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Degradation Kinetics in Aqueous Solution of Cefotaxime Sodium, a Third-Generation Cephalosporin

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Abstract □ The degradation kinetics of a 3-acetoxymethylcephalosporin, cefotaxime sodium salt, in aqueous solution investigated by HPLC under different conditions (pH, ionic strength, temperature) and using different buffers. The scheme of degradation involves a cleavage of the β-lactam nucleus and the deacetylation of the side chain. In highly acidic medium, the deacetylated derivative is easily converted to the lactone. The degradation rate constants were calculated at three pH values (1.9, 4.0, and 9.0) by measuring the residual cephalosporin and the main decomposition products. The degradation pathway is both supported by the results of a primary salt effect and by the agreement between the theoretical pH-rate profile and the experimental values. In the pH range from 3.0 to 7.0, the main process is a slow water-catalyzed or spontaneous cleavage of the β-lactam nucleus with intramolecular participation of the side chain amido fraction in the 7-position. In alkaline or strongly acidic medium, the hydrolysis is a base- or acid-catalyzed reaction. Of the buffer systems investigated, carbonate buffer (pH 8.5) and borate buffers (pH 9.5 and 10.0) are found to increase the degradation rates, while acetate buffer decreases the degradation rates. The apparent activation energies determined at different pH values are compatible with a solvolysis mechanism and similar to those previously given in the literature for other cephalosporins. Cefotaxime in aqueous solution is slightly less stable than the main cephalosporin derivatives, despite its high resistance to the β-lactamases and its remarkable biological activity.

Keyphrases □ 3-Acetoxymethylcephalosporins—stability in aqueous solutions, HPLC, pH effect, primary salt effect, buffer effect, temperature effect □ Cefotaxime—kinetics and mechanism of degradation, pH-rate profile □ Antibiotics—cefotaxime, stability, degradation profile, kinetics

Systematic studies on the degradation of cephalosporin derivatives are of interest for several reasons: (*a*) a correlation between degradation and antibiotic activity has been shown in first- and second-generation cephalosporins (1), (*b*) some degradation products may be involved in allergic reaction (2), and (*c*) the stability of the compounds has to be known for the synthesis of derivatives (3) and the formulation of drugs. The kinetics of first- and second-generation cephalosporins have been reported in a few instances (1, 3–6). These studies concern a quantitative analysis of the antibiotic itself and sometimes the kinetics of the major degradation product (1, 3). In this report, a systematic kinetic study of a recently commercially available third-generation cephalosporin, cefotaxime sodium salt[1], has been carried out. The quantitation of this cephalosporin and its major decomposition products allowed the proposal of a degradation pathway.

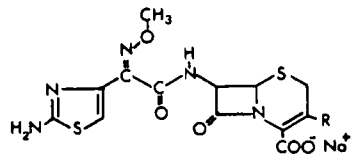
BACKGROUND

Cefotaxime sodium (I) is sodium [6(*R*)-[6α,7β(*Z*)]]-3-[(acetyloxy)-methyl]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate]. The possible *in vitro* degradation products are deacetylcefotaxime (II), deacetoxycefotaxime (III), deacetylcefotaxime lactone (IV), thiazoximic acid (V), and 7-aminocephalosporanic acid (VI). The *anti* isomer which could be expected as a potential degradation product of I has been shown to be formed only in nonaqueous medium (7) and has not been taken into consideration in this study.

Cefotaxime sodium (I) is an original cephalosporin derivative with a 2-amino-4-thiazolyl side chain and an α-methoximino group in the *syn* position. The former is probably responsible for the very great affinity for transpeptidase, involved in the construction of the bacterial wall, and of the great activity against Gram-negative bacilli. The latter is probably responsible for the stability of the drug against most β-lactamases (8, 9). An exhaustive review of the bacteriological and clinical properties of I has been presented (10).

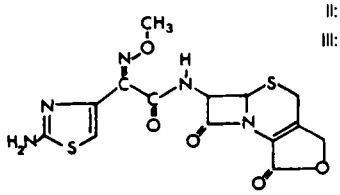
EXPERIMENTAL

Materials—Compounds I–VI were used as received[2]. UV, IR, and NMR spectra were used to confirm the structure of these compounds. All other chemicals were analytical reagent grade.

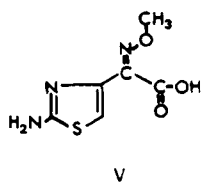


R

I: R=CH_2-O-CO-CH_3
II: R=CH_2-OH
III: R=CH_3



IV



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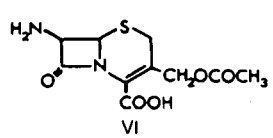

VI

[1] Trade names: Claforan, Tarivid, Zariviz, and Primafen.

[2] Gifts from Roussel UCLAF Laboratories.

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Table I—Pseudo-First-Order Rate Constants for I Degradation at Different Buffer Concentrations and Different pH

Buffer	pH	k_{obs}, $h^{-1} \times 10^3$					k_{pH}, $h^{-1} \times 10^3$
		0.05 M	0.10 M	0.15 M	0.20 M	0.30 M	
HCl–KCl	1.6	—	—	—	60.987	—	60.987
	1.9	—	—	—	38.129	—	38.129
Citrate	2.2	—	25.868	—	26.087	26.093	25.790
	3.0	—	14.290	—	14.720	15.056	13.930
Acetate	4.0	—	—	—	13.056	—	—
	5.0	—	13.020	—	12.106	10.921	14.125
Phosphate	6.0	—	14.460	—	15.019	16.528	13.267
	7.5	16.445	17.046	17.316	—	—	16.065
Carbonate	8.5	—	53.153	—	60.840	68.684	45.362
Borate	9.0	—	—	—	117.046	—	—
	9.5	—	341.016	—	359.424	383.465	318.840
	10.0	—	858.726	—	876.720	894.720	840.720

Buffer Solutions—The buffers used in the kinetic studies were hydrochloric acid–potassium chloride (pH 1.6 and 1.9), citrate (pH 2.2 and 3.0), acetate (pH 4.0 and 5.0), phosphate (pH 6.0 and 7.5), carbonate (pH 8.5), and borate (pH 9.0, 9.5, and 10.0). The buffer concentrations were 0.1, 0.2, and 0.3 M (except at pH 7.5, where concentrations were 0.05, 0.10, and 0.15 M, and at pH 4.0 and 9.0, where buffer concentrations were 0.2 M) and the ionic strength was adjusted to 0.5 with potassium chloride. Primary salt effects were investigated at 0.2 M buffer concentration at pH 2.2, 6.0, and 9.5 with ionic strengths of 0.3, 0.5, and 0.7. The pH of buffer solutions was controlled with a pH meter[3] standardized with pH 2.0, 7.0, and 9.0 solutions.

Kinetic Procedures—Experiments were performed in volumetric flasks. Compound I was dissolved in the buffer solution previously equilibrated at 4°C, 23°C, or 37°C. For the kinetics followed by HPLC, the concentrations used were $\sim 2 \times 10^{-3}$ or $\sim 1 \times 10^{-3}$ M according to the solubility. The samples were assayed immediately after a suitable dilution. For the kinetics followed by UV, the concentrations used were $\sim 2 \times 10^{-5}$ M. All experiments were conducted in the dark in a constant-temperature water bath with a thermoregulator at ±0.2°C or in a refrigerator for the trials at 4°C. pH Values were controlled at the end of each experiment; no significant change was observed.

Analytical Procedures—*Liquid Chromatography*—The liquid chromatograph[4] was equipped with a variable-wavelength UV detector[5] set at 235 nm, an RP 18 column[6], and a 10-μl injection loop. The mobile phase was phosphate buffer (pH 7.6)–methanol (83:17); the flow was 1.5 mL/min.

A mixed stock standard solution was prepared in the mobile phase using I–VI (75 μg of each/mL). This solution was suitably diluted with the mobile phase to give a concentration range from 7.5 to 37.5 μg of each compound/mL.

Ultraviolet—UV spectral changes as a function of time were followed at pH 1.9, 4.0, and 9.0 with a spectrophotometer[7].

pK_a Determinations—The pK_a values of I were determined by the potentiometric titration of a 2.8×10^{-3} M cefotaxime aqueous solution with 0.1 M NaOH, at 20°C, under nitrogen. The two functional groups corresponding to the terminal amino group of the side chain at C-7 and the carboxylic group at C-3 of the cephem ring were acidified with a stoichiometric amount of 0.1 M HCl before titration.

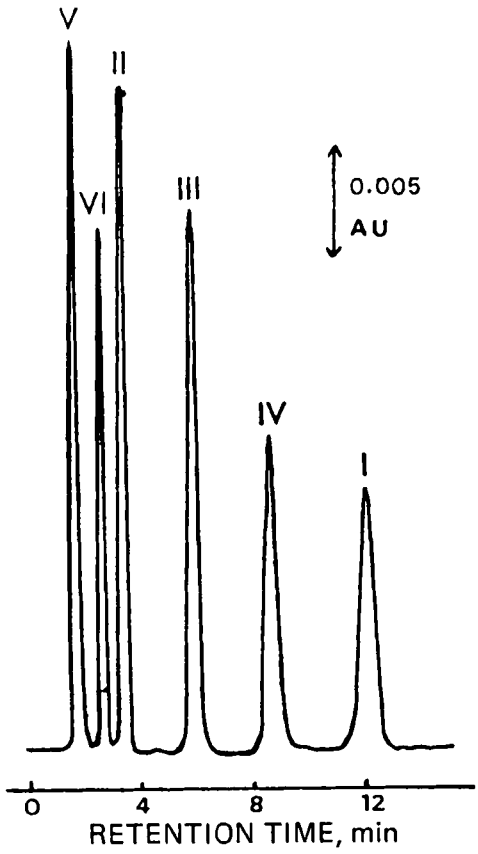


Figure 1—*Chromatogram of a standard solution of I (24.90 μg/mL), II (21.90 μg/mL), III (22.20 μg/mL), IV (23.25 μg/mL), V (14.00 μg/mL), and (26.40 μg/mL). The mobile phase was phosphate buffer (pH 7.6)–methanol (83:17); the flow was 1.5 mL/min and pressure was 175 bars. Detector sensitivity was 0.005 AUFS, chart recorder speed was 0.5 cm/min, and λ = 235 nm.*

[3] Potentiograph Metrohm E 436.

RESULTS AND DISCUSSION

Validity Tests of the Liquid Chromatographic Procedure—A chromatogram of a standard solution recorded at 235 nm (suitable wavelength for a simultaneous determination of I and its degradation products) is shown in Fig. 1. A calibration graph was plotted of the peak areas (or the height measure-

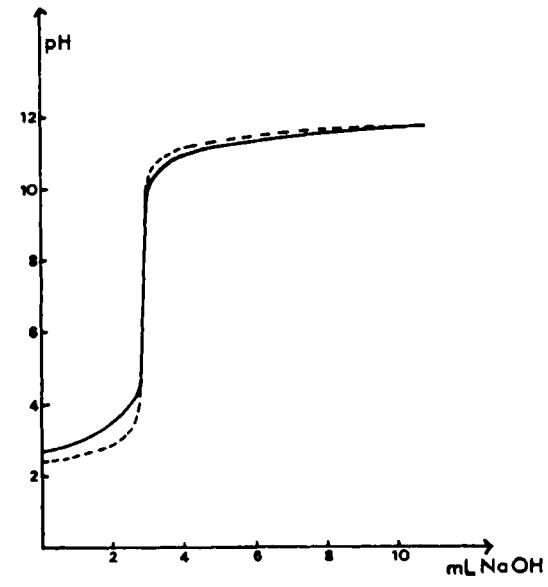
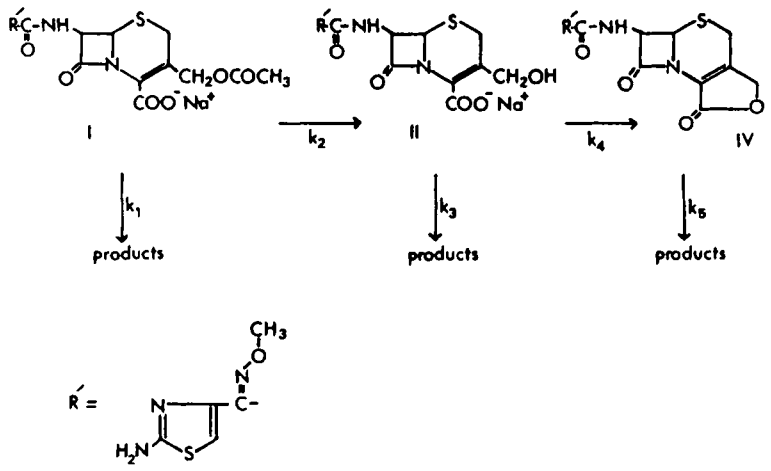


Figure 2—*Titration curve for 2.8×10^{-3} M cefotaxime sodium salt solution at 20°C. Dashed lines represent the blank determination. Two equivalents of acid have been added before titration.*

[4] Spectra-Physics SP 8000.
[5] Schoeffel, model SF 770.
[6] Merck, 7 μm, 25 cm × 0.4 cm.
[7] Beckman, model 25.



Scheme I

ments) of each solute against solute concentration. The correlation coefficients of the linear regression analysis were always >0.999 by peak areas (as well as height measurements). The repeatability, tested by six replicates and evaluated by the coefficient of variation, was 0.22% (I), 1.15% (II), 0.47% (III), 1.89% (IV), 0.60% (V), and 2.49% (VI). The recovery studies were carried out with a solution of cefotaxime with 1.0% and 3.0% (with respect to I) of II–VI added. The percentage recovered (average of two determinations) was found to be 98.17% for II, 98.64% for III, 107.52% for IV, 99.46% for V, and 98.86% for VI in the former case and 97.10% for II, 98.03% for III, 99.21% for IV, 105.66% for V, and 99.33% for VI in the latter case. Applying the same procedure to the determination of I using a suitable dilution, the recovery was 102.01 and 99.76%, respectively. The minimum amounts detectable were found to be 3.5×10^{-3} μg (I), 1.8×10^{-3} μg (II), 2.0×10^{-3} μg (III), 4.0×10^{-3} μg (IV), 0.8×10^{-3} μg (V), and 2.5×10^{-3} μg (VI). The proposed HPLC procedure therefore allows a satisfactory determination of I–VI. It is a more convenient procedure than TLC (11) for the kinetic studies.

Ionization Constants—The titration curve and the blank determination carried out under the same conditions are shown in Fig. 2. Apparent pK_a values were calculated (12) with a computer. Potentiometric comparative determination with III permitted us to attribute $pK_{a_1} = 2.1$ to the dissociation of the carboxylic group and $pK_{a_2} = 3.4$ to the terminal amino group of the side chain in the 7-position. The pK_{a_3} value of 10.9 may be referred to as the dissociation of the amino group in the α-carbonyl position on the same side chain. It must be noted that the pK_a values in acidic media are approximate values, because these pK_a values are very close to the logarithm of dilution (12).

Kinetic Results—The scheme proposed (Scheme I) for cephalosporin derivatives with an acetoxymethyl group should be valid for I in the entire pH range. The first step in the degradation involves deacetylation. The second step, in acidic medium is the conversion of the deacetylated compound (II) in its lactone form. In addition, I, II, and IV give products resulting from cleavage of the β-lactam ring. To check this postulated pathway, the kinetics of I, II, and IV between pH 1.6 and 10.0 were simultaneously followed by HPLC. Typical chromatograms of acidic, neutral, and basic solutions, withdrawn at ~30–40% cefotaxime degradation (0.2 M buffer concentration, 37°C, and $\mu = 0.5$ ionic strength) are shown in Fig. 3.

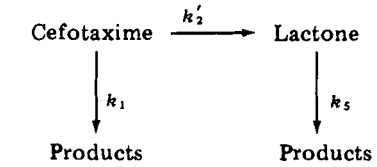
Reaction Order and HPLC Observed Constants—The example of the semilogarithmic plots of the data at various pH values (37°C, $\mu = 0.5$, and 0.2 M buffer concentration) illustrated in Fig. 4 show that the degradation of I follows a pseudo-first-order reaction. The results obtained at all the other pH, temperatures, ionic strengths, and buffer concentrations investigated gave the same order reaction. The observed constants, k_{obs}, for I calculated from the slopes are given in Table I.

According to Scheme I, in the alkaline and neutral pH media where lactone is not formed, the degradation of I may be illustrated by:

$$[I] = C_0 e^{-(k_1+k_2)t} \qquad \text{(Eq. 1)}$$

$$[II] = C_0 \frac{k_2}{(k_1+k_2)-k_3}[e^{-k_3 t} - e^{-(k_1+k_2)t}] \qquad \text{(Eq. 2)}$$

where [I] is the concentration of I at times t, C_0 is the initial concentration of I, and [II] is the concentration of II at time t. In a highly acidic medium, where the deacetylated derivative is very easily converted to lactone, the simplified degradation pathway (Scheme II[8]) was used:



Scheme II

with corresponding equations similar to Eqs. 1 and 2.

In the entire pH range, the kinetics of I, II, and IV were followed for one half-life, but to determine the rate constants k_1–k_5, the kinetics were followed at three different pH values (1.9, 4.0, and 9.0) at 37°C, 0.2 M buffer concentration, and $\mu = 0.5$, for 4–5 half-lives (Fig. 5). The rate constants obtained at these three pH values, analyzed using Eqs. 1 and 2 and B.M.D.P. program P_3R, are listed in Table II.

Reaction Order and UV Observed Constants—Spectral changes for the degradation of I as a function of time at pH 1.9, 4.0, and 9.0 (37°C, 0.2 M buffer concentration, and $\mu = 0.5$) were recorded for 4–5 half-lives (Fig. 6). Absorbance at 260 nm is characteristic of the β-lactam linkage (13). Applying Eq. 3:

$$\ln \frac{A_t - A_\infty}{A_0 - A_\infty} = -k_1' \cdot t \qquad \text{(Eq. 3)}$$

where A_t is the absorbance at time t measured at $\lambda = 260$ nm; A_∞ is the absorbance at infinite time at $\lambda = 260$ nm; A_0 is the absorbance at time zero at $\lambda = 260$ nm; and k_1' is the rate constant determined by UV measurements. Semilogarithmic plots of $(A_t - A_\infty)/(A_0 - A_\infty)$ *versus* time were obtained with a linear correlation of $r > 0.98$. The UV data ascertain for I the first-order reaction determined from HPLC results. The UV values of the pseudo-first-order constants k_1' are listed in Table II.

Table II—Rate Constants Calculated from HPLC Results and UV Measurements [a]

pH	k, $h^{-1} \times 10^3$					
	k_1 [b]	k_1' [c]	k_2 [b]	k_3 [b]	k_2' [b]	k_5 [b]
1.9	24	36	—	—	15	39
4.0	10	13	3	6	—	—
9.0	75	83	43	29	—	—

[a] At 0.2 M buffer concentration, $\mu = 0.5$, and 37°C. [b] From HPLC results. [c] From UV measurements.

Table III—Effect of Ionic Strength on the Pseudo-First-Order Rate Constant of I Degradation [a]

Buffer	pH	k_{obs}, $h^{-1} \times 10^3$		
		$\mu = 0.3$	$\mu = 0.5$	$\mu = 0.7$
Citrate	2.2	25.395	26.087	26.381
Phosphate	6.0	14.669	15.019	15.606
Borate	9.5	321.684	359.424	383.016

[a] At 0.2 M buffer concentration and 37°C.

[8] k_2' is the approximate value of the deacetylation constant k_2.

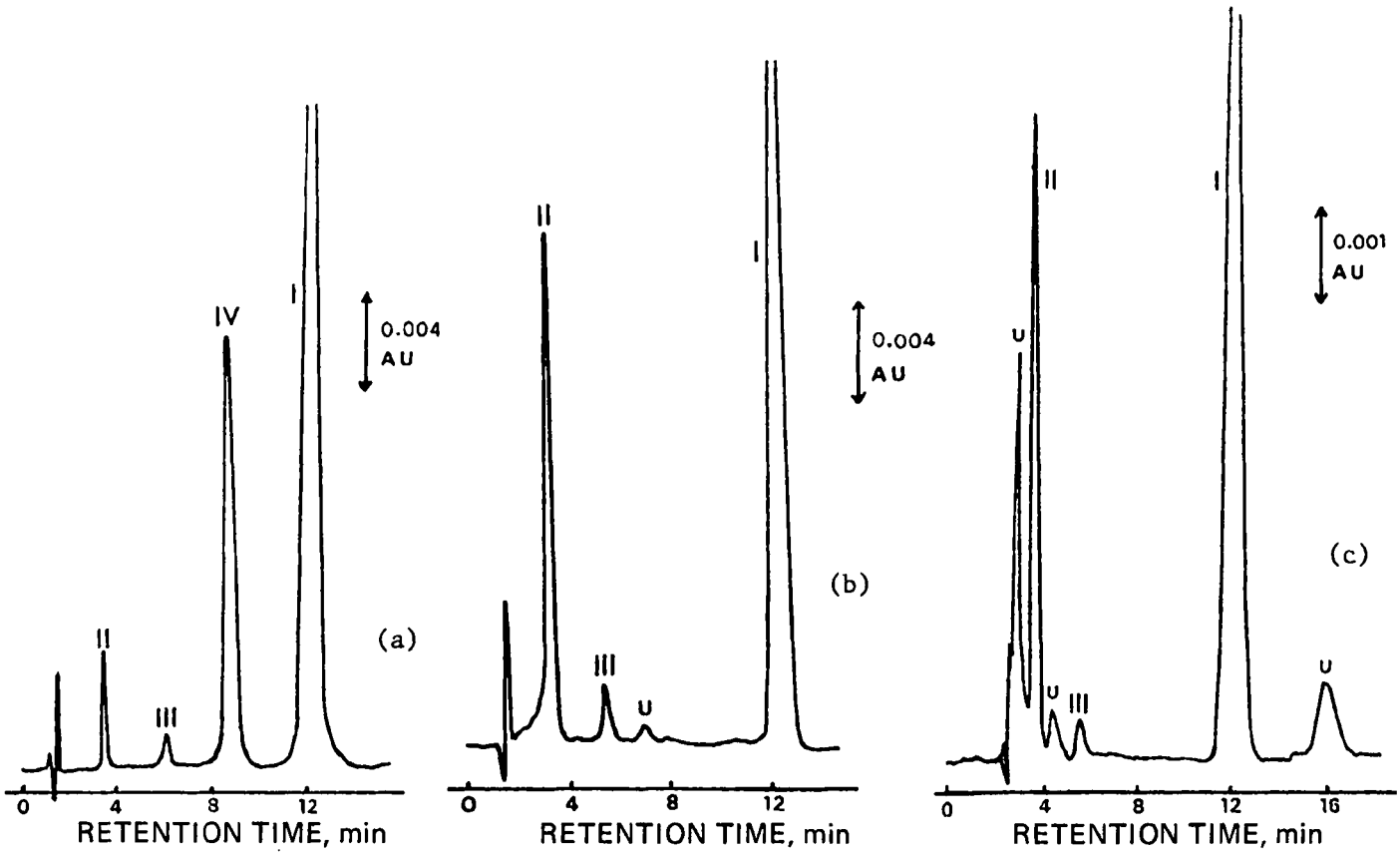


Figure 3—*Chromatogram of a 2 × 10^{-3} M degraded cefotaxime sodium salt solution withdrawn at ~30–40% degradation (0.2 M buffer concentration, 37°C, μ = 0.5) at pH 1.6 (a), pH 6.0 (b), and pH 10.0 (c). Key: (U) unknown peak.*

The UV rate constants may be considered as an estimation of the β-lactam opening rate of the cephalosporins (1, 3, 14) and may be compared with the k_1 values obtained using HPLC. Since the specific extinction coefficients at 260 nm of II ($E^{1\%}_{1cm}$ = 406 at pH 1.9, $E^{1\%}_{1cm}$ = 349 at pH 4.0, and $E^{1\%}_{1cm}$ = 340 at pH 9.0) and IV ($E^{1\%}_{1cm}$ = 418 at pH 1.9, $E^{1\%}_{1cm}$ = 361 at pH 4.0, and $E^{1\%}_{1cm}$ = 353 at pH 9.0) are very similar to those of I ($E^{1\%}_{1cm}$ = 445 at pH 1.9, $E^{1\%}_{1cm}$ = 390 at pH 4.0, and $E^{1\%}_{1cm}$ = 382 at pH 9.0), an approximate estimation of the destruction of the nucleus should be obtained with UV measurements, except if an appreciable production of an absorbing compound is noted. The k'_1 values obtained from UV determination and the k_1 values obtained from HPLC present a rather good concordance, as noted in Table II, in spite of the presence of small amounts of absorbing UV products, probably resulting from the β-lactam cleavage.

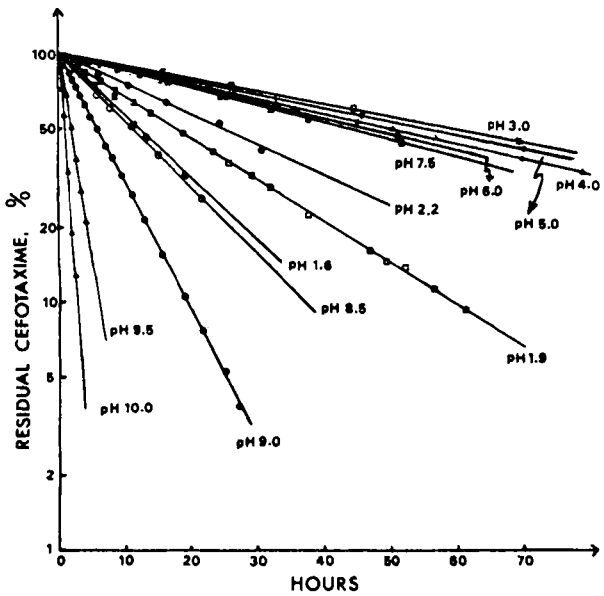


Figure 4—*Apparent first-order reaction of cefotaxime sodium salt solution at various pH values, 37°C, μ = 0.5, and 0.2 M buffer concentration (except at pH 7.5 where the buffer concentration was 0.15 M).*

Buffer Effect and General Acid–Base Catalysis—The values of k_{obs} obtained at constant pH, constant ionic strength (μ = 0.5), constant concentration of I, and constant temperature (37°C), varying only the buffer concentration, are given in Table I. The Student's *t* test was used to determine the significance of differences between the k_{obs} obtained for each buffer concentration. The catalytic effects of the buffers were estimated with a probability p = 95%.

Citrate buffer at pH 2.2 and 3.0 and phosphate buffer at pH 6.0 and 7.5 do not have a catalytic effect. At pH 2.2 and 3.0, the predominant species are H_3A and H_2A^- for citrate buffer (5); at pH 6.0 and 7.5, the predominant species are $H_2PO_4^-$ and HPO_4^{2-}. These species are not general acid–base catalysts.

Carbonate buffer at pH 8.5 and borate buffer at pH 9.5 and 10.0 give a significant catalytic effect on the degradation of cefotaxime. At these pH values, the dominant species, HCO_3^- and $H_2BO_3^-$ are general acid–base catalysts which increase the rate constants. The rate constant k_{obs} is given by:

$$k_{obs} = k_{pH} + k' \cdot [B] \qquad \text{(Eq. 4)}$$

where [B] is the buffer concentration, $k' = k_{HCO_3^-} \cdot f_{HCO_3^-}$ or $k_{H_2BO_3^-} \cdot f_{H_2BO_3^-}$, and $k_{HCO_3^-}$ and $k_{H_2BO_3^-}$ are second-order rate constants of HCO_3^- ions and $H_2BO_3^-$ ions, respectively. $f_{HCO_3^-}$ is the buffer HCO_3^- fraction (#1) at pH

Table IV—Values of Rate Constants at Different Temperatures and the Arrhenius Activation Parameter for I at Different pH [a]

Buffer	pH	Temp., °C	k_{pH}, $h^{-1} \times 10^3$	E_a, kcal·M^{-1}
Citrate	3.0	23	1.938	25.687
		37	13.930	
Acetate	5.0	23	1.607	28.307
		37	14.125	
Phosphate	7.0	4	0.194	
		23	1.679	21.755
		37	13.810	
Borate	10.0	4	3.034	
		23	31.825	28.732
		37	840.720	

[a] At 0.2 M buffer concentration, μ = 0.5.

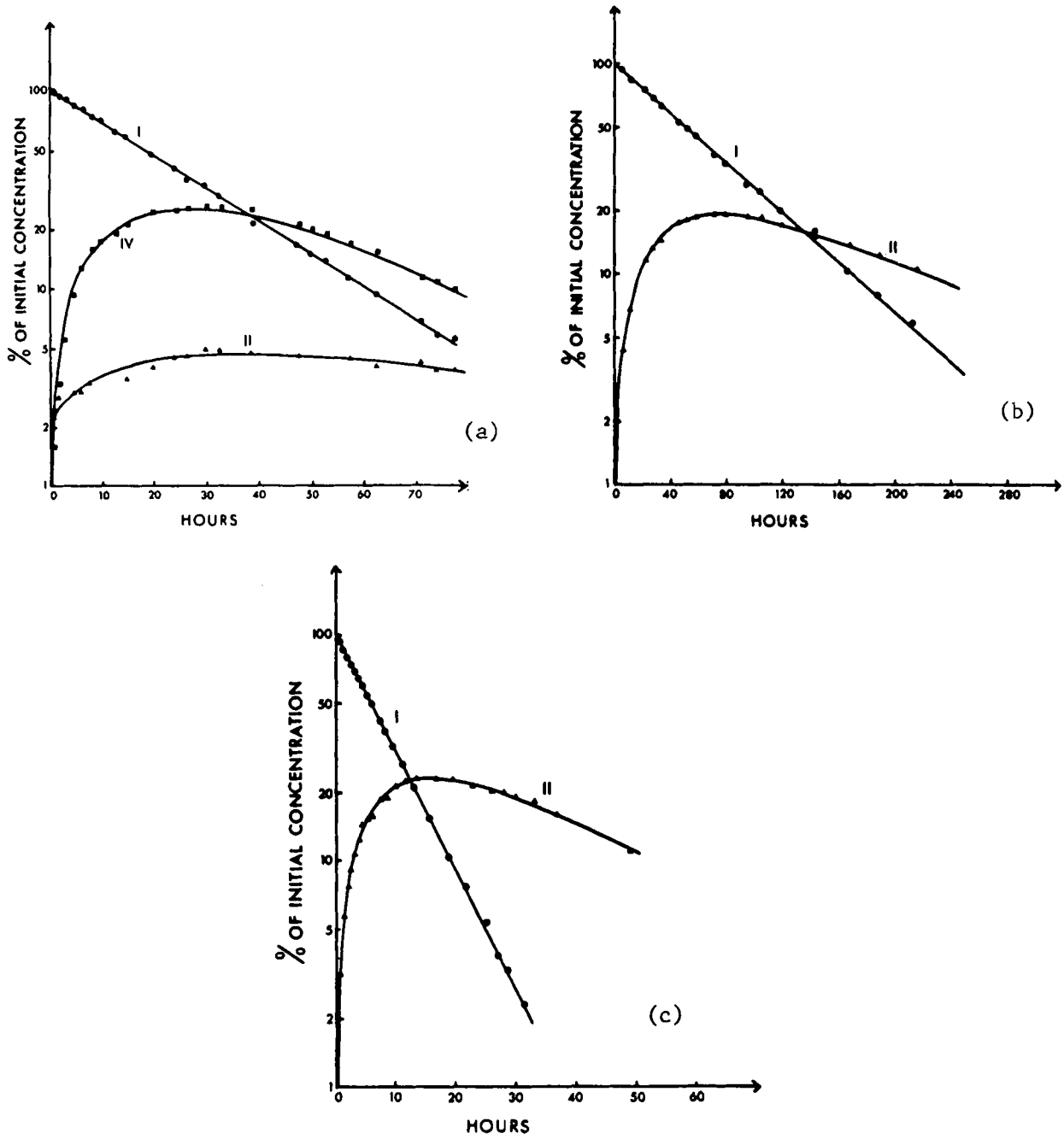


Figure 5—*Kinetics for cefotaxime (●), deacetylcefotaxime (▲), and deacetylcefotaxime lactone (■) during the degradation of a cefotaxime sodium salt solution at 37°C (μ = 0.5 and 0.2 M buffer concentration) at pH 1.9 (a), pH 4.0 (b), and pH 9.0 (c).*

8.5, $f_{H_2BO_3^-}$ is the buffer $H_2BO_3^-$ fraction (# I) at pH 9.5 and 10.0, and k_{pH} is the rate constant at zero buffer concentration.

An estimation of the catalytic effect was made from the plots of k_{obs} against the buffer concentration. The second-order rate constants were determined as $k'_{HCO_3^-} = 77.6 \times 10^{-3}$ $h^{-1}\cdot M^{-1}$ at pH 8.5; $k'_{H_2BO_3^-} = 212.28 \times 10^{-3}$ $h^{-1}\cdot M^{-1}$ at pH 9.5; and $k'_{H_2BO_3^-} = 187.25 \times 10^{-3}$ $h^{-1}\cdot M^{-1}$ at pH 10.0. k' in all cases is increasingly dependent on the buffer concentration. The rate constant, k_{pH} at zero buffer concentration, obtained by the intercept with the y-axis, gives the value of the rate constant independently of the buffer effect (Table I).

With acetate buffer, at pH 5.0 where CH_3COOH and CH_3COO^- species are present, k_{obs} decreases with the buffer concentration. At this pH, the observed rate constant is given by:

$$k_{obs} = k_{pH} + [B]\frac{k_{CH_3COOH}\cdot a_H + k_{CH_3COO^-}K_a}{a_H + K_a} \qquad \text{(Eq. 5)}$$

where K_a is the dissociation constant of acetic acid at 37°C, a_H is the hydrogen ion activity potentiometrically measured, and k_{CH_3COOH} and $k_{CH_3COO^-}$ are the second-order rate constants of CH_3COOH and CH_3COO^- ion, respectively. The catalytic negative effect of acetate buffer at this pH may be estimated by the value of the slope obtained when k_{obs} is plotted against buffer concentration (-10.5×10^{-3} $h^{-1}\cdot M^{-1}$); k_{obs} is decreasingly dependent on the buffer concentration. The protective effect against degradation observed with acetate buffer may be related to the presence of a 3-acetoxymethyl group in the molecular structure of I.

Primary Salt and Ionic Strength Effects—The primary salt effect was investigated at 37°C and pH 2.2, 6.0, and 9.5 for 0.2 M buffer concentration. Ionic strength was varied with potassium chloride solution. The data of k_{obs} for ionic strengths μ = 0.3, 0.5, and 0.7 are given in Table III. The influence of ionic strength on rate constants was demonstrated (15) by Bronsted and Bjerrum. Debye-Hückel theory applied to the kinetics shows that for ionic strengths <0.05, a linear relationship can be obtained between log k_{obs} and $\mu^{1/2}$ according to:

$$\log k_{obs} = \log k_{0,\mu_0} + 2QZ_AZ_B\sqrt{\mu} \qquad \text{(Eq. 6)}$$

where Q is a constant depending on the temperature and the dielectric constant

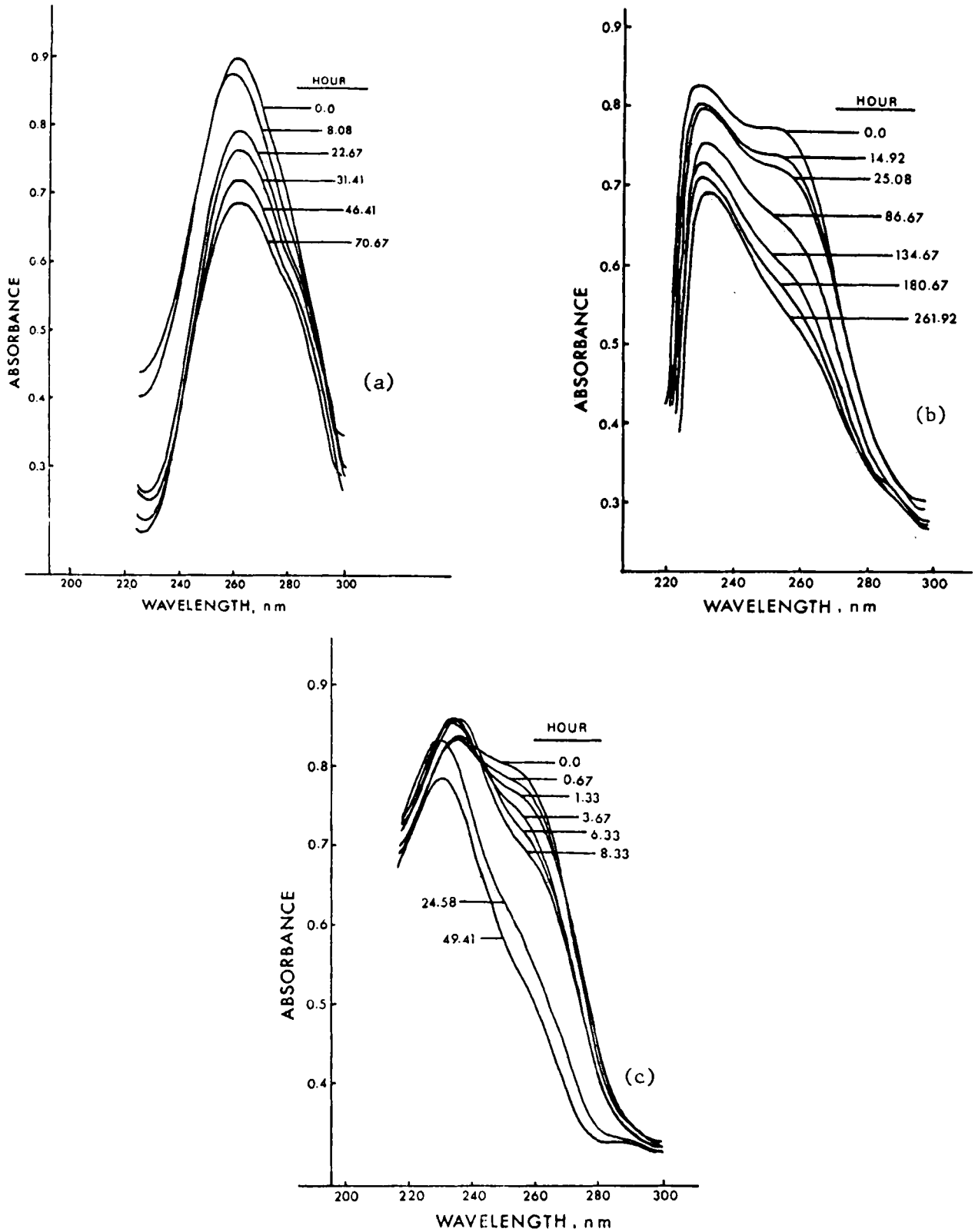


Figure 6—*Spectral changes during the degradation of a 2.0 × 10^{-5} M cefotaxime sodium salt solution (0.2 M buffer concentration, 37°C, μ = 0.5) at pH 1.9 (a), pH 4.0 (b), and pH 9.0 (c).*

of the solvent, Z_A and Z_B are the electric charges of the reactants conducting to the activated complex, and k_{0,μ_0} is the rate constant at zero ionic strength. At higher ionic strength, the second Debye-Hückle (16) relationship gives:

$$\log k_{obs} = \log k_{0,\mu_0} + \frac{2QZ_AZ_B\sqrt{\mu}}{1+\sqrt{\mu}} \qquad \text{(Eq. 7)}$$

The two ways of representation are given in Fig. 7.

A linear representation is obtained when log k_{obs} is plotted against $\mu^{1/2}$ and $\mu^{1/2}/1+\mu^{1/2}$. At pH 2.2 and 6.0 kinetic primary salt effects were negligible, in contrast to pH 9.5 where a kinetic primary salt effect was observed. At pH 2.2 and 6.0, the rate constants are almost independent of the ionic strength because one of the reactants is noncharged. At pH 9.5, the positive slope observed shows that the two reactant species have the same charge.

pH-Rate Profile—The pH-rate profile for the degradation of I was ob-

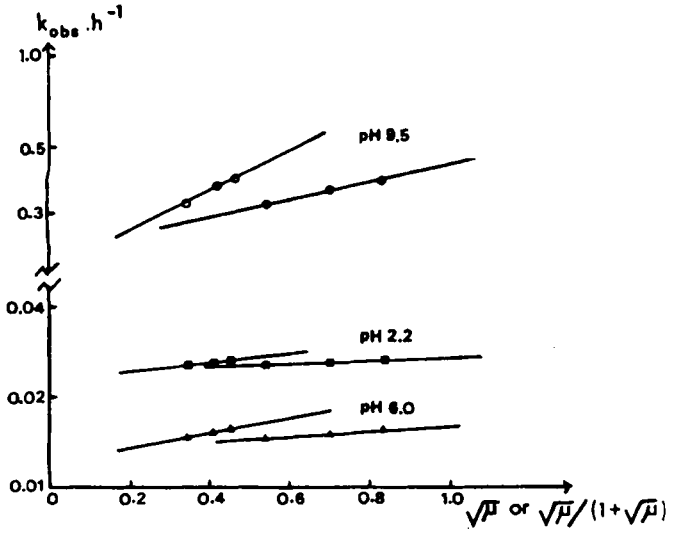


Figure 7—*Plots of log* k_{obs} versus $\sqrt{\mu}$ (●, ■, ▲) *or* $\sqrt{\mu}/1+\sqrt{\mu}$ (○, □, △) *at different pH values (0.2 M buffer concentration, 37°C) for a cefotaxime sodium salt solution.*

tained by plotting the values of log k_{pH} against pH, independently of any buffer effects (except for pH 4.0 and 9.0), at 37°C and $\mu = 0.5$ (Fig. 8). Three distinct regions can be seen: (*a*) a region where the reaction is hydrogen ion catalyzed, between pH 1.6 and 3.0, (*b*) a plateau where the reaction is not pH dependent, between pH 3.0 and 7.0, and (*c*) a region where the reaction is hydroxide ion catalyzed, above pH 7.0. The U-shape of the experimental curve, without inflexion in the pK_a zones (2.1 and 3.4), is characteristic of a reaction both hydrogen ion catalyzed and hydroxide ion catalyzed. The log k_{pH}–pH profile for I was fitted with:

$$k_{pH} = k_H \cdot a_H + k_0 + k_{OH}\frac{K_w}{a_H} \qquad \text{(Eq. 8)}$$

where k_H is the second-order rate constant for the hydrogen ion-catalyzed degradation, identical for the ionized and un-ionized cefotaxime species; a_H is the activity of hydrogen ions potentiometrically determined; k_0 is the first-order rate constant for the spontaneous or water-catalyzed reaction; k_{OH} is the second-order rate constant for the hydroxide ion-catalyzed degradation; and K_w is the autoprotolysis constant of water, $\sim 2.09 \times 10^{-14}$ at 35°C.

From the experimental values at pH 1.60, 6.00, and 9.50, k_H, k_0, and k_{OH} were calculated from Eq. 8 to be $k_H = 1.90\ h^{-1}\cdot M^{-1}$, $k_0 = 0.013\ h^{-1}$, and $k_{OH} = 4626.5\ h^{-1}\cdot M^{-1}$. These constants were used to generate the theoretical pH-rate profile given in Fig. 8, using a computer. A satisfactory fit of the data was obtained.

The good agreement between the experimental data, the theoretical profile, and the results of primary salt effects suggest the following reactions (Scheme III) to occur in the pH region investigated:

$$\text{Cef} + H^+ \xrightarrow{k_H} \text{products}$$

$$\text{Cef} \xrightarrow{k_0} \text{products}$$

$$\text{Cef} + OH^- \xrightarrow{k_{OH}} \text{products}$$

Scheme III

At pH 2.2, due to the overall average charge of the species in solution, the salt effect is not visible. At pH 6.0, I is ionized, the main process is a water attack, and no salt effect is observed. At pH 9.5, where a general base-catalytic effect has been shown with borate ions, a positive salt effect is obtained because I is negatively charged and reacts with hydroxide and borate ions.

Temperature Effect—The effect of temperature was studied with an ionic strength of 0.5 in 0.2 M buffer solutions, at pH 7.0 and 10.0, by measuring the degradation rates at 4°C, 23°C, and 37°C. Other determinations (pH 3.0 and 5.0) were conducted at only two temperatures (23°C and 37°C). The values of rate constants at different temperatures and the Arrhenius activation parameter are given in Table IV. These values are very similar to those obtained for other cephalosporins (1, 5, 6) and correspond to the values generally observed for solvolysis.

Comparative Stability and Reaction Mechanism—In Fig. 8, a stability comparison of I and other cephalosporins is plotted. This graph lists cephalosporins with an acetoxymethyl group in the C-3 position (cephalothin and

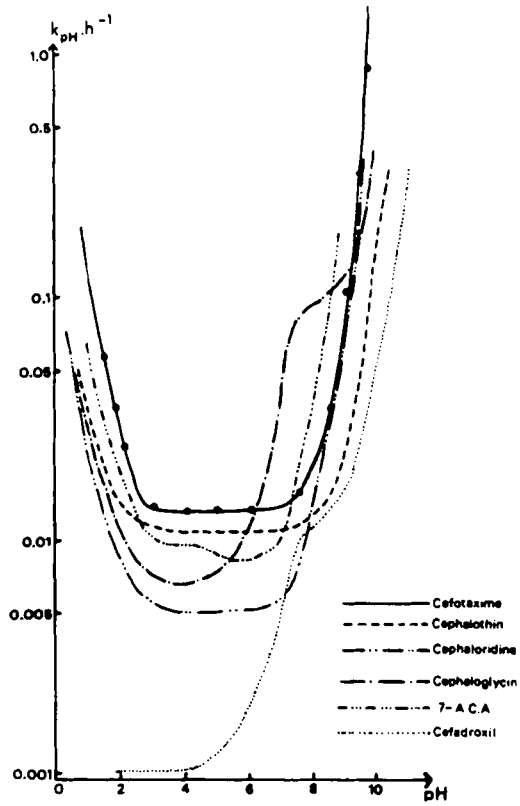


Figure 8—*log* k_{pH}*–pH profile for cefotaxime sodium salt solution at 37°C,* $\mu = 0.5$*. The points are experimental values. The solid lines were generated from Eq. 8 with an analog computer. Comparative stability study with other cephalosporins [cephalothin, cephaloridine, cephaloglycin, and 7-amino-cephalosporanic acid (7-A.C.A). from Ref. 1, cefadroxil from Ref. 6].*

cephaloglycin), cephalosporins without an acetoxymethyl group (cephaloridin and cefadroxil), and 7-aminocephalosporanic acid (7-A.C.A).

In the pH 3.0–7.0 range, where the degradation rates are not pH dependent, the rate constant $k_0 = 13 \times 10^{-3}\ h^{-1}$ calculated for I is similar to the k_0 values obtained from cephalothin and cephaloglycin. As suggested (1) for these two acetoxymethyl cephalosporins, the plateau in this pH range may correspond both to a hydrolytic cleavage of the β-lactam moiety and to an intramolecular attack with a side chain amino group participation. This intramolecular attack should be facilitated by the electron-donating effect of the *O*-methyloxime substituent on the side chain.

In acidic and alkaline media, the rate constants observed are higher than the rate constants given for cephalothin and cephaloglycin. This enhanced reactivity of I should imply a side-chain participation in the degradation, in contrast with cephalothin and cephaloglycin.

From the present results, the scheme of degradation of I in the entire pH range has been found similar to the other acetoxymethyl cephalosporins of the first generation (cephalothin and cephaloglycin). The chemical stability of I under alkaline conditions has not been proven to be reliable for β-lactamase hydrolysis and consequently for the activity against Gram-negative bacteria, as suggested for the cephalosporins of the first and second generations (1). Cefotaxime sodium (I) has a remarkable resistance to hydrolysis of these enzymes (17), in contrast to a fair stability under alkaline conditions. The enhanced resistance to the β-lactamase deactivation has been attributed to the presence of a rigid *syn O*-methyloxime group (17). The biological activity of I has also been related to the original structure of the 7-substituent aminothiazole ring, which plays an important role in the affinity of the molecule against transpeptidase activity in the synthesis of the bacterial wall.

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Ocular Delivery of Pilocarpine from Erodible Matrices

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Received January 13, 1983, from the *College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68105.* Accepted for publication March 21, 1983. Present addresses: *School of Pharmacy, University of Wisconsin, Madison, WI 53706; ‡ICI Pharma, 16 Falconer Drive, Mississauga, Ontario, L5N 3M1 Canada; §School of Pharmacy, Creighton University, Omaha, NE 68178.

Abstract □ The present study examined the feasibility of sustaining the release of a water-soluble drug, pilocarpine, to the tear film. Both gels and dried films were utilized as drug delivery systems. *In vitro* studies demonstrated significant prolongation of drug release from these systems as compared with simple aqueous or viscous solutions. The *in vitro* results were supported by *in vivo* miosis studies in albino rabbits.

Keyphrases □ Pilocarpine—sustained release, polymeric delivery systems, *in vitro* release studies, *in vivo* miosis in rabbits □ Drug delivery systems—polymeric gels and films, pilocarpine, sustained release to the cornea, rabbits □ Polymeric matrices—sustained release of pilocarpine to the cornea, *in vivo* miosis in rabbits, *in vitro* release studies

It is well established that ocular drug bioavailability from topical dosing is dependent on several factors, one of the most prominent being the contact time of drug with tissues in the precorneal area of the eye (1). Several investigators (2, 3) have reported that the relatively low-viscosity ophthalmic solutions, while able to somewhat improve the amount of drug that penetrates the cornea to the anterior chamber, generally do not provide a sustaining effect. Conversely, Schoenwald *et al.* (4) and Patton and Robinson (2) have demonstrated that moderately viscous systems, such as gels, can sustain drug delivery to the cornea and anterior segment of the eye. Furthermore, it is known that slowly dissolving lamellae remain in the eye for extended periods of time. Therefore, it is of interest to examine and assess these systems for their potential in ocular drug delivery.

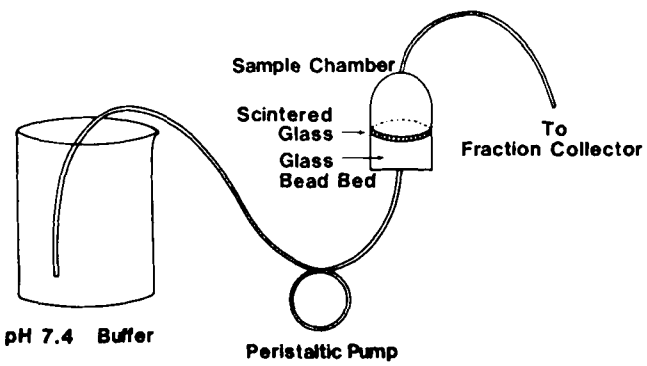

Figure 1—In vitro *dissolution apparatus used to measure appearance of pilocarpine from solutions and lamellae.*

A chronic problem with dissolving hydrogels is their usual high water content, which allows low molecular weight substances to diffuse quickly out of the gel, leaving a "ghost" without active ingredients. Extensive cross-linking of polymers, or dehydration of the gel to a dry film state, could measurably improve the release characteristics of the system.

Due to the need for frequent dosing of pilocarpine from commercial solution preparations, the expense of currently marketed controlled-release devices, and because extensive work has been published regarding ocular absorption of pilocarpine from both solutions and various gel systems, this drug was selected as a model compound for release from several types of ocular polymeric systems.

EXPERIMENTAL

Materials—Reagent-grade polyvinyl alcohol (PVA)[1], with an average mol. wt. of 16,000, and carboxyvinyl copolymer (carbomer 934)[2] were used as received. Tritiated pilocarpine[3], with a specific activity of 20 mCi/mg, was purified by vacuum evaporation immediately prior to use (5). All other chemicals were reagent or analytical grade.

Male albino rabbits[4], weighing 2.5–3.5 kg, were used throughout the study. No special dietary restrictions were used for the animals, and they were maintained in a normal lighting and auditory environment.

Methods—*Preparation of Solutions and Films*—A 4% w/v solution of pilocarpine nitrate, made isotonic with sodium chloride, was prepared in pH 7.4 Sorensen's phosphate buffer. To 10 mL of this solution, tritiated pilocarpine (10 μL) was added to yield a specific activity of ~1.5 μCi/mL. A negligible change in pilocarpine solution concentration occurred through this spiking procedure.

Pilocarpine, 2% in 10% PVA in phosphate buffer, was prepared with a specific activity of ~0.9 μCi/mL. Solutions were refrigerated between uses and were discarded within 7 d.

Erodible polymer films containing drug were prepared in the following manner. The 10% PVA solution in distilled water (40 mL) was poured into

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Carbopol 934; B. F. Goodrich, Cleveland, Ohio.
[3] New England Nuclear, Boston, Mass.
[4] Sasko, Omaha Neb.

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