac{1}{2}}$ equation was determined by a pH plot.

A linear slope was recorded between pH 3.5 and pH 13, no polarographic waves were recorded below pH 3.5.

$$E_{\frac{1}{2}} = +0.09 - 0.022 \text{ pH}.$$

Benzylpenillic acid (2.45×10^{-4} M), the acid rearrangement product of benzylpenicilloic acid, was subjected to a pH plot in citrate-phosphate buffer.

No polarographic waves were recorded between pH 2 and pH 12.

Penicillamine is confirmed as an acid degradation product of penicillin G, penicillin V and ampicillin at 100°C in the absence of dissolved oxygen. The penicillamine-containing solutions so produced from each degradation were compared by pH plots. The compounds examined had $E_{\frac{1}{2}}$ equations very similar to penicillamine. The penicilloic acid of these compounds is not seen to be formed under the same conditions.

26. Mild alkaline hydrolysis followed by mild acid hydrolysis at 100°C produces pyrazine derivatives from α-aminobenzyl-β-lactams. A study was carried out to see if stronger alkaline hydrolysis alone could produce pyrazines.

Cephalexin 100 ppm (2.88×10^{-4} M) was degraded at 100°C for 1 hour in NaOH (0.1 M). A pH plot was carried out on a wave that had a reduction potential of -0.85 V at pH 5, quite near that of pyrazine derivatives. During this pH plot a wave corresponding to a carbonyl compound (-1.26 V)

and independent of pH was observed (see Table 91).

Table 91.

pH	wave 1		wave 2	
	V (-)	µA	V (-)	µA
2.1	0.63	2.8	-	0.0
3.1	0.70	2.6	-	0.0
4.1	0.78	2.6	-	0.0
5.0	0.85	2.8	-	0.0
6.1	0.92	2.4	1.26	0.4
7.3	0.98	2.2	1.26	0.7
8.1	1.05	1.2	1.26	0.6
9.4	1.12	0.8	1.26	0.7
10.3	-	0.0	1.26	0.7

A graph was plotted of pH versus reduction potential for wave 1 (see fig. 46). A linear slope was observed in the pH range 2 - 9. The $E_{\frac{1}{2}}$ equation was

$$E_{\frac{1}{2}} = (-0.47 - 0.074\ pH).\ V.$$

Comparing this result with that from synthesised 2-hydroxy-3-phenyl-6-methylpyrazine $E_{\frac{1}{2}} = (-0.50 - 0.065\ pH).\ V$ shows that a pyrazine in small yield is produced from cephalexin under strong alkaline hydrolysis. No pyrazine is observed when stronger alkaline hydrolysis is used (NaOH (0.5 M) 100°C, 30 minutes). Any pyrazine formed may be degraded by these conditions.

The DKP derivative of cephalexin was found previously not to give pyrazine derivatives after combined mild alkaline/mild acid hydrolysis. This compound (2.9×10^{-4} M) was degraded as cephalexin (above) (NaOH, 0.1 M) for 30 minutes and 1 hour.

No wave corresponding to a pyrazine derivative was observed. A wave at -1.26 V (independent of pH) was recorded in the range pH 6 - 10. The DKP derivative of cephalexin gave a signal of 0.7 µA for this carbonyl

compound. The current height was independent of pH.

Pyrazine derivatives are not formed from the DKP (cephalexin) under alkaline conditions. Equal amounts of a carbonyl compound in low yield are formed from the alkaline hydrolysis of cephalexin and its DKP.

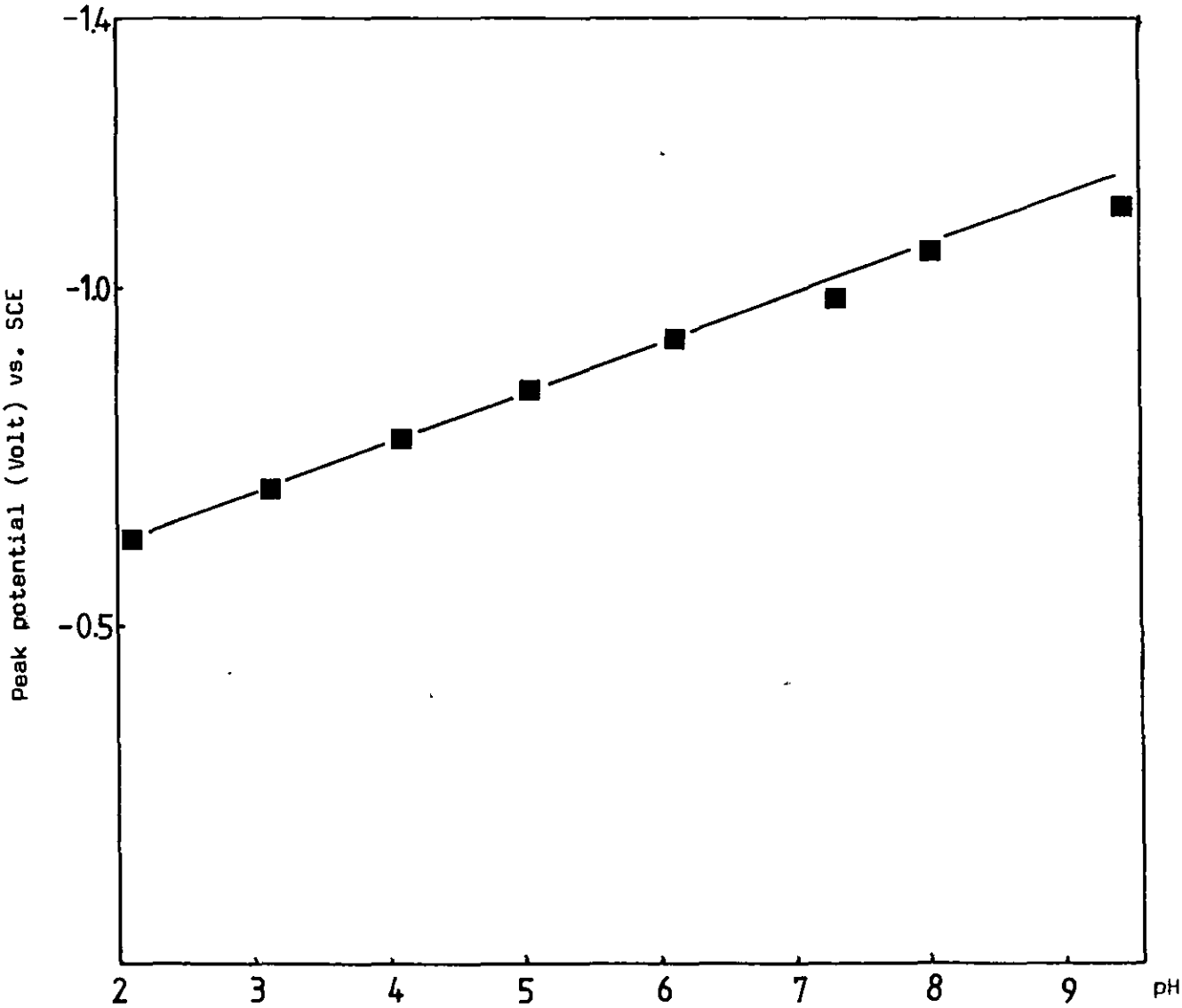


Fig. 46 Plot of the change in the reduction potential for a pyrazine derivative of cephalexin produced from alkaline hydrolysis, NaOH (0.1M), 100°C, for 1 hour.

C. DEGRADATION OF CEPHALEXIN TO FORM A THIOPHENONE AND A DIKETOPIPERAZINE ALDEHYDE[207].

1. Cephalexin (0.0422 g) was dissolved in 100 ml of distilled water. The pH of this solution (1.27×10^{-3} M) was adjusted to pH 3.3 with 0.2 M HCl. This unbuffered solution was degraded at 75°C for 90 minutes. At the end of this time period the solution was cooled and extracted with two 100 ml portions of chloroform (S.L.R.). This procedure separated the reaction mixture into a polar layer and a non-polar layer. The latter was evaporated at room temperature to give an orange-coloured amorphous solid which weighed 0.0094 g.

2. Examination of the reaction products

(i) From the non-polar layer.

The orange solid (0.0094 g) was dissolved in phosphate buffer (pH 7.4) (100 ml). On DPP a peak at -0.96 V, corresponding to a pyrazine derivative, was observed. A plot of E versus pH revealed an $E_{\frac{1}{2}}$ equation experimentally identical to previously isolated (VI) -6-methylpyrazine. ($E_{\frac{1}{2}} =$ (-0.48 - 0.068 pH) V). On heating this solution at 100°C and pH 7.4 and examining it after 1 hour and 4 hours, five peaks were recorded (Table 92).

Table 92. Changes of DP peak heights of non-polar solid degradation products with heating time at 100°C in phosphate buffer solution pH 7.4.

Time/hrs	Peak height/μA				
	Peak at -0.13 V	Peak at -0.37 V	Peak at -0.56 V	Peak at -0.96 V	Peak at -1.26 V
0.0	0.0	0.0	0.0	2.4	0.0
1.0	2.2	3.8	4.3	3.0	15.8
4.0	4.5	0.4	1.2	3.6	3.4

On carrying out a DC scan the peaks at -0.13 V and -0.37 V gave anodic waves. On the addition of the thiol inhibitor, para-hydroxy-mercuricbenzoate, the peaks at -0.13 V and -0.37 V disappeared. It is concluded that these two peaks are due to thiol-type compounds. For ease of identification the peak at -0.13 V will be called thiol II, whilst that at -0.37 V will be called thiol I. The peak at -0.56 V is due to hydrogen sulphide, the peak at -0.96 V is due to a pyrazine derivative and the peak at -1.26 V is due to a carbonyl.

(ii) Examination of the polar (aqueous) layer.

The aqueous portion was polarographed at pH 7.4, then heated at 100°C and sampled after 1 hour and 4 hours. Four peaks were recorded (Table 93).

Table 93. Changes of DP peak height of polar layer degradation products with heating time at 100°C in phosphate buffer solution pH 7.4.

Time/hrs	Peak height/μA			
	Peak at -0.37 V	Peak at -0.56 V	Peak at -0.96 V	Peak at -1.49 V
0	62.0	0.0	0.0	66.0
1	12.0	0.0	2.3	130.0
4	0.5	2.2	3.1	0.5

The thiophenone is formed from the unbuffered cephalexin solution at pH 3.3 and is extracted into the non-polar layer. On heating in phosphate buffer solution at pH 7.4 the thiophenone breaks up to form the carbonyl compound (-1.26 V) and hydrogen sulphide (H_2S). Thiols I and II are formed in the boiling solution. A pyrazine derivative is formed in the unbuffered cephalexin solution at 75°C and crosses into the non-polar layer. A precursor to pyrazine is partioned between the two phases as on heating they both increase their production of the pyrazine derivative. Thiol I is formed in relatively high yield from cephalexin at 75°C and

degrades on heating and is almost gone from the polar layer after heating for four hours.

3. Time study of the degradation of cephalexin at pH 3.3 and 75°C.

Cephalexin (0.05 g in 100 ml) (1.44×10^{-3} M) was dissolved in distilled water and the pH adjusted to pH 3.3 with 0.2 M hydrochloric acid. The solution was heated in a water bath at 75°C. Periodically samples were removed and polarographed at pH 7.4.

Four peaks were recorded (see fig. 47).

After 75 minutes (the time quoted by Dinner[207] for maximization of the thiophenone (XVII) and the diketopiperazine aldehyde (XII)), thiol I, the -1.49 V compound, H_2S and the pyrazine compounds are at their maximum levels. After this time period they all decrease to steady levels. There is no sign of thiol II (-0.13 V).

4. Degradation of the thiophenone to form the carbonyl at -1.26 V (pH 7.4).

The thiophenone (XVII) was isolated after degradation of cephalexin and extraction with chloroform as above[207]. Thiophenone (21 ppm) (0.0021 g/100 ml) was dissolved in phosphate buffer (pH 7.4). This solution was heated at 100°C for 3 hours, during which time samples were periodically removed, cooled and polarographed at pH 7.4 (see figs. 48 and 49). The carbonyl compound at -1.26 V is produced rapidly from the degradation of the thiophenone at pH 7.4.

5. Degradation of cephalexin at pH 3.3 to produce uncontaminated thiophenone[207].

Cephalexin (1.2 g) was dissolved in 100 ml of distilled water. The resultant pH was 3.3. This solution was heated at 75°C in a water bath for 30 minutes. At the end of this time period the cooled solution under-

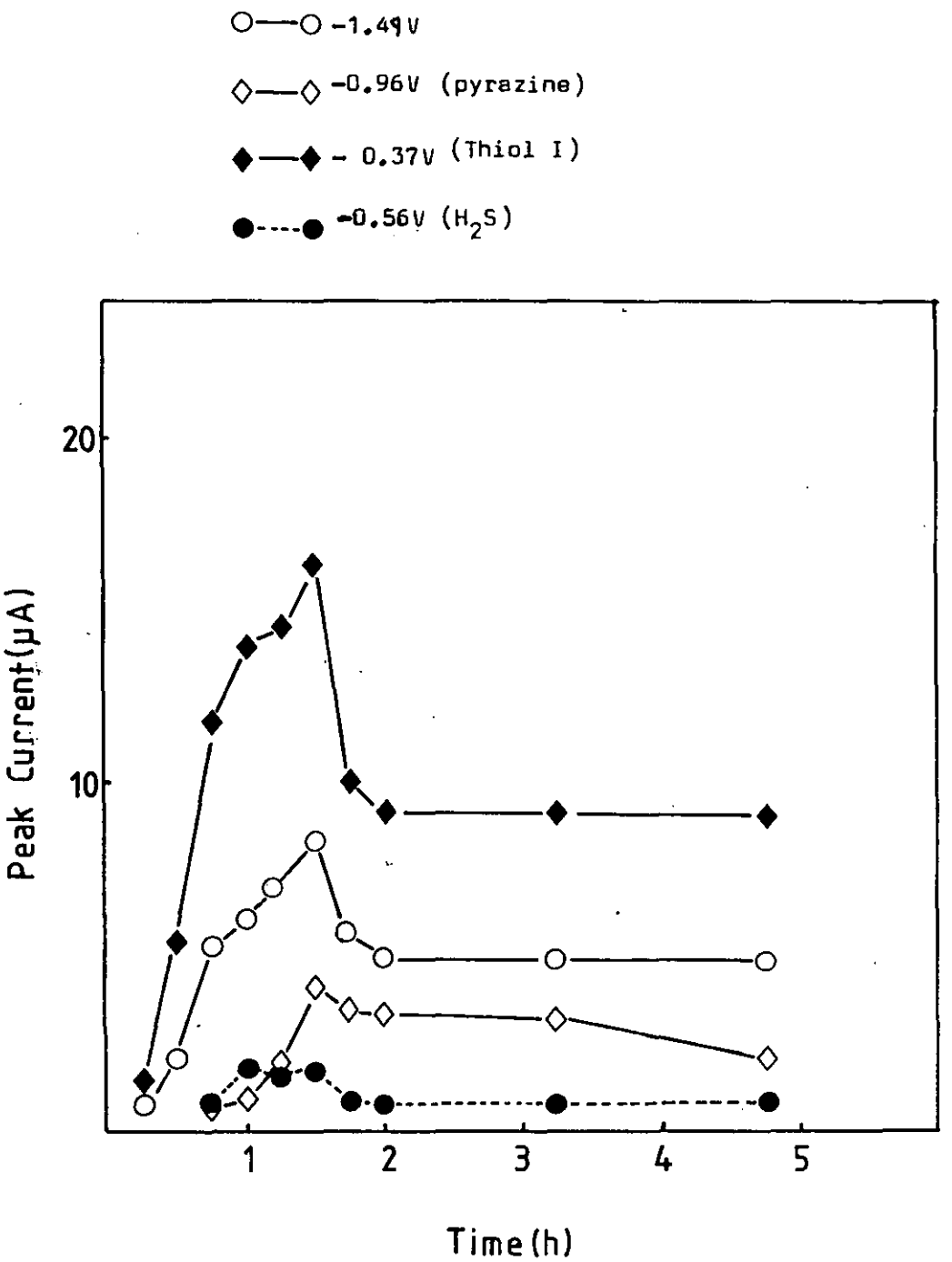


Fig. 47 Plot of the peak heights of the degradation products of cephalexin. Degradation at 75°C and pH3.3. Polarography at pH7.4.

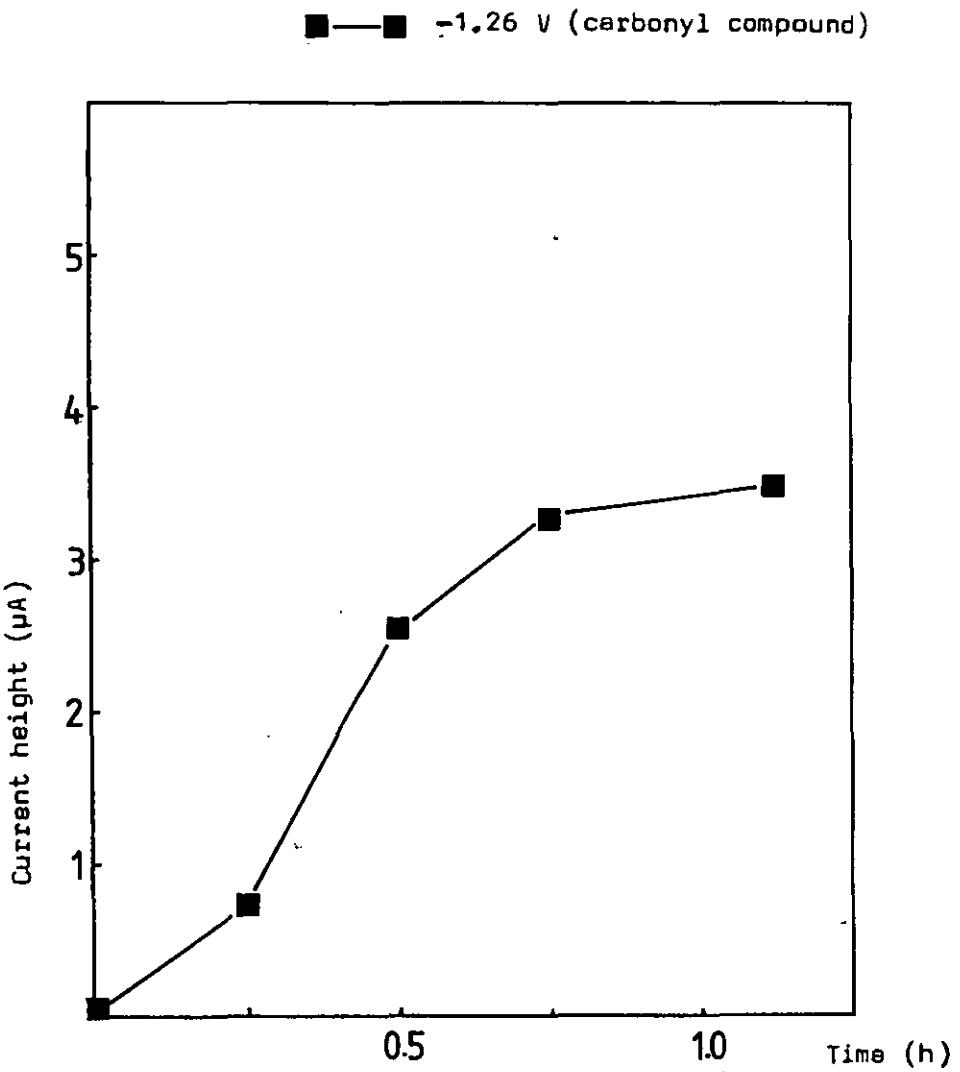


Fig. 48 Formation of the carbonyl compound(-1.26V) from the degradation of the thiophenone(XVII) (21ppm) at 100°C in phosphate buffer (pH7.4).

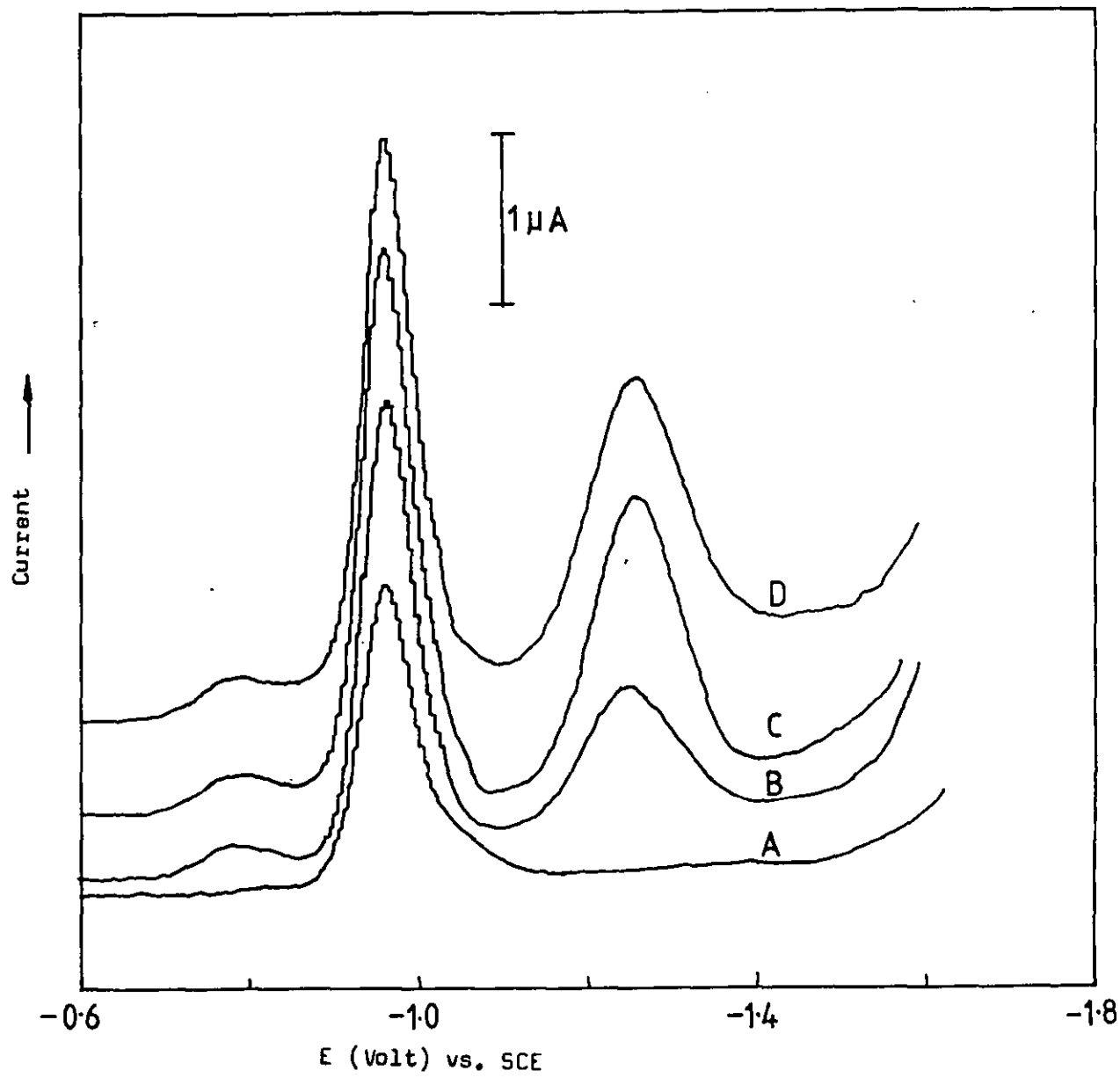


Fig. 49 DP polarograms of the degradation of the thiophenone (XVII) (21ppm) at 100°C in phosphate buffer solution (pH7.4). A (0 hours), B (0.25 hours), C (0.75 hours), D (3 hours).

went solvent extraction with two 100 ml portions of chloroform. The organic layer was left to evaporate at room temperature and yielded a very small amount of an orange amorphous solid of unknown weight.

Examination of the reaction products.

(i) From the non-polar layer.

The orange solid was dissolved in 100 ml of phosphate buffer (pH 7.4). On polarographing this solution a DP peak at -0.96 V corresponding to a pyrazine derivative was observed. This was the only wave observed. The solution was boiled for 3 hours. On polarographing the cooled solution the pyrazine compound (-0.96 V) and the carbonyl compound (-1.26 V) were observed. Thiols I and II were only just visible.

(ii) From the polar (aqueous) layer.

A DP scan at pH 7.4 revealed two waves, thiol I (33.0 µA) and the -1.51 V wave (20.0 µA).

The pyrazine derivative is seen to form readily at pH 3.3 and 75°C. This compound crosses over in the non-polar layer and contaminates the thiophenone solid.

6. Degradation of the diketopiperazine derivative of cephalexin at pH 3.3.

The DKP (VIII) (4.29×10^{-4} M), a 100 ml solution, was adjusted to pH 3.3 with 0.2 M HCl. This unbuffered solution was heated in a water bath at 75°C; periodically samples were removed, cooled and polarographed at pH 7.4. Two waves were recorded (see fig. 50).

The wave at -0.90 V is due to the diketopiperazine derivative of cephalexin. This compound is seen to degrade quickly in pH 3.3. After 2 hours' hydrolysis it has almost completely disappeared. Hydrogen sulphide (-0.56 V) is seen to be produced from the degradation of the DKP derivative. No thiols, and in particular thiol I (which was formed in high

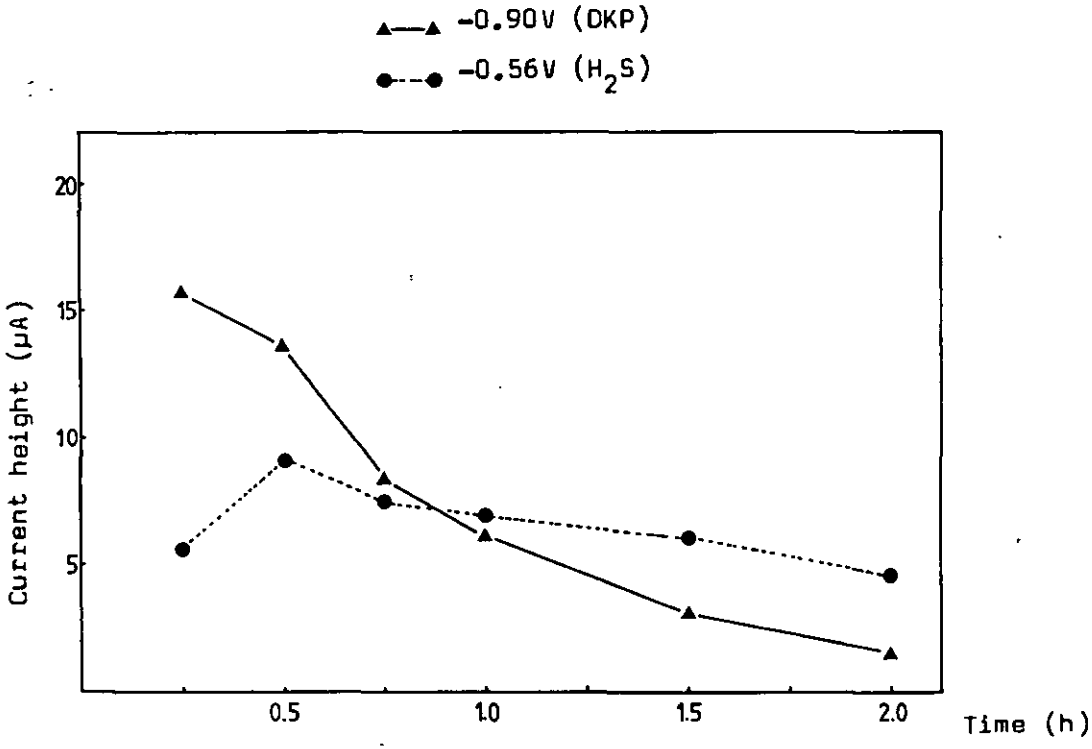


Fig. 50 plot of the peak heights of the degradation products of the DKP derivative of cephalexin, degradation at 75°C and pH3.3. Polarography at pH 7.4.

yield from cephalexin under these conditions), are observed. No pyrazine derivatives are observed as well as no large wave at -1.50 V (both these compounds formed from cephalexin under the same conditions).

Previously in a study of the degradation of cephalexin at pH 3.3 and 75°C no DKP derivative was observed. If this compound is formed in cephalexin solutions then it degrades almost immediately.

7. pH plot for thiol I.

A plot of E against pH was obtained for the -0.37 V wave (thiol I) produced when cephalexin (260 ppm) (7.48×10^{-4} M) was degraded at 75°C at pH for 90 minutes.(see Table 94 and fig. 51).

Table 94. Changes in DP peak potential of thiol I with pH.

pH	peak potential V(-)
2.0	0.15
3.0	0.18
4.1	0.22
5.2	0.26
6.3	0.31
7.3	0.36
8.4	0.42
9.3	0.46
10.5	0.51
11.8	0.52
13.0	0.53

The $E_{\frac{1}{2}}$ equation of this wave in the pH range 2 - 10 was calculated as

$E_{\frac{1}{2}} = (-0.04 - 0.046\ pH)\ V$

At pH values greater than 10 this wave is independent of pH. A similar $E_{\frac{1}{2}}$ equation is obtained from a thiol degradation product of cephalosporins when they are subjected to alkaline hydrolysis (wave G) (see Table 36).

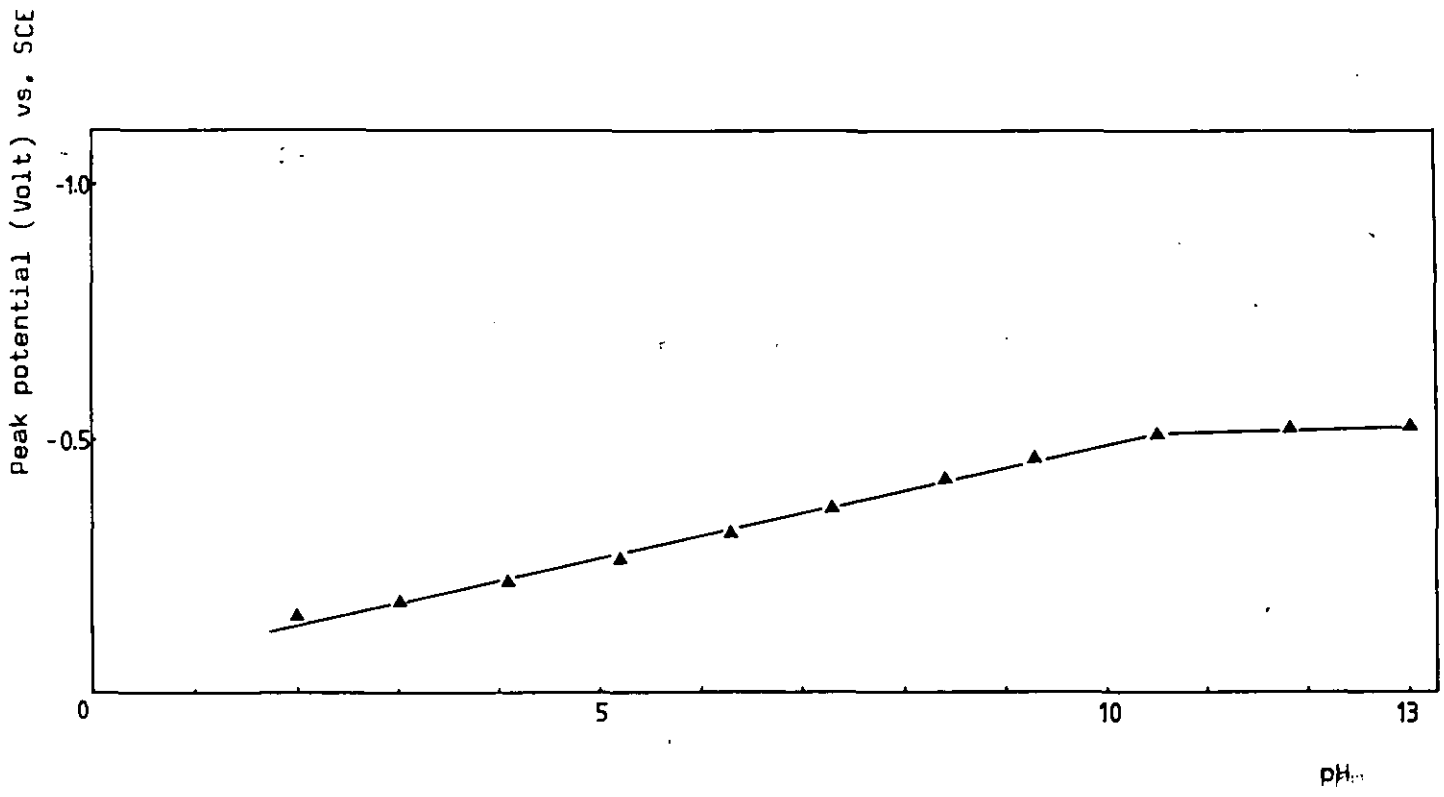


Fig. 51 Plot of the change in the reduction potential with pH of Thiol I.

8. Degradation of the aminomethylene derivative of cephalexin (XIII) at pH 3.3.

This compound may be a precursor to the diketopiperazine aldehyde (XII). The aminomethylene derivative of cephalexin (100 ppm) (4.6×10^{-4} M), a 100 ml solution, was degraded at pH 3.3 (unbuffered) in a water bath at 75°C for 90 minutes. The solution was cooled and polarographed at pH 7.4. A very small wave at -0.83 V was observed. On heating this solution (pH 7.4) at 100°C for 1 hour very small waves at -0.61 V, -0.83 V, -1.10 V and -1.44 V were observed. No pyrazine peaks were recorded.

9. Determination of the amount of H_2S produced from the degradation of cephalexin at pH 3.3.

Using the normal pulse polarographic method for determination of sulphide (previously described) the production of H_2S from the degradation of cephalexin (385 ppm) (1.11×10^{-3} M) a 100 ml solution, at pH 3.3 (unbuffered) and 75°C was quantified. The degrading solution was purged with nitrogen gas and any H_2S that was formed was blown off and passed through a solution of sodium hydroxide (0.5 M) (15 ml) where it was retained as the bisulphide ion. Periodically this NaOH solution was replaced with a fresh solution, the old solution being polarographed (see Table 95 and fig. 52).

Table 95. Amount of sulphide produced from the degradation of cephalexin at pH 3.3 and 75°C.

Time/hrs	Current obtained μA	% sulphur produced as S^{2-}	Accumulated % S^{2-} produced
1.5	0.4	0.23	0.23
3.2	1.5	0.86	1.09
4.8	0.3	0.17	1.24
6.3	0.4	0.23	1.49
7.3	2.0	1.14	2.63
9.0	1.2	0.69	3.32
24.0	3.2	1.83	5.15
32.0	0.4	0.23	5.38
48.5	0.2	0.10	5.48
54.0	0.1	0.05	5.54

The total accumulated current is 9.7 μA. From the calibration graph of the normal pulse method for sulphide (fig. 37) this is equivalent to 4.1×10^{-4} M of S^{2-}, or as a percentage of sulphur in the cephalexin molecule forming H_2S this is equivalent to (4.1×10^{-4} M/1.11×10^{-3} M) = 36.94%. When corrected for the dilution factor (purging from a 100 ml solution to a 15 ml solution), 36.94 (0.15)% = 5.54%. Therefore in 54 hours' hydrolysis only 5.54% of the available sulphur in the cephalexin molecule is converted to sulphide. The majority of this conversion has occurred after 25 hours of hydrolysis. After 90 minutes hydrolysis, when according to Dinner[207] no more intact cephalexin is left, the conversion is only 0.23%.

10. Degradation of cephalexin at pH 7.4 to produce the thiophenone (XVII) by the method of Bundgaard[208].

Cephalexin (2.0 g) was dissolved in pH 7.4 phosphate buffer (100 ml) and this solution degraded for 48 hours at 35°C. At the end of this time period a precipitate had formed. This was due to the aminomethylene derivative of cephalexin (XIII). The solution was filtered and the pH of

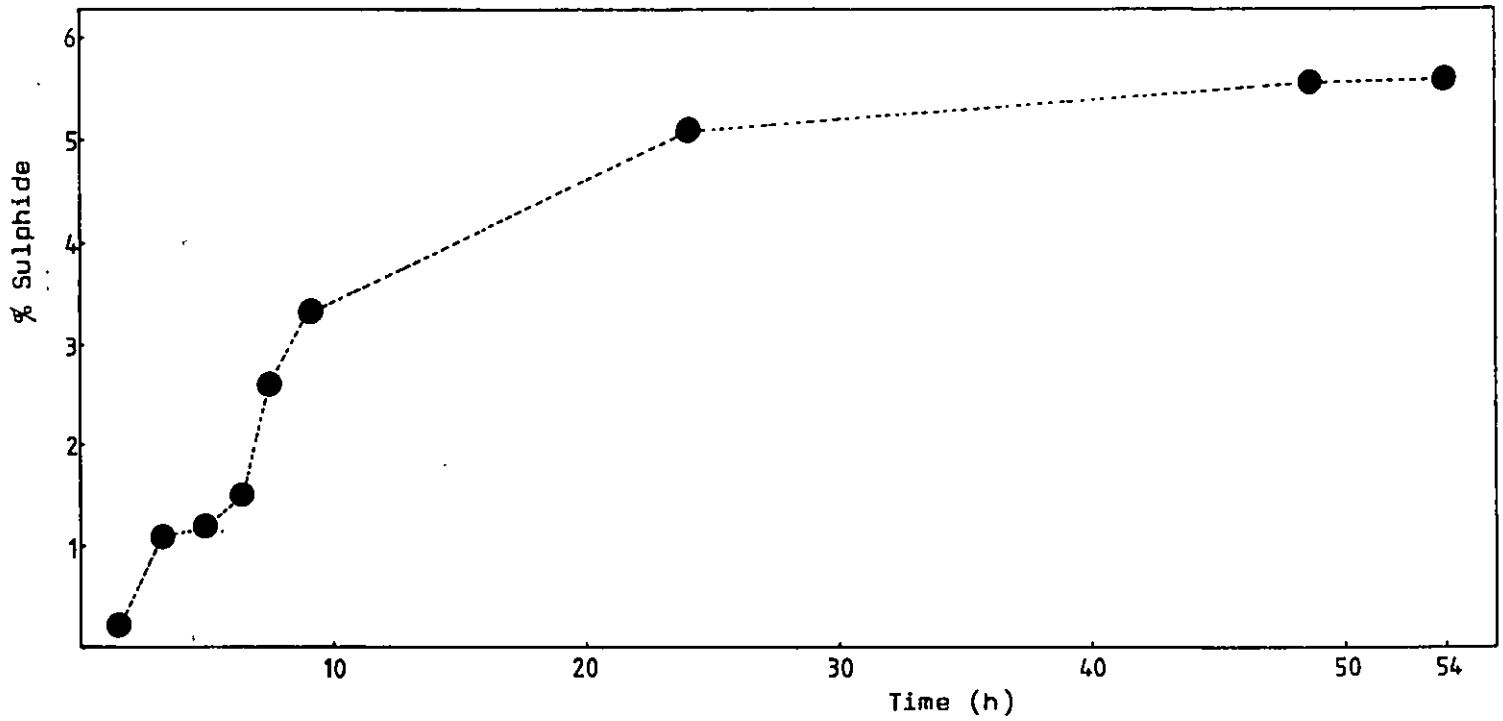


Fig. 52 Amount of H_2S (as accumulated %) produced from the degradation of cephalexin at 75°C and pH3.3 as determined by the normal pulse polarographic method.

the filtrate was brought down to pH 3.5. The solution underwent solvent extraction with two 100 ml portions of chloroform. After rotary evaporation and recrystallization from ethanol the thiophenone had the appearance of a yellow amorphous powder. The compound gave a melting point of 206°C which was in close agreement with that previously reported, 205-206°C[207].

11. Degradation of the thiophenone at 100°C.

Thiophenone (55 ppm) (4.23×10^{-4} M) was dissolved in phosphate buffer (pH 7.4) (100 ml). This solution was heated at 100°C in a water bath for one hour, cooled and polarographed at pH 7.4 (see Table 96).

Table 96. Changes in the DP peak heights of thiophenone degradation products with heating time at 100°C and pH 7.4.

Time/hrs	Peak height/μA			
	Peak at -0.13 V	Peak at -0.37 V	Peak at -0.56 V	Peak at -1.26 V
0.0	0.0	0.0	0.0	0.0
1.0	3.6	1.2	3.6	9.5

The thiophenone so produced gives a clear polarogram at pH 7.4 with no hydrolysis, showing it to be a purer compound than that obtained by Dinner's method[207] at pH 3.3.

A comparison was made of the amount of the carbonyl compound (-1.26 V) produced by degrading the two thiophenones as current (μA) obtained per mole, at 100°C and in pH 7.4 phosphate buffer (see Table 97).

Table 97.

Method	Peak height of -1.26 V wave/No. of moles (μA/mole)
Bundgaard[208]	22 518.2
Dinner[207]	21 867.4

Bundgaard's thiophenone is seen to produce more of the carbonyl compound at -1.26 V per mole, or rather, it seems to be less contaminated.

12. Determination of the amount of H_2S produced by the degradation of the thiophenone at 100°C and pH 7.4.

The thiophenone (XVII), produced by Bundgaard's method[208], (100 ppm) (7.68×10^{-4} M) was degraded at 100°C in 100 ml of phosphate buffer pH 7.4. The hydrogen sulphide so produced was swept into a 100 ml solution of sodium hydroxide (0.5 M). This solution was polarographed in the normal pulse mode.

A reading of 9.2 µA was obtained, corresponding to 3.9×10^{-4} M of hydrogen sulphide. This means that 51% of the sulphur present in the thiophenone forms hydrogen sulphide.

D. DEGRADATION OF α-AMINOBENZYL CEPHALOSPORINS AT NEUTRAL pH

1. Cefaclor

Cefaclor is similar to cephalexin in structure except that at the C-3 position there is a chlorine atom instead of a methyl group (see general structural table).

The neutral degradation (pH 7.4) of cefaclor in phosphate buffer at various temperatures was studied using polarography. In all cases a solution of 100 ppm (2.72×10^{-4} M) was used. Periodically samples were removed and polarographed at 25°C (see Tables 98 to 101 and figs. 53, 54 and 55).

Cefaclor on degrading produces a compound that polarographs at -0.96 V and is thought to be a DKP derivative (primary degradation product). Hydrogen sulphide is a degradation product (-0.56 V) but does not seem to be present in significant amounts. A wave that was observed previously by Fogg et al.[79] in the neutral degradation of cephalexin and absent when no molecular oxygen was present in the system was recorded at -0.78 V. From Tables 98 to 101 it is seen to form as the DKP derivative (-0.96 V) degrades, which suggests that it is a degradation product of the DKP. Unlike the cephalexin study[79] the DKP of cefaclor seems to reach a steady level after some partial degradation. This can be explained by the fact that the DKP of cefaclor and the pyrazine derivative of cefaclor both polarograph at the same potential (-0.96 V) so in fact the latter part of the -0.96 V values in Tables 98 to 101 is due to pyrazine formation. pH plots assigning the dependence of the half-wave potential ($E_{\frac{1}{2}}$ equation) of DKP of cefaclor and its pyrazine derivative have been carried out (see later) and show that at

pH 7.4 both these compounds reduce at -0.96 V.

It is seen that at all cases of temperature studied that the pyrazine derivative reaches a steady stable level. The -0.13 V wave is only significant in the 37°C study (Table 98, fig. 53). This wave gives an anodic DC signal and disappears on the addition of the thiol inhibitor para-hydroxy-mercuricbenzoate, demonstrating that it may be due to a thiol. The -1.23 V wave was thought to be analogous to the -1.26 V wave (carbonyl) found in large yields from the neutral (pH 7.4) degradation of cephalexin. However, addition of sodium metabisulphide did not displace this wave and it is thought not to be similar to the carbonyl produced from cephalexin.

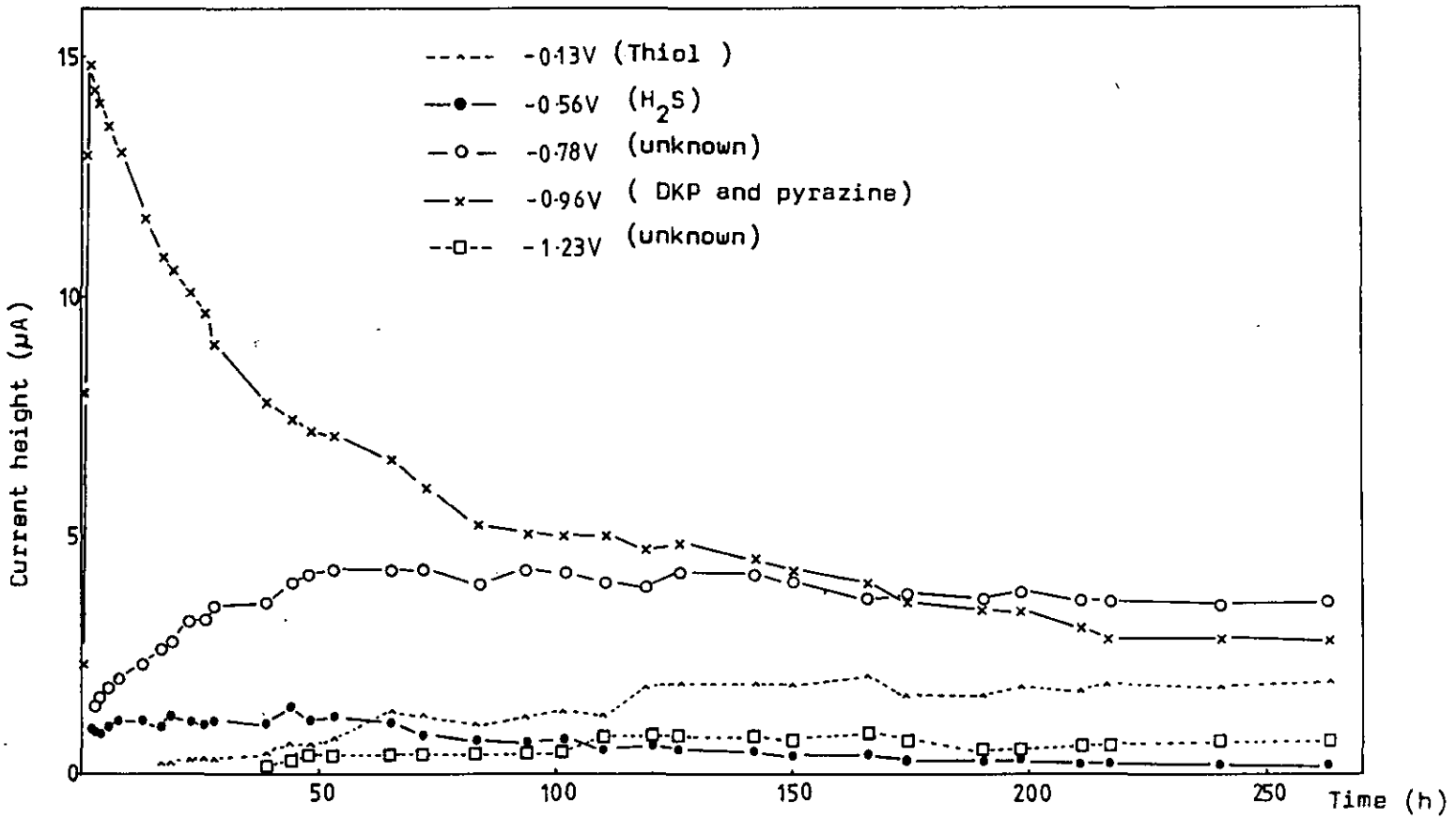


Fig. 53 Plot of the changes in current height of the neutral degradation products of cefaclor. Degradation in phosphate buffer (pH7.4) at 37°C. Polarography at pH7.4 .

Key as for Fig. 53

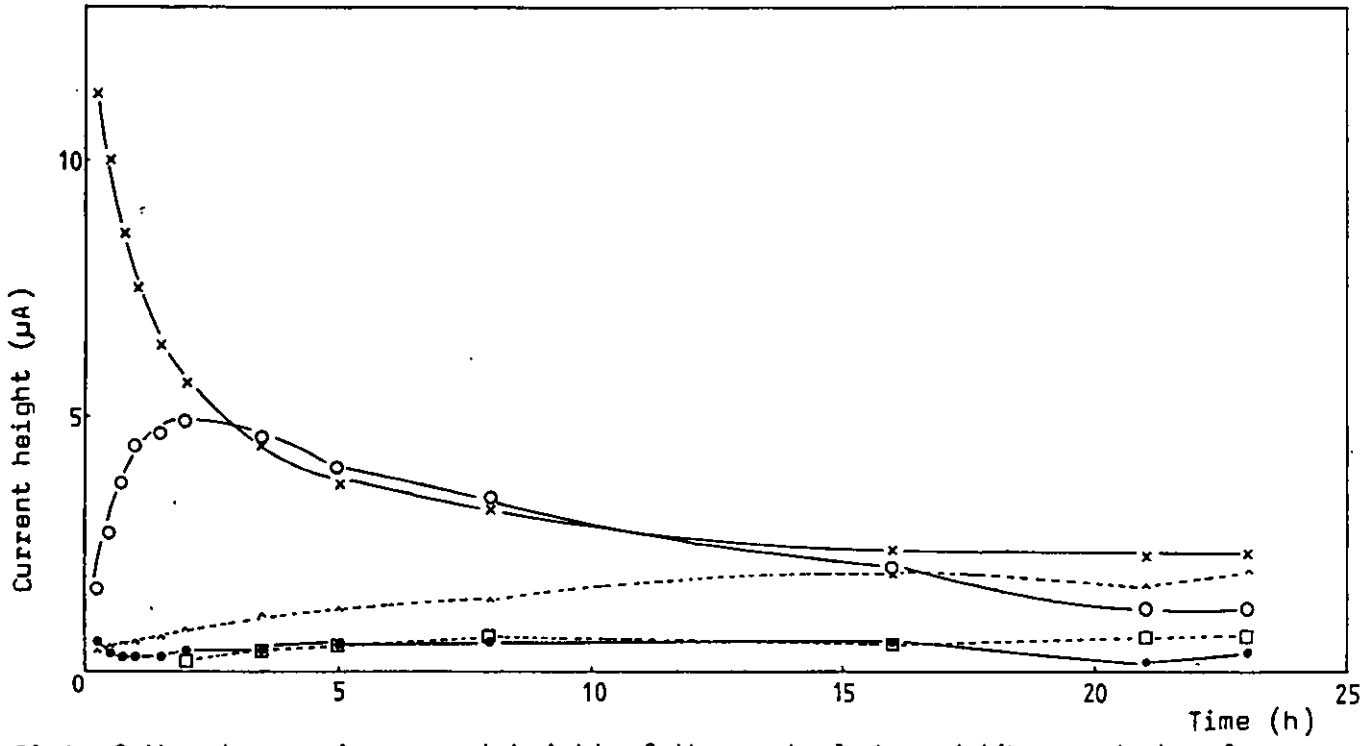


Fig. 54 Plot of the changes in current height of the neutral degradation products of cefaclor. Degradation in phosphate buffer (pH7.4) at 80°C, polarography at pH7.4 .

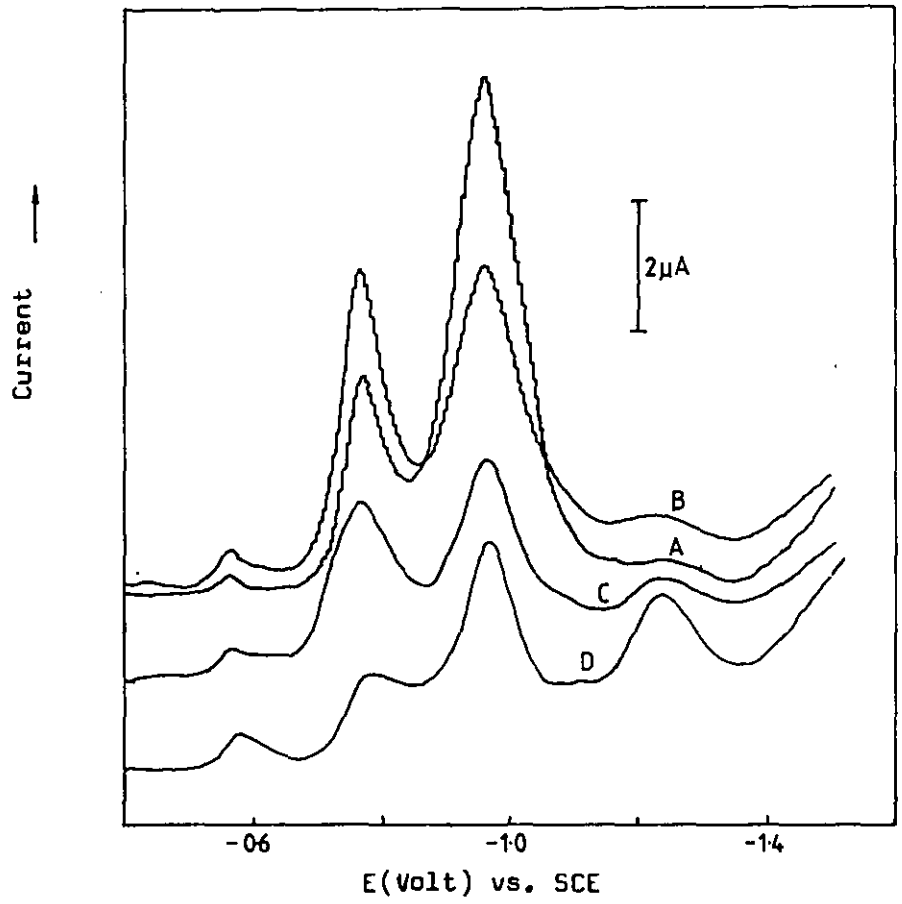


Fig. 55 DP polarograms of the neutral degradation of cefaclor (100ppm) in phosphate buffer (pH7.4) at 100°C, polarography at pH7.4 .

A (0.25 hours), B (1 hour), C (3.5 hours), D (8 hours).

Table 98. Changes of DP peaks of cefaclor degradation products with heating time at 37°C in phosphate buffer solution pH 7.4.

Time/hrs	Peak height/μA				
	Peak at -0.96 V	Peak at -0.78 V	Peak at -0.56 V	Peak at -0.13 V	Peak at -1.23 V
0.25	2.2	0.0	0.0	0.0	0.0
0.60	8.0	0.0	0.0	0.0	0.0
1.00	12.9	0.0	0.0	0.0	0.0
2.00	14.8	0.0	1.0	0.0	0.0
3.00	14.3	1.4	0.9	0.0	0.0
4.00	14.0	1.6	0.9	0.0	0.0
6.00	13.5	1.8	1.0	0.0	0.0
8.00	13.0	2.0	1.1	0.0	0.0
13.00	11.6	2.3	1.1	0.0	0.0
17.00	10.7	2.6	1.0	0.2	0.0
19.00	10.5	2.7	1.2	0.2	0.0
23.00	10.0	3.2	1.1	0.25	0.0
26.00	9.6	3.2	1.0	0.3	0.0
28.00	9.0	3.5	1.1	0.3	0.0
39.00	7.8	3.6	1.1	0.4	0.2
44.00	7.4	4.0	1.4	0.6	0.3
48.00	7.2	4.2	1.1	0.6	0.4
53.00	7.1	4.3	1.2	0.7	0.4
65.00	6.6	4.3	1.1	1.3	0.4
72.00	6.0	4.3	0.8	1.2	0.4
84.00	5.2	4.0	0.7	1.0	0.5
94.00	5.0	4.3	0.7	1.2	0.5
102.00	5.0	4.2	0.7	1.3	0.5
110.00	5.0	4.0	0.5	1.2	0.8
119.00	4.7	3.9	0.6	1.3	0.6
126.00	4.8	4.2	0.5	1.8	0.8
142.00	4.5	4.2	0.5	1.9	0.8
150.00	4.3	4.1	0.4	1.8	0.7
166.00	4.0	3.6	0.4	2.0	0.8
174.00	3.6	3.7	0.3	1.6	0.7
190.00	3.4	3.5	0.3	1.6	0.5

continued ...

Table 98. (continued)

Time/hrs	Peak height/μA				
	Peak at -0.96 V	Peak at -0.78 V	Peak at -0.56 V	Peak at -0.13 V	Peak at -1.23 V
198.00	3.4	3.8	0.3	1.8	0.5
211.00	3.0	3.6	0.2	1.7	0.6
217.00	2.8	3.6	0.2	1.8	0.6
240.00	2.8	3.5	0.2	1.8	0.7
263.00	2.8	3.6	0.2	1.9	0.7

Table 99. Changes of DP peaks of cefaclor degradation products with heating time at 60°C in phosphate buffer solution pH 7.4

Time/hrs	Peak height/μA				
	Peak at -0.96 V	Peak at -0.78 V	Peak at -0.56 V	Peak at -0.13 V	Peak at -1.23 V
0.25	8.6	0.0	1.8	0.0	0.0
0.50	13.1	0.9	1.2	0.0	0.0
0.75	13.5	1.3	1.2	0.0	0.0
1.00	12.9	1.5	1.0	0.3	0.0
1.50	12.3	1.9	0.9	0.3	0.0
2.00	11.7	2.2	0.8	0.4	0.0
3.50	9.8	3.0	0.5	0.6	0.0
5.00	8.4	3.9	0.4	0.7	0.0
8.00	6.5	4.6	0.4	0.9	0.0
16.00	4.4	4.2	0.4	0.9	0.4
19.50	4.2	4.0	0.4	1.2	0.4
23.50	3.9	3.3	0.4	1.4	0.8
46.00	2.6	2.8	0.2	1.4	1.4
53.50	2.4	2.5	0.1	1.4	1.5
67.00	2.4	2.3	0.1	1.9	1.4
71.00	2.4	2.2	0.1	1.1	1.6
95.00	2.2	1.9	0.0	1.0	1.9

Table 100. Changes of DP peaks of cefaclor degradation products with heating time at 80°C in phosphate buffer solution pH 7.4.

Time/hrs	Peak height/μA				
	Peak at -0.96 V	Peak at -0.78 V	Peak at -0.56 V	Peak at -0.13 V	Peak at -1.23 V
0.25	11.3	1.6	0.6	0.4	0.0
0.50	10.0	2.7	0.4	0.5	0.0
0.75	8.6	3.7	0.3	0.6	0.0
1.00	7.5	4.4	0.3	0.6	0.0
1.50	6.4	4.7	0.3	0.7	0.0
2.00	5.7	4.9	0.4	0.8	0.2
3.50	4.5	4.6	0.4	1.1	0.4
5.00	3.7	4.0	0.5	1.2	0.5
8.00	3.2	3.4	0.6	1.4	0.7
16.00	2.4	2.1	0.6	1.9	0.6
21.00	2.3	1.3	0.2	1.7	0.7
23.00	2.3	1.3	0.4	2.0	0.7
27.50	2.3	1.0	0.0	1.8	0.6

Table 101. Changes of DP peak heights of cefaclor degradation products with heating time at 100°C in phosphate buffer solution pH 7.4.

Time/hrs	Peak height/μA				
	Peak at -0.96 V	Peak at -0.78 V	Peak at -0.56 V	Peak at -0.13 V	Peak at -1.23 V
0.25	7.9	3.3	0.2	0.6	0.0
0.30	6.2	4.4	0.2	0.8	0.0
0.45	5.2	4.8	0.2	1.0	0.2
1.00	4.5	5.2	0.4	1.1	0.2
1.50	3.9	4.1	0.6	1.5	0.3
2.00	3.4	3.5	0.6	1.7	0.4
2.50	3.0	3.1	0.4	1.9	0.4
3.50	2.8	2.4	0.4	2.2	0.4
5.00	2.6	1.6	0.4	2.4	0.8
8.00	2.6	1.1	0.3	2.4	1.4

At 60°C the same pattern was observed as that at 37°C (see Table 99), although degradation is faster. The DKP derivative of cefaclor reaches its maximum value after 0.75 hours and is degraded thereafter.

Similar results were recorded from the 80°C (see fig. 54) and 100°C studies (see fig. 55). As the temperature of degradation was increased so the maximum value obtained for the DKP (-0.96 V) decreased (see Table 102).

Table 102. Comparison of the maximum current heights obtained of the DKP (cefaclor) (-0.96 V) with variation in degradation temperature at pH 7.4.

Temperature (°C)	Peak height at -0.96 V (μA)	Time of maximum height (μA)
37	14.8	2.00
60	13.5	0.75
80	11.3	0.25
100	7.9	0.25

Comparison with cephalexin[79] shows that the neutral degradations of cefaclor yield slightly greater amounts of DKP derivative, of the compound responsible for the wave at -0.78 V and of the pyrazine derivative.

2. Cephaloglycin.

Cephaloglycin differs from cephalexin in that at the C-3 position the methyl group has an acetoxy ($-OC(O)CH_3$) group attached. The neutral degradation of this compound was carried out as for cefaclor at 37, 80 and 100°C. In all cases the concentration of cephaloglycin used was 2.47×10^{-4} M (100 ppm).

(i) Degradation at 37°C

The degradation was followed over a period of 244 hours (see Table 103 and fig. 56). Cephaloglycin itself does not give a polarographic wave at pH 7.4 but it does in pH 2 solutions where the wave at -0.90 V

corresponds to the reductive elimination of the C-3 acetyl group. The loss of intact cephaloglycin at 37°C (pH 7.4) was followed by adjusting aliquots of the degrading solution to pH 2 and measuring the height of the -0.90 V wave (see Table 104 and fig. 57).

Table 103. Changes of DP peaks of cephaloglycin degradation products with heating time at 37°C in Britton-Robinson buffer solution pH 7.4.

Time/hrs	Peak height/μA			
	Peak at -0.96 V	Peak at -0.70 V	Peak at -0.56 V	Peak at -0.20 V
0.40	0.00	1.2	0.00	0.00
0.60	0.00	1.4	0.00	0.00
1.40	0.00	6.3	0.00	0.00
4.00	0.00	13.9	0.00	0.00
9.00	0.80	17.2	0.00	0.50
24.00	1.20	12.6	0.00	0.40
28.00	2.60	11.6	0.00	1.60
59.00	1.50	6.3	0.05	1.50
81.00	1.40	3.3	0.08	1.40
101.00	1.20	1.4	0.15	1.40
125.00	1.00	0.7	0.60	1.30
145.00	0.80	0.4	0.90	1.00
170.00	0.80	0.30	1.00	0.90
190.00	0.80	0.30	1.20	0.00
244.00	0.70	0.20	2.20	0.00

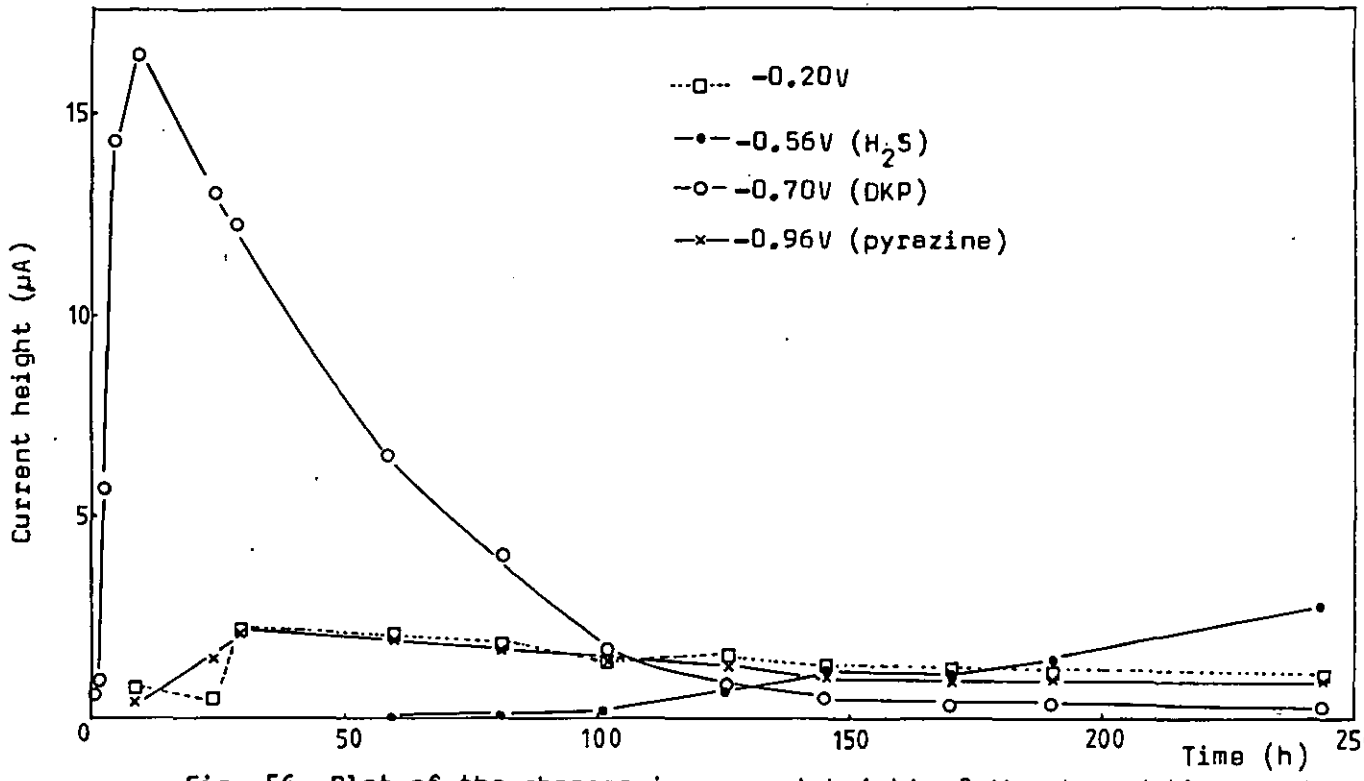


Fig. 56 Plot of the changes in current height of the degradation products of cephaloglycin. Degradation in phosphate buffer (pH7.4) at 37°C, polarography at pH7.4 .

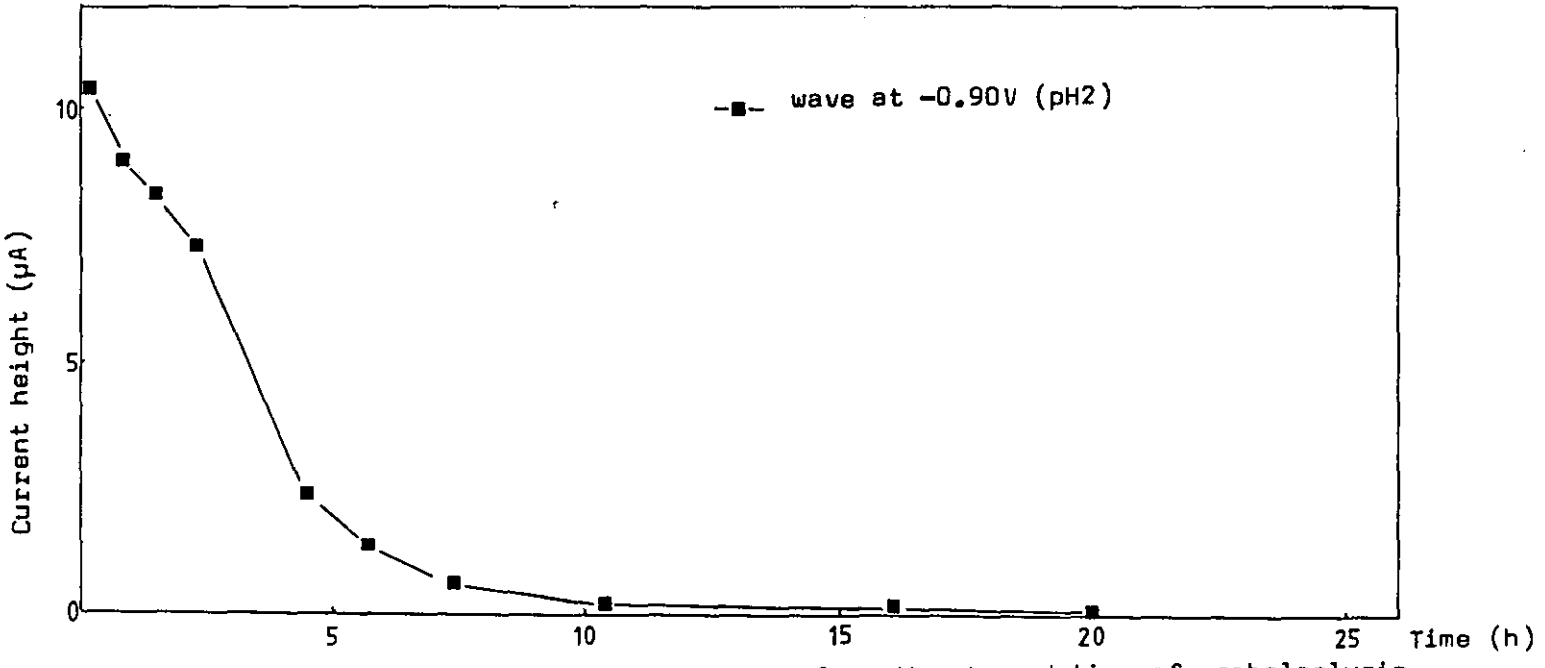


Fig. 57 Decline of the -0.90V wave produced from the degradation of cephaloglycin.
Degradation at 37°C in phosphate buffer (pH7.4), polarography at pH2.

Table 104. Changes of the DP peak at -0.90 V, pH2, corresponding to the reductive elimination of C-3 acetyl group of cephaloglycin as cephaloglycin is being degraded at pH 7.4 (37°C).

Time/hrs	Peak height/μA
	Peak at -0.90 V
0.167	10.4
0.833	10.0
1.50	8.3
3.30	7.3
4.50	2.40
5.72	1.36
7.40	0.65
10.4	0.20
16.0	0.15
20.0	0.10
25.0	0.05

Table 105. Changes in DP peaks of cephaloglycin degradation products with heating time at 80°C in Britton-Robinson buffer pH 7.4.

Time/hrs	Peak height/μA			
	Peak at -0.96 V	Peak at -0.70 V	Peak at -0.56 V	Peak at -0.20 V
0.12	0.00	2.65	0.00	0.00
0.50	1.00	13.50	0.00	0.00
0.83	1.50	11.00	0.00	0.00
1.17	1.90	9.45	0.00	0.20
1.67	2.10	6.80	0.00	0.30
2.50	2.10	3.10	0.30	0.30
3.50	2.10	2.40	0.30	0.40
5.50	2.20	1.45	1.00	0.30
7.50	1.90	0.90	1.50	0.35
21.50	1.90	0.10	1.40	0.40
29.50	2.05	0.00	1.40	0.50
45.50	1.90	0.00	1.25	0.50
75.00	1.90	0.00	1.20	0.60
168.50	1.95	0.00	0.60	0.50
192.00	1.85	0.00	0.60	0.00

Table 106. Changes in DP peaks of cephaloglycin degradation products with heating time at 100°C in Britton-Robinson buffer pH 7.4.

Time/hrs	Peak height/μA			
	Peak at -0.96 V	Peak at -0.70 V	Peak at -0.56 V	Peak at -0.20 V
0.167	0.6	10.6	0.00	0.00
0.333	2.0	4.6	0.10	0.00
0.560	1.75	2.2	0.20	0.20
0.833	1.8	0.9	0.25	0.20
1.50	1.8	0.45	0.60	0.25
2.00	1.5	0.0	1.30	0.35
2.50	1.5	0.0	1.90	0.35
3.00	1.5	0.0	1.80	0.35
4.00	1.5	0.0	1.30	0.35
5.00	1.5	0.0	1.00	0.45

Table 107. Changes in DP peaks of cephaloglycin degradation products with heating time at 100°C in phosphate buffer pH 8.5 (Solution aliquots polarographed after adjustment to pH 7.4).

Time/hrs	Peak height/μA				
	Peak at -0.96 V	Peak at -0.70 V	Peak at -0.56 V	Peak at -0.13 V	Peak at -1.07 V
1 min	0.0	2.1	0.0	0.30	0.0
0.25	1.6	4.1	0.6	0.40	1.0
0.50	2.2	1.0	1.3	0.50	2.4
1.00	4.1	0.1	1.9	0.80	3.0
1.25	5.0	0.0	1.9	0.70	2.9
1.50	5.3	0.0	1.9	0.65	2.2
1.75	5.9	0.0	1.9	0.80	2.1
2.00	6.2	0.0	1.8	0.75	1.9
3.00	7.6	0.0	1.5	0.85	1.8
3.25	7.8	0.0	1.5	1.00	1.8
3.50	8.2	0.0	1.5	1.30	1.8
4.00	8.2	0.0	1.5	1.20	1.8
5.00	8.2	0.0	1.4	1.30	1.8

Cephaloglycin degrades rapidly at 37°C (pH 7.4) being almost totally absent after 10 hours. During this time period a peak at -0.70 V appeared. This peak reached a maximum current value at 9 hours of hydrolysis and then became smaller as the compound responsible for it degraded and had almost disappeared after 250 hours. This compound is thought to be the diketo-piperazine derivative of cephaloglycin. Small waves at -0.20 V (unknown), at -0.56 V (H_2S) and at -0.96 V (pyrazine) are also observed. As in the cefaclor study at 37°C (Table 98), the amount of H_2S produced was small, as was the amount of pyrazine.

No wave corresponding to the carbonyl produced from cephalexin (-1.26 V, pH 7.4) was recorded. There was no sign of the -0.78 V wave that was present in degradation of cefaclor and cephalexin and that was absent when cephalexin was degraded in absence of molecular oxygen[79].

(ii) Degradation at 80°C and 100°C.

A similar pattern to the one at 37°C was seen when cephaloglycin was degraded at 80°C (see Table 105 and fig. 58). The peaks responsible for pyrazine (-0.96 V and H_2S (-0.56 V) increased. The DKP derivative degraded faster, having completely disappeared by 22 hours. The unknown wave at -0.13 V remains at a low level throughout the study which was carried out for 192 hours.

With hydrolysis at 100°C the DKP derivative degrades rapidly and is absent after 2.5 hours (see Table 106 and fig. 59). Again increased yields of H_2S and of the pyrazine derivative are observed.

3. Degradation of cephaloglycin at pH 8.5.

Cephaloglycin (100 ppm) (2.47×10^{-4} M) was degraded at 100°C in phosphate buffer (pH 8.5). Periodically samples were removed, cooled and polarographed at pH 7.4 for ease of comparison of the waves produced (see Table 107 and fig. 59).

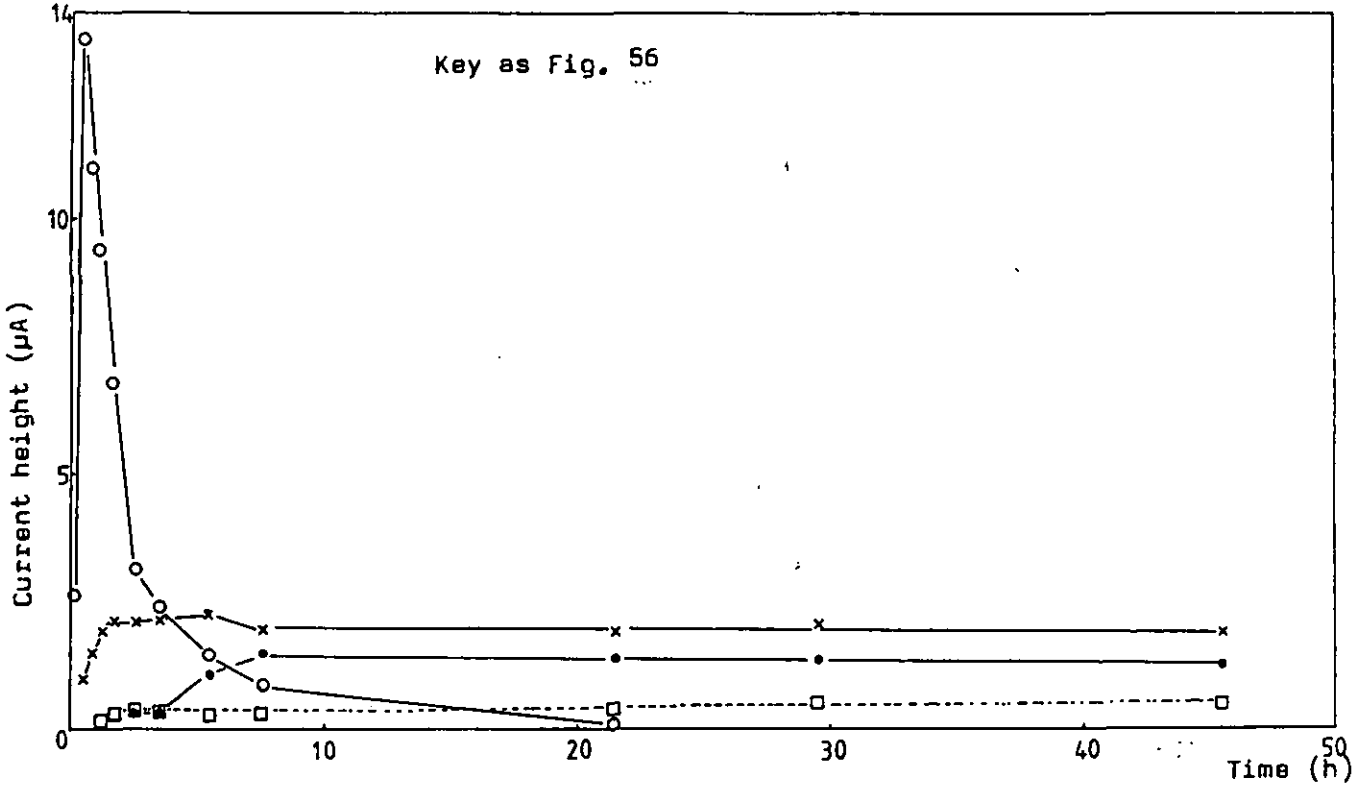


Fig. 58 Plot of the changes in the current height of the neutral degradation products of cephaloglycin. Degradation in phosphate buffer (pH7.4) at 80°C, polarography at pH7.4 .

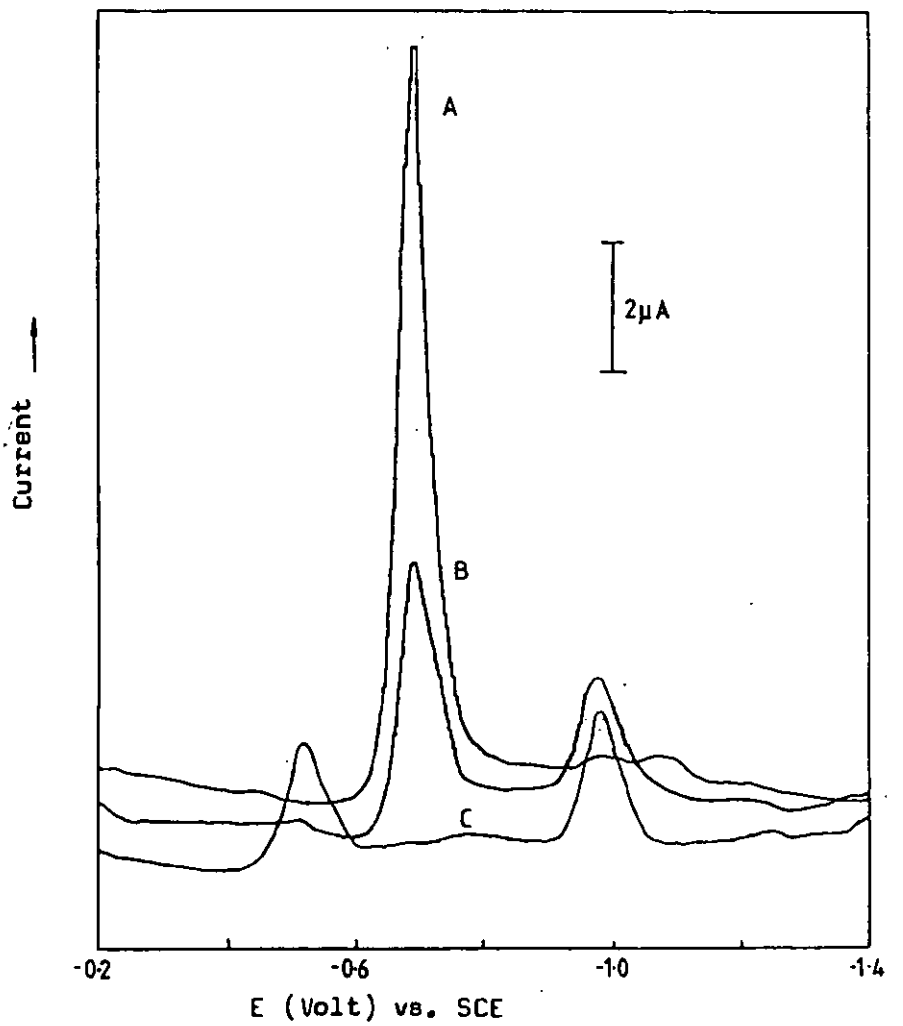


Fig. 59 DP polarograms of the neutral degradation of cephaloglycin (100ppm) in phosphate buffer (pH7.4) at 100°C, polarography at pH7.4 .

A (10 mins), B (30 mins), C (2.5 hours).

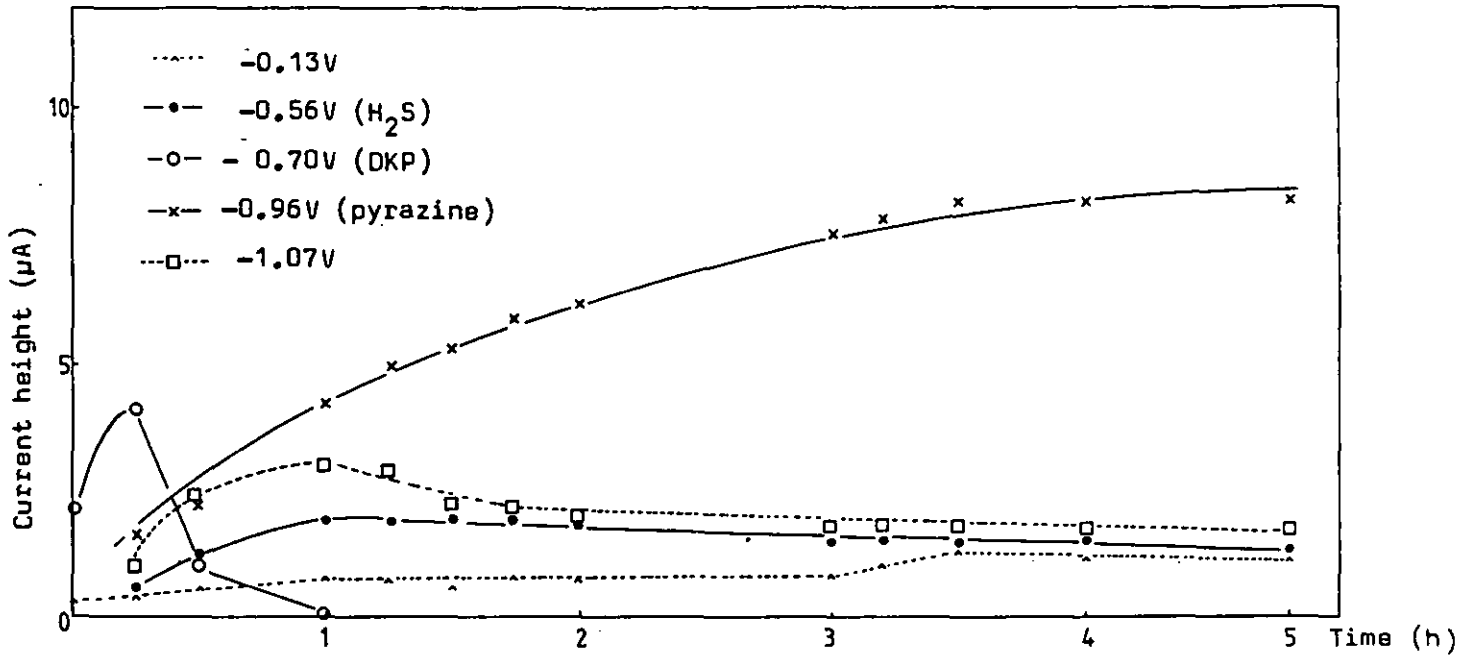


Fig. 60 Plot of the changes in the current height of the near neutral degradation products of cephaloglycin. Degradation in phosphate buffer (pH8.5) at 100°C, polarography at pH7.4 .

As with cephalexin[79], degradation of cephaloglycin at pH 8.5 and 100°C gave a greatly increased yield of pyrazine derivative (see fig. 60). Over five times more pyrazine is produced than in the study at pH 7.4. Both cephalexin[79] and cephaloglycin yielded a small wave at -1.07 V. The DKP derivative of cephaloglycin did not form to the same extent and was completely absent after 1 hour.

4. A number of pH plots were carried out on partially degraded solutions of some α-aminobenzylcephalosporins to ascertain the dependence on pH of the half-wave potential of some of their degradation products.

Cefaclor (2.72×10^{-4} M) was degraded at 80°C for 15 minutes in phosphate buffer (pH 7.4). Tables 108 and 109 show the data obtained for the changes in reduction potential for the DKP derivative and the pyrazine derivative respectively.

Table 108. The DKP derivative of cefaclor.

pH	Reduction potential V (-)	Current height (μA)
1.9	0.40	20.4
2.8	0.49	19.4
3.8	0.60	17.6
5.1	0.75	12.2
6.2	0.86	12.9
7.4	0.95	11.2
8.2	1.06	3.2
9.5	-	0.0

A graph was plotted of these results (fig. 61). A linear slope was observed in the pH range 2 - 8. The $E_{\frac{1}{2}}$ equation obtained was

$$E_{\frac{1}{2}} = (-0.20 - 0.042\ pH)\ V$$

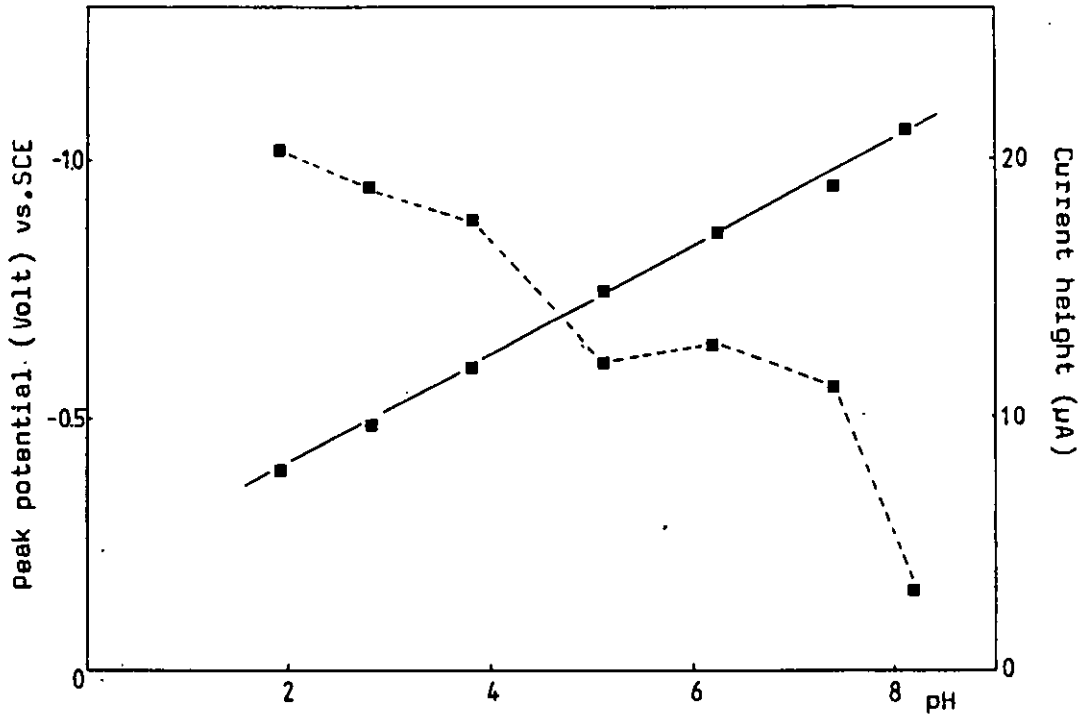


Fig. 61 Plot of the changes in the reduction potential and current height (dashed line) with pH for the DKP derivative of cefaclor.

This compound gave larger current signals as the pH of the solution was decreased.

Table 109. The pyrazine derivative of cefaclor.

pH	Reduction potential V (-)	Current height (μA)
2.0	0.63	1.45
3.0	0.70	1.70
4.0	0.76	1.90
5.0	0.83	1.70
6.0	0.91	1.40
6.9	0.98	1.25
8.1	1.05	0.80
9.0	1.11	0.60
10.1	1.20	0.30

A graph was plotted of these results (see fig. 62). A linear slope was observed in the pH range 2 - 10. The $E_{\frac{1}{2}}$ equation obtained was

$$E_{\frac{1}{2}} = (-0.49 - 0.070\ pH)\ V.$$

Cephaloglycin (100 ppm) was partially degraded, Tables 110 and 111 showing the data obtained for the changes in reduction potential for the DKP derivative and pyrazine derivative respectively.

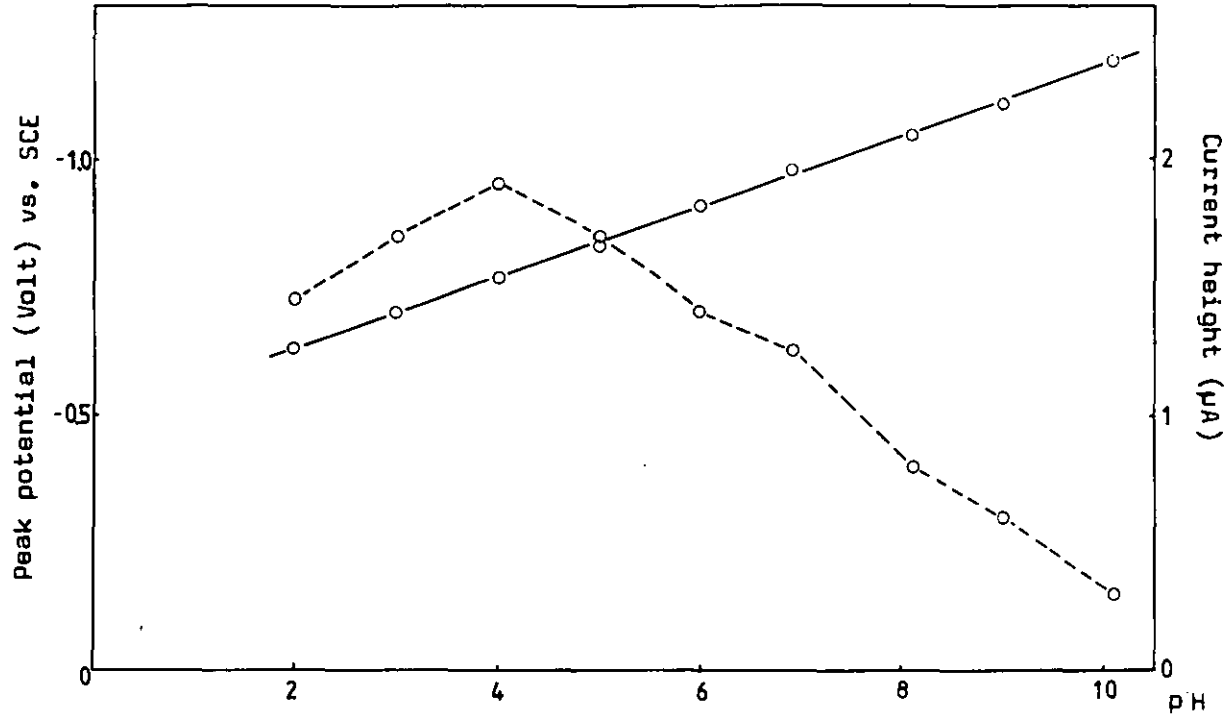


Fig. 62 Plot of the changes in the reduction potential and current height (dashed line) with pH for the pyrazine derivative of cefaclor.

Table 110. The DKP derivative of cephaloglycin.

pH	Reduction potential V (-)	Current height (μA)
1.90	0.36	2.1
3.05	0.42	3.2
4.40	0.54	3.6
5.25	0.60	5.3
6.40	0.64	5.2
7.40	0.72	5.1
7.85	0.75	5.1
8.50	0.80	5.1
8.90	0.82	5.1
10.45	0.91	1.3
11.40	0.94	1.0

A graph was plotted of these results, (fig. 63). A linear slope was observed in the pH range 2 - 11. The $E_{\frac{1}{2}}$ equation obtained was

$$E_{\frac{1}{2}} = (-0.25 - 0.063\ pH)\ V.$$

The greatest current values were obtained in the middle pH range 5 - 9.

Table 111. The pyrazine derivative of cephaloglycin.

pH	Reduction potential V (-)	Current height (μA)
2.1	0.62	4.5
3.1	0.70	4.5
4.3	0.77	4.5
5.7	0.87	4.1
6.3	0.92	4.0
7.4	1.00	3.8
8.1	1.03	2.5
9.0	1.08	2.4
9.8	1.13	1.2

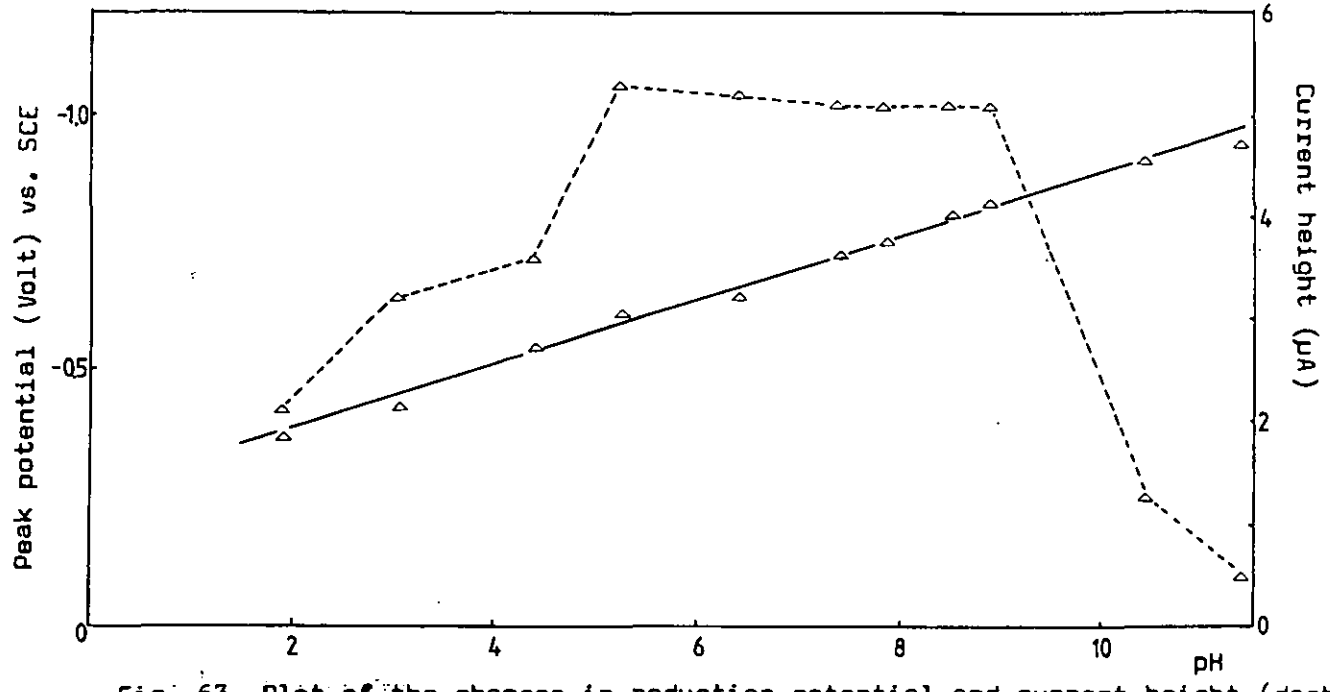


Fig. 63 Plot of the changes in reduction potential and current height (dashed line) for the DKP derivative of cephaloglycin.

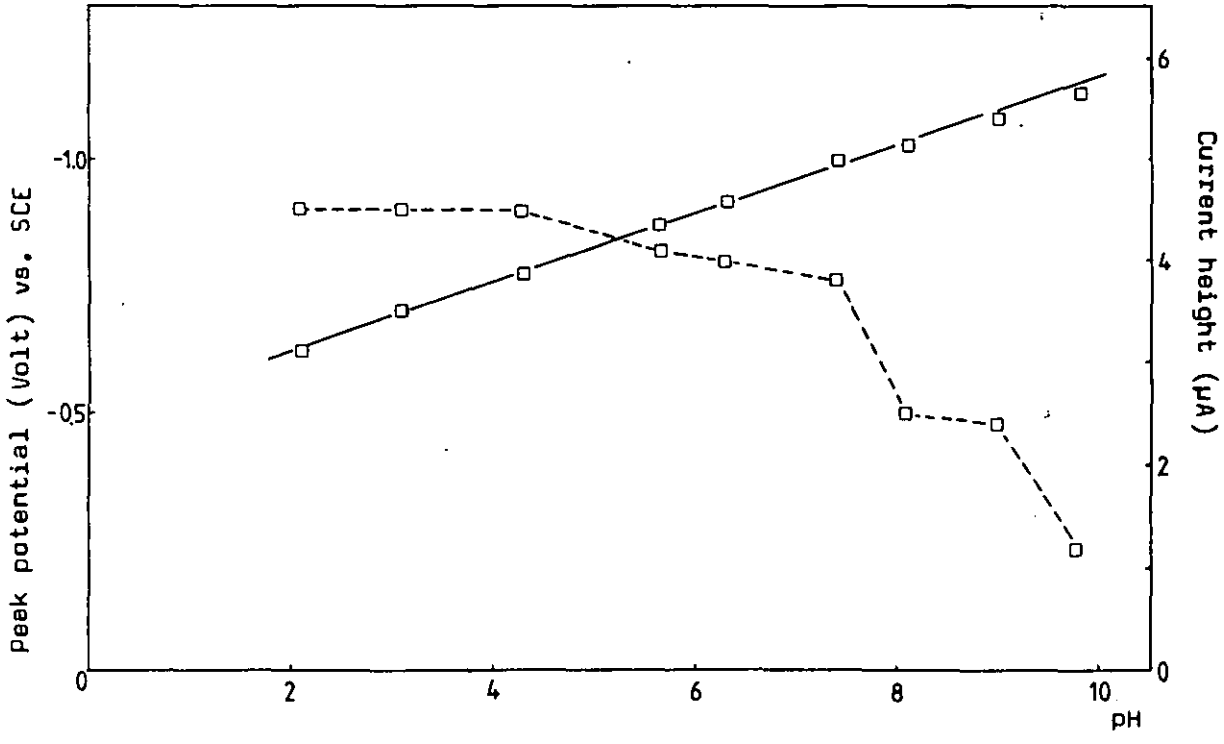


Fig. 64 Plot of the changes in reduction potential and current height (dashed line) with pH for the pyrazine derivative of cephaloglycin.

A graph was plotted of these results (see fig. 64). A linear slope was obtained in the pH range 2 - 10. The $E_{\frac{1}{2}}$ equation obtained was

$$E_{\frac{1}{2}} = (-0.48 - 0.068\ pH)\ V.$$

The -1.26 V wave (carbonyl) from degraded cephalexin was found to be independent of pH, the $E_{\frac{1}{2}}$ equation being

$$E_{\frac{1}{2}} = -1.26\ V.$$

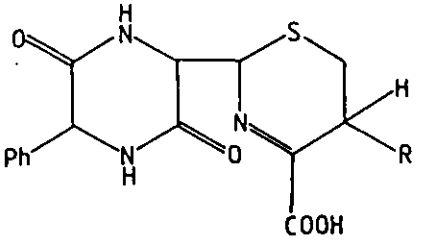
The height of the carbonyl peak was also found to be independent of pH.

A comparison of the $E_{\frac{1}{2}}$ values for the DKP derivatives of the α-amino-benzylcephalosporins examined was made (see Table 112).

Table 112.

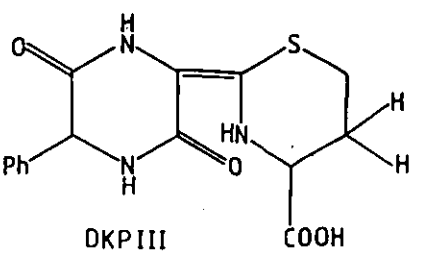
Compound	($E_{\frac{1}{2}}$ equation).V
Cephalexin	-0.15 - 0.103 pH
Cephaloglycin	-0.25 - 0.063 pH
Cefaclor	-0.20 - 0.042 pH

The differences in the $E_{\frac{1}{2}}$ equations are due to the different substituents at the C-3 position as the other parts of the molecules are equivalent. The structure of the DKP derivatives are shown below,



R = $-CH_3$, (DKPI)

R = -OH, (DKPII)



DKPIII

In the case of cephaloglycin the acetyl group at C-3 is easily hydrolysed to give the desacetyl derivative which undergoes intramolecular nucleophilic attack to DKP II. With cefaclor breakage of the β-lactam ring results in the expulsion of the chlorine atom at C-3[206] which results in DKP III.

The reduction processes occur at the $\Delta^{4,5}$ double bond in the thiazine ring for DKP I and DKP II. The constituent at C-3 exerts an inductive effect on the double bond at this $\Delta^{4,5}$ position. In the case of DKP II the -OH function withdraws electrons from the system and therefore destabilises the $\Delta^{4,5}$ double bond, making its reduction easier. In the case of DKP I the methyl group pushes electrons into the system and makes the double bond harder to reduce. With DKP III the site where reduction takes place is unknown.

A comparison of the $E_{\frac{1}{2}}$ equations of the pyrazine derivatives from the α-aminobenzylcephalosporins examined are shown below (see Table 113).

Table 113.

Compound	($E_{\frac{1}{2}}$ equation).V
Cephalexin	-0.50 - 0.065 pH
Cephaloglycin	-0.48 - 0.068 pH
Cefaclor	-0.49 - 0.070 pH

The table shows that the $E_{\frac{1}{2}}$ equations are identical within experimental error.

5. 7-ADCA is similar to cephalexin in all but the C-7 side-chain (see general tables). 7-ADCA (119 ppm) was subjected to heating at 100°C for 1 hour in phosphate buffer (pH 7.4).

Polarography at 25°C showed a wave at -1.26 V (3.0 μA) corresponding to the carbonyl compound from cephalexin and a wave at -0.56 V (H_2S). Addition of sodium metabisulphide resulted in the disappearance of the

wave at -1.26 V. This peak was also found to be independent of pH, $E_{\frac{1}{2}}$ = -1.26 V. The molar production of the carbonyl compound (-1.26 V) after 1 hour of hydrolysis was 0.54 x 10^4 µA/mole.

As 7-ADCA does not form a DKP derivative, the carbonyl compound and therefore the thiophenone (XVII) that is its suspected precursor, need not be formed via a DKP type derivative as stated by Dinner[207].

6. The molar production of the carbonyl compound was calculated from cephalexin and its DKP derivative.

Cephalexin (299 ppm) was degraded at 100°C for 1 hour in phosphate buffer (pH 7.4). Polarography at 25°C gave a reading of 8.5 µA for the -1.26 V wave. This is equivalent to 0.99 x 10^4 µA/mole.

The DKP derivative of cephalexin (100 ppm) (2.89 x 10^{-4} M) was degraded as cephalexin (above). A reading of 0.7 µA was recorded for the -1.26 V wave. This is equivalent to 0.24 x 10^4 µA/mole. A wave at -0.56 V (H_2S) was also recorded.

A comparison was made of the molar production of the carbonyl compound from the compounds examined (see Table 114).

Table 114.

Compound	molar production of -1.26 V wave (x 10^4 µA/mole)
Cephalexin	0.99
DKP (cephalexin)	0.24
Thiophenone	2.52
7-ADCA	0.54

From Table 114 it seems that the thiophenone is a key intermediate in formation of the carbonyl compound. Roughly 4 times as much cephalexin goes to the thiophenone as its DKP derivative. Formation of the carbonyl (-1.26 V) from 7-ADCA shows that a DKP type derivative is not necessary

for thiophenone formation. Failure of cephaloglycin and cefaclor to form the carbonyl compound (-1.26 V) in pH 7.4 suggests that a methyl group at C-3 is essential for the production of thiophenone and the carbonyl compound. It can be suggested that under the above conditions 43.8% of cephalexin, 10.7% of its DKP derivative and 24% of 7-ADCA degrade via thiophenone to the carbonyl compound.

7. The NP polarographic method was used to determine the amount of sulphide produced at 100°C, pH 7.4 for cephalexin and its DKP derivative..

Cephalexin (156 ppm) (4.49×10^{-4} M) was heated at 100°C in phosphate buffer pH 7.4. The solution was sampled for sulphide at regular intervals (see Table 115).

Table 115. Build up of S^{2-} from the degradation of cephalexin at pH 7.4, 100°C

Time (h)	NP signal (μA)	% S^{2-} accumulated
1	9.30	88.0
2	0.75	97.0
3	-	97.0
4	-	97.0
5	-	97.0

Almost all the sulphur in the cephalexin molecule (97%) is generated as H_2S, the majority of which (88%) is formed after 1 hour's hydrolysis, no more H_2S being generated after 2 hours.

The DKP derivative of cephalexin (130 ppm) (3.76×10^{-4} M) was investigated as cephalexin (above) (see Table 116).

Table 116. Build up of S^{2-} from the degradation of the DKP (cephalexin) at pH 7.4, 100°C

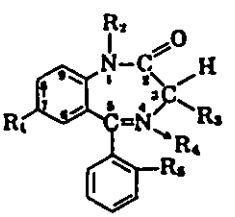
Time (h)	NP signal (μA)	% S^{2-} accumulated
1	4.2	46.8
2	0.1	47.9
3	-	47.9
4	-	47.9
5	-	47.9

Roughly half of the DKP used afforded H_2S compared with almost all of the cephalexin molecule.

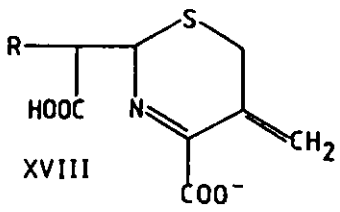
GENERAL DISCUSSION

Cephalosporins are reported to degrade in alkaline solution to their unstable cephalosporoic acids[188]. These in turn degrade to "fragmentation products". Alkaline degradation of a number of cephalosporins at room temperature, with polarography at pH 2 showed the formation and decline of two polarographically active degradation products common to almost all cephalosporins studied. These degradation products reduce at -0.39 V (pH 2) and -0.50 V (pH 2). The general pattern is that the -0.39 V wave forms first and as it declines the -0.50 V wave then begins to form. The rate of formation and decline of the two waves varies from compound to compound, although generally the -0.39 V wave (the initial alkaline degradation product) reaches its maximum peak current value after 1 to 2 hours of hydrolysis and the -0.50 V wave (the secondary alkaline degradation product) reaches its i_p maximum value after 2 to 5 hours. In a polarographic study of the alkaline hydrolysis of cephalosporin C derivatives Hall[68] observed the presence of two small waves at -0.35 V and -0.48 V with polarography at pH 2.

1,4-Benzodiazepines which contain a carbon-nitrogen double bond

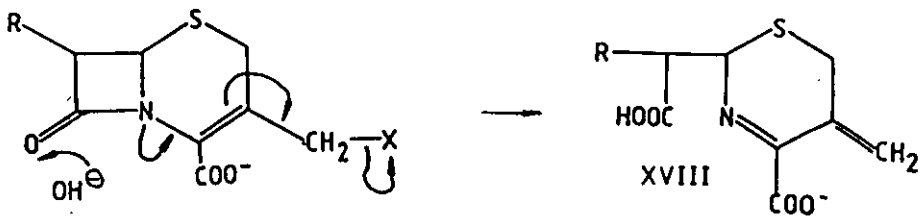


reduce in this potential range[214-6] and it is possible that the cephalosporin degradation products contain this bond. It is proposed here that the structure of the compound responsible for the -0.39 V wave in the case of the substituted C-3 position methyl compounds is an exo-cyclic methylene compound with a general structure of XVIII (see below).



XVIII

Formation of such a compound in alkaline solutions would follow the reaction scheme II (see below).

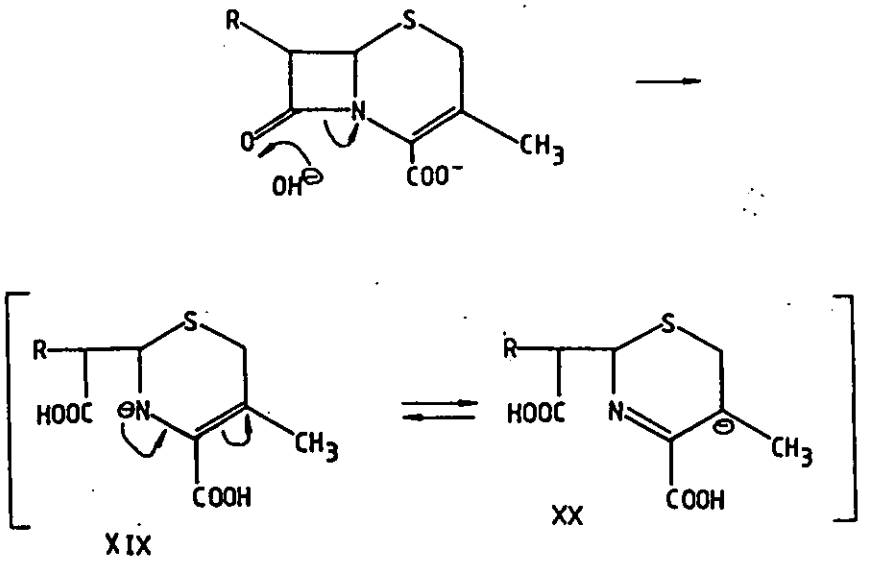


XVIII

Scheme II

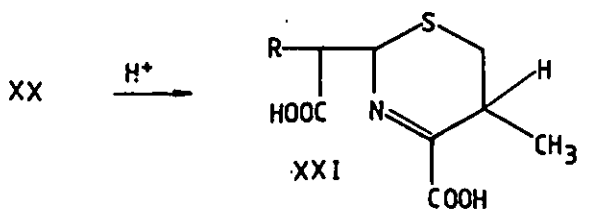
This compound (XVIII) may then reduce at -0.39 V (pH 2) at the $\Delta^{4,5}$ double bond.

Cephalosporins with an unsubstituted methyl group at the C-3 position (e.g. cephalexin) may still form a compound with a carbon-nitrogen double bond as an alkaline degradation product. A mechanism allowing this is given in scheme III (see below).

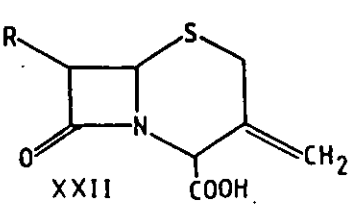


Scheme III

Normally one would expect the enamine ion (XIX) to pick up a proton from the aqueous solution but if resonance between it and the less stable imine ion (XX) does occur then it is equally possible for the imine ion to pick up a proton and form the imine (XXI)



which could undergo polarographic reduction at the $\Delta^{4,5}$ double bond. Exo-cyclic methylene derivatives of cephalosporins with general structure (XXII) have been shown not to reduce in the potential range 0 to -2.0 V[70].

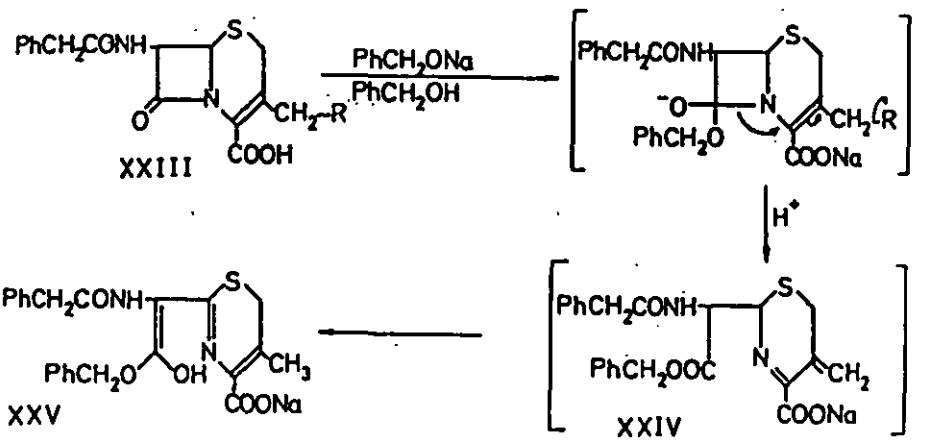


The $E_{\frac{1}{2}}$ equation obtained for the -0.39 V wave is approximately

$$E_{\frac{1}{2}} = (-0.20\ V - 0.070\ pH)\ V.$$

The dependence on pH is 70 mV per pH unit change. This is consistent for an irreversible process and for an equal number of electrons and protons involved in the reduction process.

Eggers et al.[217] proposed that in the production of a "cephalosporoate" from 7-phenylacetamidocephalosporanic acid (XXIII) (cephaloram) with sodium benzyloxide in benzyl alcohol, an exo-cyclic methylene compound (XXIV) is formed as an intermediate compound (see scheme IV below).



Scheme IV

Hamilton-Miller et al.[193] have suggested that when cephalosporins undergo aminolysis a semi stable exo-cyclic methylene compound (as structure XVIII) is formed which then degrades to penaldates. Alkaline degradation of the proposed initial alkaline hydrolysis products with structures (XVIII) and (XXI) may then result in compounds still retaining the carbon-nitrogen double bond which reduce at -0.50 V (pH 2), such as compound (XXV) the "cephalosporoate".

Alkaline degradation of cephaloglycin in the absence of dissolved molecular oxygen (see fig. 22) resulted in increased yields of the compounds responsible for the waves at -0.39 V (pH 2) and -0.50 V (pH 2), suggesting

that these compounds may be prone to oxidation.

The compound responsible for the wave at -0.50 V was observed only in the case of cephalosporin C in the 0.1 M, sodium hydroxide, $E_{\frac{1}{2}}$ versus pH plots. Increasing the severity of the alkaline hydrolysis conditions, e.g. increasing the concentration to 0.5 M and heating, results in the appearance of this compound from degraded solutions of cephalexin, cephaloglycin, cephalonium, cephalothin, cephradine and cefaclor.

Alkaline degradation of the DKP derivative of cephalexin (VIII) produced the initial alkaline degradation product (-0.39 V, pH 2) and a wave corresponding to the DKP at -0.35 V (pH 2). The -0.39 V wave was observed immediately from the DKP and may be produced by hydroxide-ion catalyzed opening of the piperazine ring of (VIII) to produce a compound similar to the protonated form of compound (XX). The -0.39 V compound so produced degrades at a faster rate than is the case for cephalexin, suggesting that the formation of this compound from the DKP of cephalexin is rapid. The -0.50 V wave forms from the DKP at a greater rate than for cephalexin, suggesting a more unstable system.

There is still confusion in the literature about the position of the double bond in the DKP derivatives of α-aminobenzylcephalosporins. Initially Bundgaard[204] reported the double bond to be at the $\Delta^{3,4}$ position for the cephalexin derivative. Indelicato *et al.*[206] reported that it was at the $\Delta^{4,5}$ position for the cephalexin derivative. Cohen *et al.*[203], from the isolated DKP derivative of cephradine observed that it was in the $\Delta^{4,5}$ position. Tsuji *et al.*[210] have isolated two different DKP compounds from the neutral and slightly alkaline degradation of cefradroxil (XI) (see page 65). A small percentage of the derivative is present as the enamine (double bond at $\Delta^{3,4}$).

Preliminary NMR studies by McGlynn[218] show that the DKP derivative of cephalexin, isolated using Bundgaard's method[204], to be mainly the compound having the double bond at $\Delta^{4,5}$ but with some $\Delta^{3,4}$ compound present. The

more stable compound is the enamine (double bond at $\Delta^{3,4}$) and the tendency will be for the double bond to move from $\Delta^{4,5}$ to $\Delta^{3,4}$ (the electroinactive compound). McGlynn[218] has indicated that when the DKP derivative of cephalexin is heated in toluene conversion of the imine to the enamine occurs.

Alkaline hydrolysis of cephalexin and cephaloglycin followed with polarography at pH 2 (figs. 9 and 10) did not reveal any waves corresponding to their DKP derivatives. If present at pH 2 the DKP of cephalexin would polarograph at -0.35 V and that of cephaloglycin at -0.38 V (as found from the $E_{\frac{1}{2}}$ versus pH plots, tables 2 and 110). This may be due either to (i) non-formation of DKP (although Bundgaard[204] quotes 40% of cephalexin and 23% of cephaloglycin going to their DKP derivatives in alkaline solutions), or (ii) formation of the imine form of the DKP which then reverts to the electroinactive enamine compound.

The variation of the substituents at C-7 does not seem to have any effect on the potential at which the alkaline hydrolysis products reduce at pH 2. The availability of a leaving group at the C-3 position did not affect the reduction potential of these compounds.

$E_{\frac{1}{2}}$ versus pH plots of degraded cephalosporin solutions after alkaline hydrolysis show many similarities in their electroactive degradation products. They show that hydrogen sulphide is produced from cephalosporins and the DKP derivative of cephalexin at room temperature. Hydrogen sulphide is a dibasic acid. pK_1 (pH 7.04) corresponds to the conversion to HS^- and pK_2 (pH 11.96) to the conversion of HS^- to the disulphide, S^{2-}. From the $E_{\frac{1}{2}}$ versus pH plot data, it is seen that the signal obtained from hydrogen sulphide is absent below pH 6 to 7, owing to the loss of hydrogen sulphide gas. The $E_{\frac{1}{2}}$ versus pH equation for hydrogen sulphide is

$$E_{\frac{1}{2}} = (-0.30 - 0.035\ pH)\ V.$$

This corresponds to one proton involved for two electrons in the reduction process, from the Nernst equation

$$E = E^{o} + (RT/nF).\ln([ox]/[red]).$$

Hydrogen sulphide was observed to form from cephalexin and its DKP derivative rapidly in alkaline solutions (sodium hydroxide, 0.1 M) at room temperature, and also in the cases of 7-ADCA and 7-ACA (see figs. 27-30).

A reliable normal pulse polarographic method has been used for the determination of sulphide from degrading cephalosporin solutions. The method is based on the reaction of mercury (Hg^{o}) and sulphide ions (S^{2-}) to form mercury(II)sulphide. The $E_{\frac{1}{2}}$ potential for the anodic current is -0.79 V with NPP in sodium hydroxide (0.5 M). A rectilinear relationship between current and sulphide concentration was observed in the range 0.3 x 10^{-4} M to .6 x 10^{-4} M of sulphide (see fig. 37).

Alkaline hydrolysis of a number of cephalosporins at 100°C in sodium hydroxide (0.5 M) produces hydrogen sulphide in reproducible yields. The yields range from 18.5% (cephalothin) to 65.6% (cephradine) as determined by the NPP method. Good agreement was observed with a spectrophotometric method[198] and a potentiometric method[213] using the same hydrolysis conditions to produce hydrogen sulphide from cephalosporins except for three compounds, cephalonium, cephoxazole and cefazolin (see Table 38). It is suggested that organic sulphur compounds produced in the alkaline hydrolysis of these cephalosporins, which subsequently degrade to hydrogen sulphide or electroinactive compounds in acidic solutions, are responsible for the high values observed for these three compounds. Thioamides, thioureas and thiobarbiturates are reported to undergo a reaction with mercury ions under anodic conditions to give partially insoluble mercury salts. A side reaction then occurs in which a carbon-sulphur bond is broken to release mercury(II) sulphide which adsorbs at the electrode surface[219]. This may

account for the high values obtained from the three compounds using polarography and why in the potentiometric method[213] normal values were observed as the organic sulphur compounds may not have reacted with the lead(II) ions.

Calculated molar yields of sulphide as determined by DPP and NPP under the same conditions (see Tables 30 and 38), do not compare well and show the unreliability of DPP for sulphide analysis[212].

The $E_{\frac{1}{2}}$ versus pH plots for the alkaline degradation of cephalosporins showed that with hydrolysis at 100°C two thiols are produced. In general no correlation was observed between the amount of thiol and hydrogen sulphide produced, i.e. a high value recorded for sulphide did not mean a low amount of thiol was produced.

Alkaline hydrolysis of cephalosporin also produced two waves independent of pH at $E_{\frac{1}{2}}$ = -1.03 V and $E_{\frac{1}{2}}$ = -1.23 V, suggesting that protons are not transferred in the reduction process. The compound that reduces at approximately -1.23 V is only formed with 100°C hydrolysis, as are the thiols.

7-ADCA and cephalexin both form a carbonyl compound whose reduction potential is independent of pH at -1.26 V.

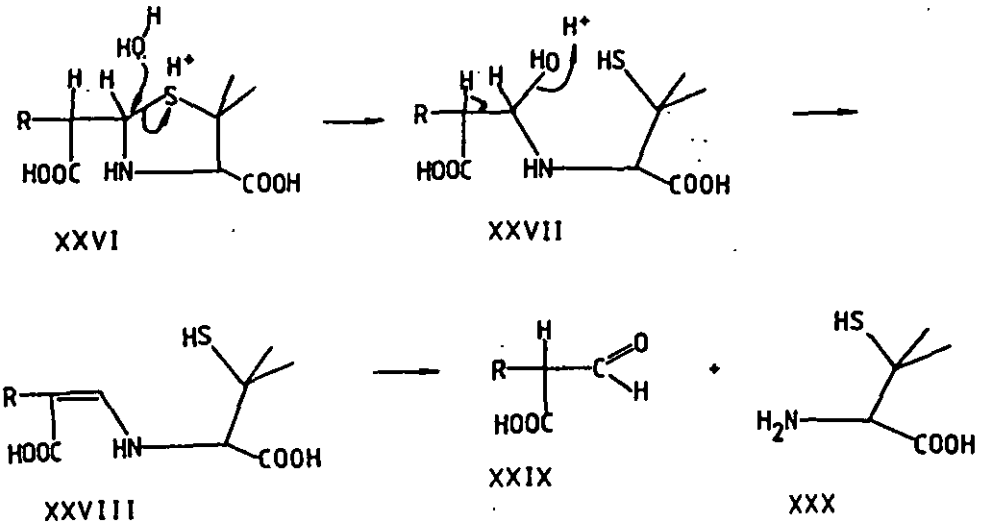
Penicillins when subjected to alkaline hydrolysis are reported to degrade via their penicilloic acid derivatives[121]. The penicillins examined and several of their isolated degradation products did not produce any polarographically active alkaline degradation products that corresponded to the compounds produced from cephalosporin degradations. Their different structure from cephalosporins precludes the formation of compounds with a carbon-nitrogen double bond and clearly penicillamine is not formed in detectable quantities although Rapson *et al.*[122] found from pK_a values that benzylpenicilloic acid forms benzylpenaldic acid and penicillamine via the penamaldic acid.

As reported by Abdalla et al.[198] penicillins were found not to release hydrogen sulphide on alkaline hydrolysis, showing a different mechanism of breakdown to cephalosporins.

Beta-lactam antibiotics have been found previously to give pyrazine derivatives under various conditions[143-151]. The addition of formaldehyde increases the rate of formation and the yield of such fluorescent derivatives. The production of pyrazines has been investigated polarographically in this study using a variety of experimental solution conditions.

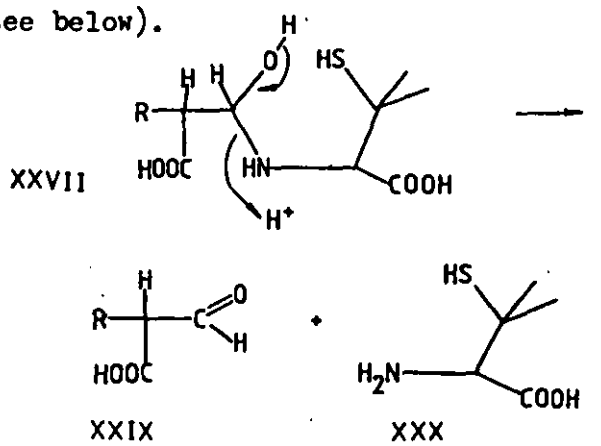
The optimum time for the beta-lactam to spend undergoing alkaline hydrolysis at room temperature prior to an acid hydrolysis step to produce pyrazine is one hour. However well over 50% of the intermediate compound found in the acid hydrolysis step which goes on to form the pyrazine is formed within one minute of alkaline hydrolysis. The most likely compound to be formed in this initial alkaline hydrolysis step is the corresponding penicilloic[121] or cephalosporoic acids[188].

When the penicilloic acid derivative of ampicillin is subjected to acid hydrolysis maximum pyrazine formation occurs at pH 5[142]. Le Belle et al.[154] has put forward a mechanism for pyrazine production from ampicillin in which the penicilloic acid derivative undergoes rearrangement at pH 5 to the pyrazine. In this mechanism (see scheme V) the penicilloic acid (XXVI) is converted to the penaldic structure (XXIX) and penicillamine (XXX) via the penamaldic acid (XXVIII).



Scheme V

The attack on the carbon at C-5 of the penicilloic acid's thiazolidine ring is feasible due to its instability with being between two hetero atoms. Penamaldic acid is formed as condensation at C_5-C_6 occurs in compound (XXVII). Formation of penaldic acid (XXIX) must then occur by initial attack by water to form compound (XXVII) and breakage of the C_5-nitrogen bond. What is more energetically feasible is that compound (XXVII) directly forms the penaldic acid (XXIX) without going through a penamaldic stage (see below).



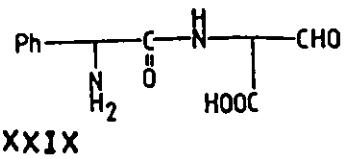
Scheme VI

The penaldic acid (XXIX) will then as a beta-keto-acid decarboxylate to penicilloaldehyde (VI).

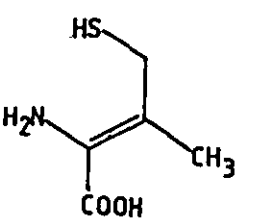
The incorrect supposition by Le Belle et al.[154] is that as pyrazines are formed from solutions known to involve the formation of the penamaldic acid derivative of ampicillin[117,142,150,152] then they must be key intermediates in penicilloaldehyde and pyrazine formation.

Penamaldic acid under various conditions[122-5] is thought to form its penaldic acid derivative and perhaps if any penamaldic acid is formed at pH 5 from penicilloic acid then it may convert to penaldic acid and to penicilloaldehyde.

This modified mechanism of Le Belle et al.[154] to form pyrazines may equally occur with cephalosporins. The carbon atom at C-6 in the thiazine ring of cephalosporoic acids is in the same unstable environment as the C-5 carbon of the penicilloic acid and will be the site for attack which may lead to penaldic acid (XXIX) and the enamine (XXXI).



XXIX



XXXI

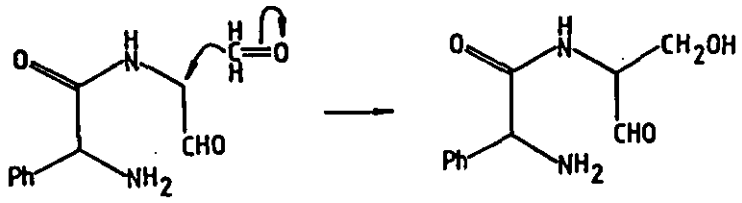
Hamilton-Miller et al.[193] have reported cephalosporins degrading to structures tentatively proposed to be penamaldates and penaldates in basic solutions when undergoing aminolysis.

The addition of formaldehyde to the mild acid step in the formation of pyrazine from α-aminobenzyl-β-lactams results in an increase of 1.5 to 2.5 times in the signal from these pyrazine derivatives. Indelicato et al. have proposed that a key intermediate in pyrazine formation is the enamine (fig. 8) formed by cyclization of penicilloaldehyde (VI)[159]. Production of a pyrazine from this enamine would normally require an oxidation step. However in the presence of an aldehyde the enamine will form an alcohol.

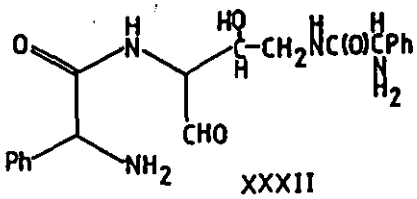
Loss of water forms an exo-cyclic methylene compound which will convert to a pyrazine (see fig. 8).

The formation of pyrazine without formaldehyde requires more energy as an oxidation step is required and this is reflected in the lower yield obtained when formaldehyde is not included in the reaction scheme.

According to Uno et al.[158] formaldehyde is incorporated into the reacting molecule before cyclization of the penicilloaldehyde.



Uno et al.[158] also suggest that in the absence of an aldehyde two penicilloaldehyde molecules will combine to form an alcohol (XXXII) which will then cyclise to form the 6-penicillopyrazine derivative (see page 53).



XXXII

A combination of the required oxidation of the enamine to pyrazine and the steric hindrance imparted by the alcohol (XXXII) to form the 6-penicillopyrazine may lead to the lower signal obtained when the acid hydrolysis is carried out in the absence of formaldehyde.

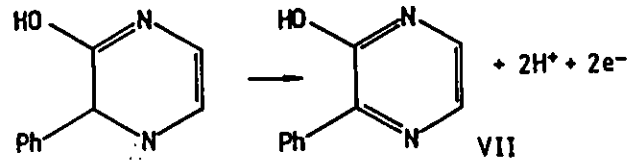
Comparison of results using formaldehyde and acetaldehyde in the acid hydrolysis step confirms the incorporation of aldehydes into the pyrazine molecule[58,158] (see Table 56), as the value for pyrazine production using acetaldehyde is lower due to steric hindrance.

The compound responsible for the initial polarographically active alkaline degradation product of cephalosporins (-0.39 V, pH 2) was observed to degrade rapidly at pH 5 (-0.61 V) when the solution was heated.

Elevated temperatures (100°C) at pH 5 are required for the formation

of high yields of pyrazine derivatives from the alkaline degradation solutions of α-aminobenzyl-β-lactams.

The absence of dissolved molecular oxygen has no effect on the mild acid hydrolysis step to form pyrazines, with or without aldehydes being present, (Table 56). Therefore, in the absence of an aldehyde the oxidation of the enamine (XXXIII) to pyrazine (VII) does not require molecular oxygen (see below).

XXXIII  VII

Larger amounts of pyrazines were formed when the acid hydrolysis step was performed in the presence of formaldehyde at pH 5 than at pH 2. The lower pH decreases the effectiveness of the formaldehyde to form the 6-methylpyrazine derivative (V) (see Table 59).

The acidic (pH 2) degradation of ampicillin itself produces pyrazine[80]. Penicillins have been reported to undergo degradation to their penicilloaldehydes in acidic (pH 2.5) solutions[41,111]. The slightly acidic (pH 5) degradation of ampicillin produces less pyrazine than at pH 2 (see Tables 57-8, 61-2). The addition of formaldehyde to degrading solutions of intact ampicillin has a detrimental effect on pyrazine production at pH 5 while at pH 2 it has no effect. The formaldehyde may be attacking the ampicillin molecule at pH 5 and preventing formation of penicilloaldehyde. As the pH is lowered the formaldehyde is inactivated and normal degradation via penicilloaldehyde occurs.

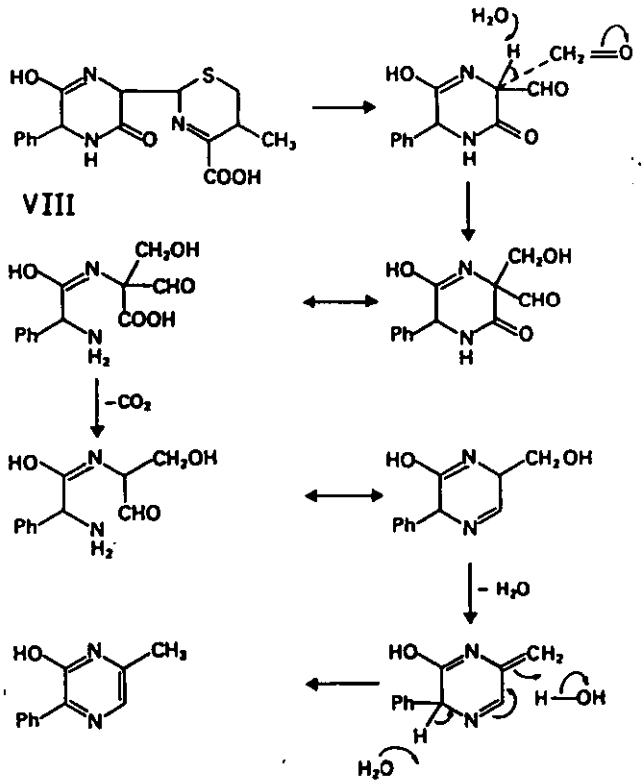
Addition of Cu(II) ions to penicillin G solutions at pH 5 results in the formation of its penicilloic acid[131]. Results from this study (Table 64) show that low amounts of pyrazines are formed from ampicillin solutions containing Cu(II) ions. α-aminobenzylpenicillins readily form polymers in aqueous solutions by intermolecular aminolysis[161-166]. Gensmantel et al.[134]

have reported that when penicillin G is coordinated with Cu(II) ions in a 1:1 complex it undergoes aminolysis by amines ca. 10^7-fold faster than uncoordinated penicillin G. The ampicillin may be undergoing intermolecular aminolysis to dimers and preventing formation of penicilloic acid.

The addition of formaldehyde to the acid hydrolysis step in pyrazine formation from cephalexin did not affect the amount of hydrogen sulphide formed at pH 5. If the formaldehyde was involved in a reaction step earlier than the penicilloaldehyde stage, e.g. formation of penaldic acid, then its presence would possibly increase the amount of sulphur released as hydrogen sulphide at pH 5. This evidence suggests that formaldehyde is only involved in the reaction when the C_6-sulphur and C_6-nitrogen bonds in the thiazine ring have been broken.

Diketopiperazine derivatives are closed ring forms of α-aminobenzyl-cephalosporoic acids. The DKP derivative of cephalexin degrades readily at 100°C and pH 5, with addition of formaldehyde accelerating this process. The open chained cephalosporoic acid is not produced as pyrazine compounds are not seen to form. Presumably formaldehyde is added to the DKP to form some product other than pyrazines.

Previously as the result of preliminary work carried out by this researcher a mechanism was published in an attempt to explain the formation of 6-methylpyrazine derivatives from cephalosporins involving formaldehyde with no oxidation step[220] (see below).



Scheme VII

This mechanism is basically the same put forward by Indelicato et al.[159] in 1981 (fig. 8), i.e. attack by formaldehyde on a closed ring system to produce an alcohol which loses water and forms the 6-methylpyrazine derivative, although further work by this researcher discounted the DKP derivative (VIII) as a key intermediate by its failure to produce pyrazine.

Alkaline hydrolysis of the DKP derivative of cephalexin (VIII) does not produce the cephalosporoic acid derivative as acid hydrolysis (pH 5) of its degraded alkaline solution did not produce pyrazines.

Glycine anhydride (X) is a diketopiperazine but is not seen to form any polarographically active compounds on degradation.

Degradation of the aminomethylene derivative of cephalexin (AMDC) (XIII) did not produce pyrazines except under concentrated acid conditions in HCl (5 M). At pH 5 and in the presence of formaldehyde the AMDC

degraded to a compound also formed from cephalosporins under the combined alkaline/acid hydrolysis steps to produce pyrazines. The identity of this compound is unknown.

The diketo-aldehyde (XII) formed from the acidic degradation of cephalexin at pH 3.3 does not form pyrazines when degraded at pH 5, and is excluded as a key intermediate in the fluorophore's formation under these conditions.

Acid hydrolysis of α-aminobenzyl-β-lactams produces pyrazines. In general the addition of formaldehyde produces a greater yield of pyrazine. The addition of acetaldehyde results in lower yields for pyrazine production in HCl (5 M) than is obtained with formaldehyde but in larger yields than without any aldehyde, which suggests steric hindrance. Beta-lactam hydrolysis in sulphuric acid at 100°C compares well with the yield obtained from the combined alkaline/acid hydrolysis steps to form pyrazines, although the time taken for maximum formation (6 h) is too long to contemplate its use as an analytical method.

Bontchev *et al.*[184] have isolated the penicilloaldehyde derivative of cephalexin and ampicillin from their sulphuric acid degradation solutions. It is assumed that cyclization of this compound, as proposed at pH 5, occurs with the presence of formaldehyde ensuring no oxidation step is required.

The absence of dissolved molecular oxygen did not make any significant difference to the amount of pyrazine produced in the HCl (5 M) study, showing that in the absence of an aldehyde, oxidation of enamine (if formed) to pyrazine does not require molecular oxygen.

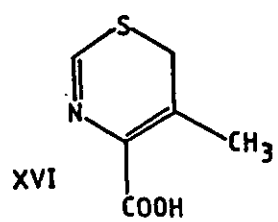
Cephalexin, alone among the α-aminobenzyl-β-lactams examined, when degraded in HCl (0.3 M) at 80°C produces low yields of pyrazine; the addition of formaldehyde has a detrimental effect. Degradation of cephalexin in HCl (5 M) at 100°C produces even higher amounts of pyrazine,

and formaldehyde has no effect. This phenomenon has not been satisfactorily explained so far.

The DKP derivative of cephalexin does not form pyrazine derivatives in hydrochloric acid (100°C) but gives small amounts in sulphuric acid (100°C), and the addition of formaldehyde increases the yield.

The degradation of a number of intact cephalosporins in sulphuric acid could be followed by observing the decline in the wave responsible for reductive elimination at the C-3 position. Addition of formaldehyde increased the rate of the degradation of these compounds.

In the sulphuric acid degradation of cephalexin at 100°C hydrogen sulphide was found not to be evolved, suggesting that the sulphur atom remains in the thiazine ring as (XVI) as suggested by Bontchev et al.[184].



XVI

Acid hydrolysis (pH 2) of penicillins at room temperature does not afford polarographically active compounds, although ampicillin degraded to produce small amounts of a pyrazine compound.

In the absence of dissolved molecular oxygen ampicillin, penicillin G and penicillin V produce penicillamine in acidic solutions (pH 2.5) at elevated temperatures (100°C). Ampicillin gives the largest yield, probably due to the formation of pyrazine forcing the reaction towards breakage of the penicillin to form penaldic acid and penicillamine. Penicillin G and penicillin V give similar yields of penicillamine. Penicillamine is however relatively unstable in acidic solution (pH 2.5) at 100°C. The effect of the absence of dissolved oxygen was to increase the amount of penicillamine formed from boiling solutions (pH 2.5) of ampicillin. These results confirm the observation of Blaha et al.[111] that the acidic

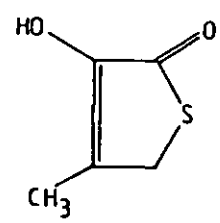
hydrolysis of penicillins produce penicillamine. Due to its instability it was not thought suited to be used for analytical purposes.

A possible explanation of the instability of penicillamine in the presence of dissolved molecular oxygen is due to oxidation to the disulphide, R-S-S-R, where R-SH is penicillamine. If it occurred the reaction would proceed as $2R\text{-}SH \xrightarrow{O_2} R\text{-}S\text{-}S\text{-}R + 2H^+ + 2e^-$, and would be irreversible as with reversibility the DPP peak signal obtained for degraded penicillamine would remain the same as undegraded penicillamine.

A pyrazine compound was produced from strong (0.5 M, 100°C) alkaline hydrolysis of cephalexin. This confirms the observations of Hamilton-Miller *et al.*[193] that cephalosporin C derivatives degrade in alkaline solutions after aminolysis to penamaldates and penaldates. Any α-amino-penaldic acid formed may go on to form a pyrazine derivative.

The DKP derivative of cephalexin (VIII) under the same alkaline conditions did not form a pyrazine. Therefore it is unlikely that ring opening occurs to form penamaldates and penaldates as derivatives.

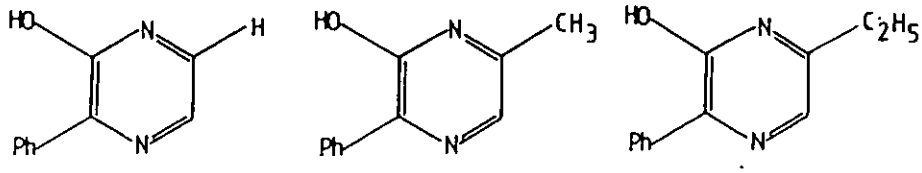
Both cephalexin and its DKP derivative produced a carbonyl compound whose reduction potential (-1.26 V) was independent of pH, and is thought to form from a thiophenone (XVII).



XVII

Alkaline hydrolysis of 7-ADCA also produces the -1.26 V wave (carbonyl compound), presumably also via the thiophenone.

The different pyrazine derivatives produced in degrading solutions cannot be distinguished by polarography.



The different contributions the substituents at the C-6 position, hydrogen atom, methyl and ethyl groups make to the reducibility of the pyrazine is negligible.

The $E_{\frac{1}{2}}$ versus pH equation for all the pyrazine derivatives produced is $E_{\frac{1}{2}} = (-0.50 - 0.065\ pH)$ V and corresponds to an equal number of protons and electrons being involved in the reduction process.

The acidic (pH 3.3) degradation of cephalexin at 75°C is seen to produce a pyrazine compound. Previously Dinner[207] reported that cephalexin degraded at pH 3.3 to a diketoaldehyde (XII) and a thiophenone (XVII). Formation of the pyrazine means that at pH 3.3 the cephalosporoic acid derivative is being formed from cephalexin, which degrades to penicilloaldehyde. Maximum time of the pyrazine formation (90 minutes) coincides with the time for complete cephalexin degradation under these conditions[207] (see fig. 47).

Synthesis of the thiophenone at pH 7.4[208] produces relatively uncontaminated thiophenone (XVII), this is because at this pH 96% of the cephalexin molecules forms its precursor the DKP derivative, and less than 4% forms the cephalosporoic acid[204].

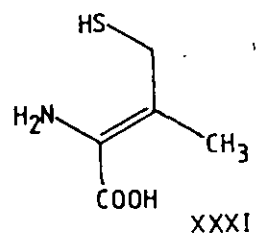
The DKP derivative of cephalexin is not observed in degrading solutions of cephalexin, at pH 3.3, by polarography. This supports Dinner's[207] observation that as soon as it is formed the DKP degrades to the diketoaldehyde (XII) and the thiophenone (XVII).

The DKP derivative (VIII) when degraded at pH 3.3 was only seen polarographically to form hydrogen sulphide.

The amount of hydrogen sulphide evolved as cephalexin degrades at

pH 3.3 (75°C) after 90 minutes, the time quoted for the total degradation of cephalexin[207], was less than 0.2%, suggesting that the rest of the sulphur present may be locked up in the thiophenone and a thiol.

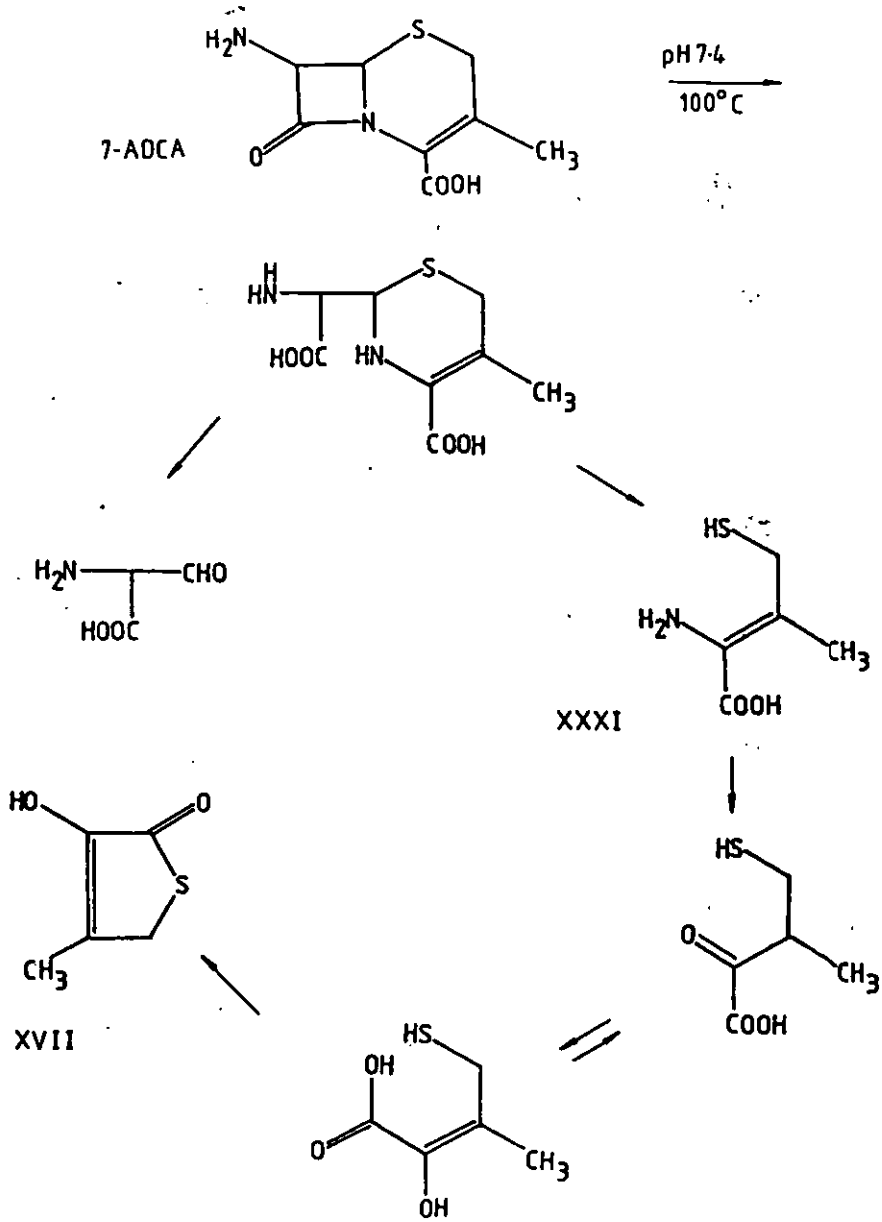
Degradation of cephalexin at pH 3.3 produces two thiols. Thiol II (-0.13 V, pH 7.4) is only formed from the degradation of the isolated thiophenone (XVII). Thiol I (-0.37 V, pH 7.4) is formed (i) in large yields from the degradation of cephalexin at pH 3.3 (75°C), (ii) in very small yields from the degradation of the thiophenone and (iii) not at all from the degradation (pH 3.3, 75°C) of the DKP (VIII). Thiol I may be a degradation product of cephalosporoic acid when it degrades to form the penaldic acid, with possible structure (XXXI).



XXXI

Degradation of cephalexin at 100°C and pH 7.4 produces large amounts of a carbonyl compound[61,79] whose reduction potential (-1.26 V) is independent of pH. Degradation of the isolated thiophenone (XVII), at pH 7.4, produced large amounts of the carbonyl compound, hydrogen sulphide (51% of total sulphur evolved as H_2S in 1 hour), thiol I and thiol II. Presence of thiol I suggests contamination by its precursor, and the degradation products proper of the thiophenone are hydrogen sulphide, the carbonyl and thiol II.

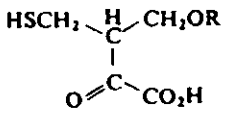
The carbonyl compound (-1.26 V) is observed from the neutral degradations of 7-ADCA, which contains an unsubstituted methyl group at C-3. The thiophenone may be formed from 7-ADCA via its cephalosporoic acid as it, unlike cephalexin, cannot form a DKP derivative (see scheme VIII).



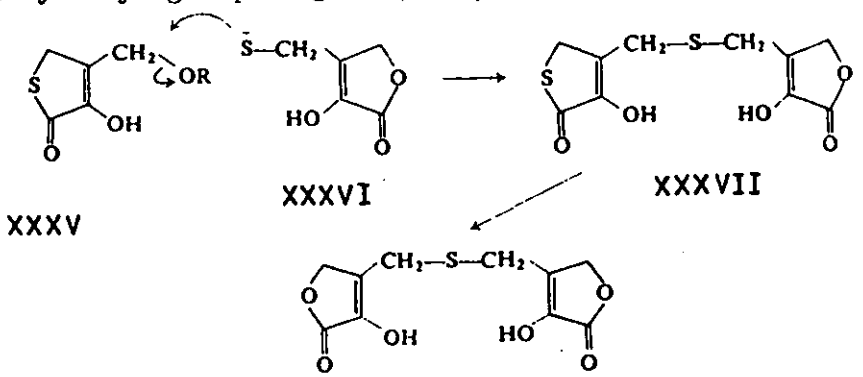
Scheme VIII

In general, penicillins and cephalosporins without a C-3 methyl group do not form the thiophenone as its breakdown product, the carbonyl compound (-1.26 V, pH 7.4), is not observed in their degrading solutions. This is because the possible precursor to any thiophenone formed would be compound (XXXIV)

XXXIV



whose carboxyl group could lactonise with either the thiol group or the potential hydroxyl group to give (XXXV) or (XXXVI).

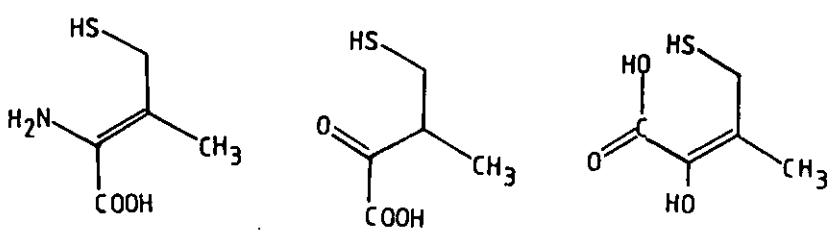


XXXV XXXVI XXXVII

XXXVIII

Condensation of (XXXV) with (XXXVI) as shown above would give compound (XXXVII). Compound (XXXVIII) could then be formed by β, γ-elimination of sulphur from the ketone form of the thiolactone ring of (XXXVII) followed by elimination of hydrogen sulphide and relactonization. Compounds (XXXVII) and (XXXVIII) have been isolated from the degraded acid solution of cephalosporin C by Abraham et al.[221] who suggested the above mechanisms.

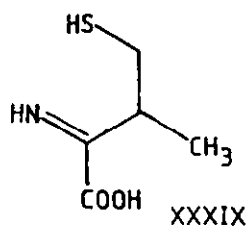
A relatively low percentage (11%) of the isolated DKP derivative of cephalexin forms the thiophenone after hydrolysis at 100°C (pH 7.4, 1 hour) compared with 44% for cephalexin and 24% for 7-ADCA. DKP has been cited as a key intermediate in thiophenone production[207]. With this key compound only present, its degradation products (see below)



could easily combine resulting in a low yield of thiophenone.

88% of the available sulphur in the cephalexin molecule forms hydrogen sulphide after 1 hour, 100°C, pH 7.4, while only 47% of the DKP does so under the same conditions, which may be connected to the side reactions of the DKP derivatives degradation products forming stable sulphur compounds.

A summary of the reactions of cephalexin at pH 3.3 and pH 7.4 discussed is shown in fig. 65. In fig. 65 the formation of the aminomethylene compound (XIII) has two routes, (i) directly from the DKP[207], and (ii) by addition of ammonia to the diketoaldehyde (XII). Bundgaard suggests that route (ii) may be possible by addition of ammonia, liberated from the hydrolysis of a thiolimine (XXXIX) to the diketoaldehyde (XII).[208]



XXXIX

Fig. 66 shows a hypothetical degradation scheme for the thiophenone (XVIII) to form a thiol, hydrogen sulphide and carbonyl compounds. The final degradation products in fig. 66 are hydrogen sulphide, glycolic acid (XXXX), glyoxal (XXXXII) and acetone (XXXXIII). A precursor to acetone and hydrogen sulphide formation is the thiol (XXXXI).

The structure of the carbonyl compound responsible for the -1.26 V wave from the neutral degradation of 7-ADCA and cephalexin is not thought to be due to these final carbonyl degradation products as none reduce in the available potential range[222], although glyoxal (XXXXII) does so at -1.41 V (pH 3.4)[222]. The carbonyl compound may be one of the precursors of these final degradation products.

Thiol II, the degradation product of the thiophenone (XVII) may have structure (XXXXI) although an authentic sample was not available for comparison.

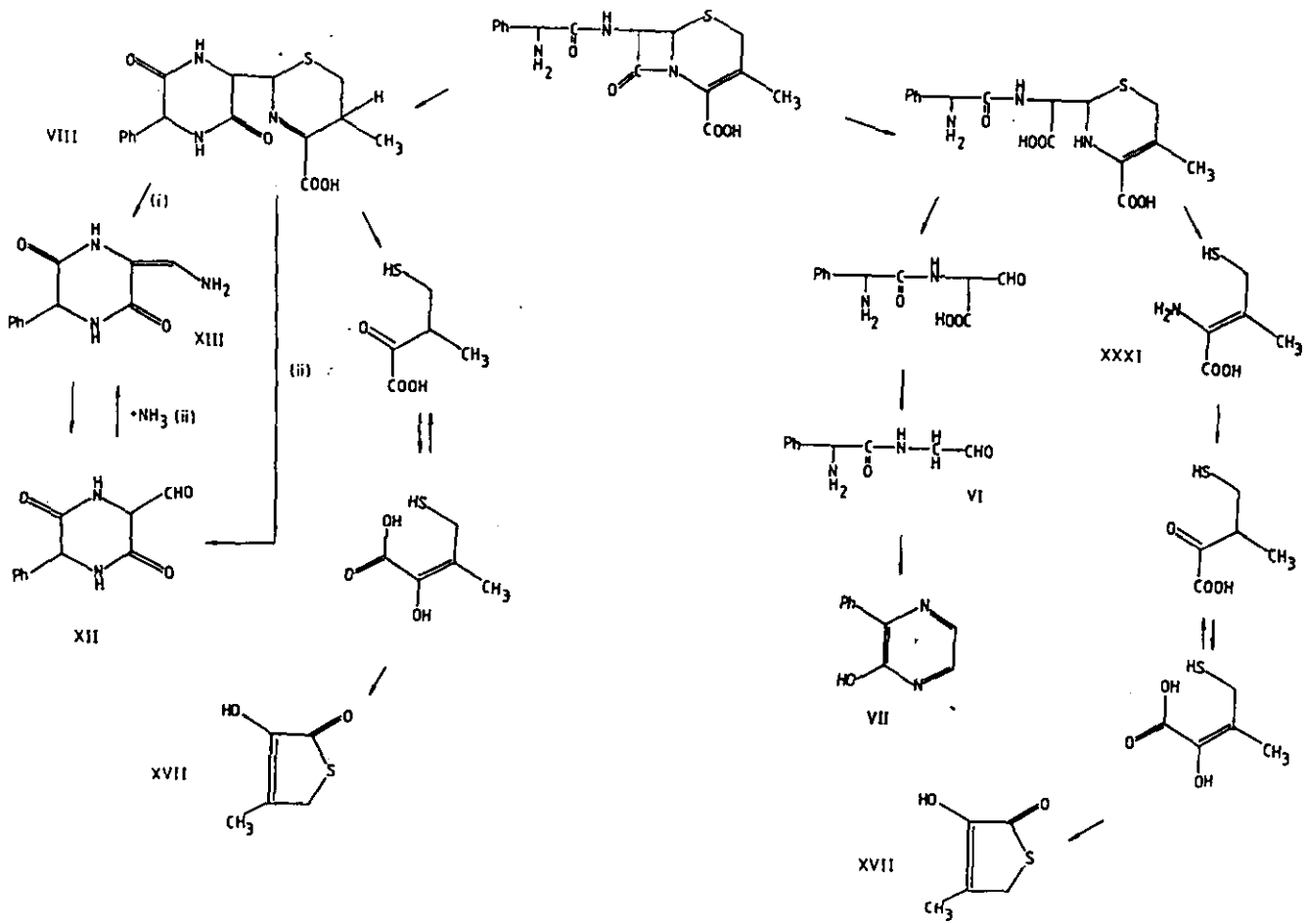


Fig. 65

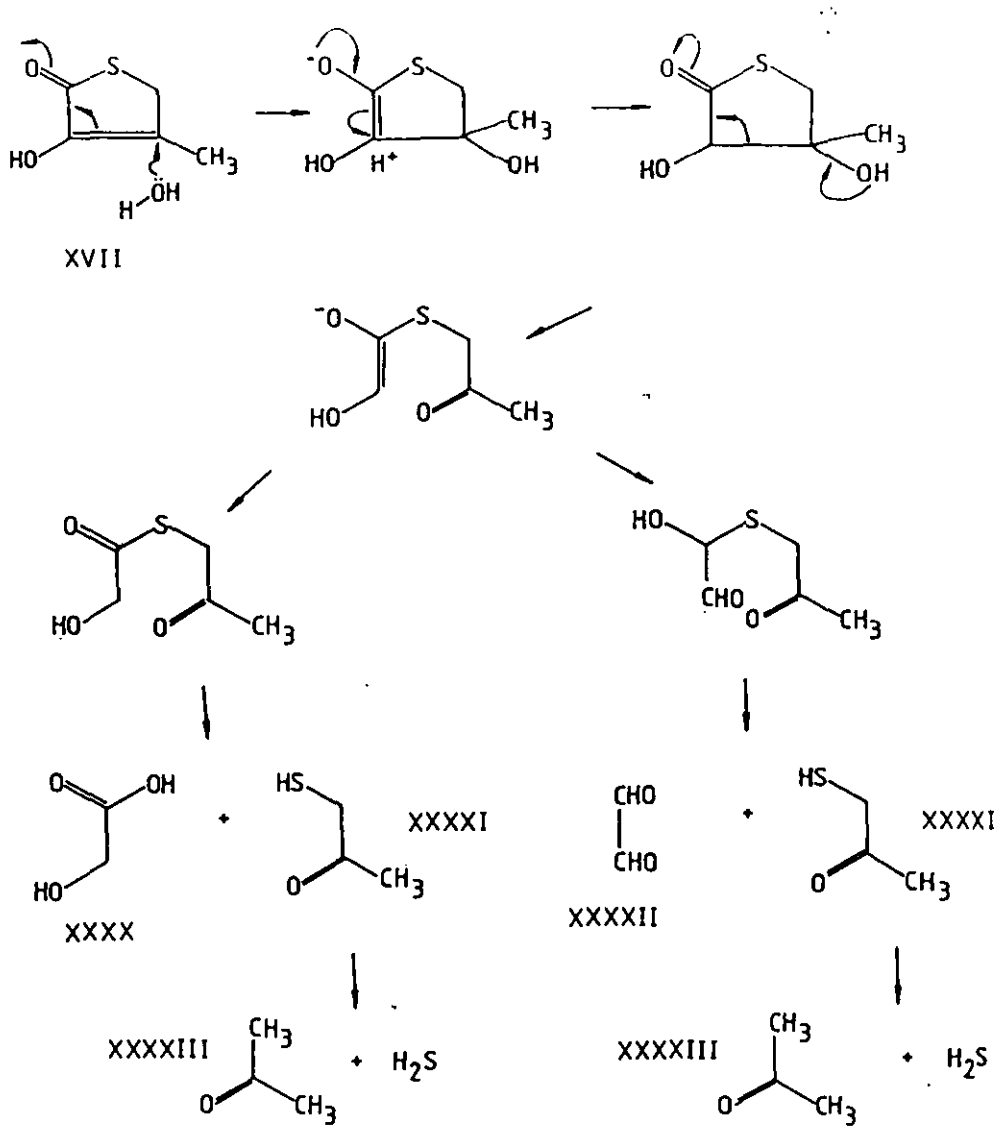


Fig. 66

Neutral degradations of cefaclor and cephaloglycin produce pyrazine and DKP derivatives. The DKP of cefaclor and its pyrazine derivative both reduce at the same potential at pH 7.4 (-0.96 V). Cephaloglycin degrades swiftly at pH 7.4, 37°C and almost no intact compound is remaining after 10 hours' hydrolysis. An increase in the temperature of degradation produces increases in the rate of decline in the DKP derivatives and the rates of formation of pyrazine and hydrogen sulphide from both antibiotics. When cephaloglycin is degraded at pH 8.5 (100°C) the amount of DKP formed is decreased and that of pyrazine is increased. This is due to the increase in the amount of cephalosporoic acid produced from the compound, as the pH increases, as less intramolecular aminolysis to the DKP occurs[204]. The same effect has previously been observed with cephalexin[79]. The three DKP derivatives from cephalexin, cephaloglycin and cefaclor may be distinguished from each other by their different $E_{\frac{1}{2}}$ versus pH equations.

The work carried out in this study on the degradations of cephalosporins and penicillins has illustrated the use of a normally quantitative analytical technique, polarography, as an instrument of qualitative analysis. Considerable information has been obtained using polarography which would have been difficult to obtain by other methods in addition to information that has confirmed previous work by other investigators using other methods.

It has led to a better understanding of the degradation chemistry of these β-lactam antibiotics and will hopefully lead to better analytical techniques to determine the parent compounds.

Polarography has also enabled the detection of some degradation intermediates, and provided valuable information about the optimum conditions for their preparation.

In the future the unanswered questions posed by this work could be solved using techniques that polarography would complement, such as U.V. spectroscopy, N.M.R. measurements of isolated compounds and H.P.L.C.

studies of degrading solutions, and work in these fields is already underway by fellow researchers in this group.

APPENDIX

The aminomethylene derivative of cephalexin (100 ppm) was dissolved in Sorensen buffer (pH 5) (5% v/v ethanol). Linear sweep cyclic voltammograms at 20 mV/second were performed at the pH values of 2.2, 5.4 and 7.0 (see Table below).

pH	V (+)	µA	nature of wave
2.2	0.70	2.6	irreversible
5.4	0.61	5.8	irreversible
7.0	0.35	5.4	irreversible

A blank containing no aminomethylene derivative gave no waves at the above pH values.

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