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著者	Tsuji Akira, Nakashima Emi, Nishide Kazunori, Deguchi Yoshiharu, Hamano Shoichiro, Yamanaka Tsukinaka
journal or publication title	Chemical & pharmaceutical bulletin
volume	31
number	11
page range	4057-4069
year	1983-11-25
URL	http://hdl.handle.net/2297/3494

[Chem. Pharm. Bull. 31(11)4057—4069(1983)]

Physicochemical Properties of Amphoteric β-Lactam Antibiotics. III.[1] Stability, Solubility, and Dissolution Behavior of Cefatrizine and Cefadroxil as a Function of pH[2]

AKIRA TSUJI,*[,a] EMI NAKASHIMA,[a] KAZUNORI NISHIDE,[a,3a)]
YOSHIHARU DEGUCHI,[a] SHOICHIRO HAMANO,[a,3b)]
and TSUKINAKA YAMANA[b]

Faculty of Pharmaceutical Sciences[a] and Hospital Pharmacy,[b] Kanazawa University, Takara-machi, Kanazawa 920, Japan

(Received January 24, 1983)

A quantitative study on the pH-dependency of the degradation, solubility, and dissolution of cefatrizine and cefadroxil was carried out at 35 or 37 °C, and at an ionic strength of 0.5. The degradation rates of cefatrizine were determined by high-performance liquid chromatography. At constant pH and temperature, the degradation followed pseudo first-order kinetics. The shape of the rate constant-pH profile resembled those for cefadroxil and other aminocephalosporins. In an acidic medium below pH 4, cefatrizine was reasonably stable with a half-life of 14 d at 35 °C. At neutral pH, cefatrizine was degraded with a half-life of about 6 h at 35 °C *via* intramolecular reaction by the nucleophilic attack of the α-amino group on the β-lactam moiety. The intramolecular reaction rate was very similar to that of cephaloglycin, but ten times faster than those for cefadroxil, cephalexin, and cefradine under the same conditions.

Both aminocephalosporins exhibited similar U-shaped solubility curves against pH. Their minimum solubilities were 4.6×10^{-2} M, close to that of cephalexin monohydrate. The dissolution rate constants from a rotating disk were determined and interpreted successfully in terms of the dissociation equilibrium reaction and the diffusion kinetic model. Temperature effects on the degradation rate, solubility, and the dissolution rate were also examined.

Keywords——cefatrizine; cefadroxil; β-lactam antibiotics; aminocephalosporin; intramolecular reaction; stability; solubility; dissolution rate; pH effect; temperature effect

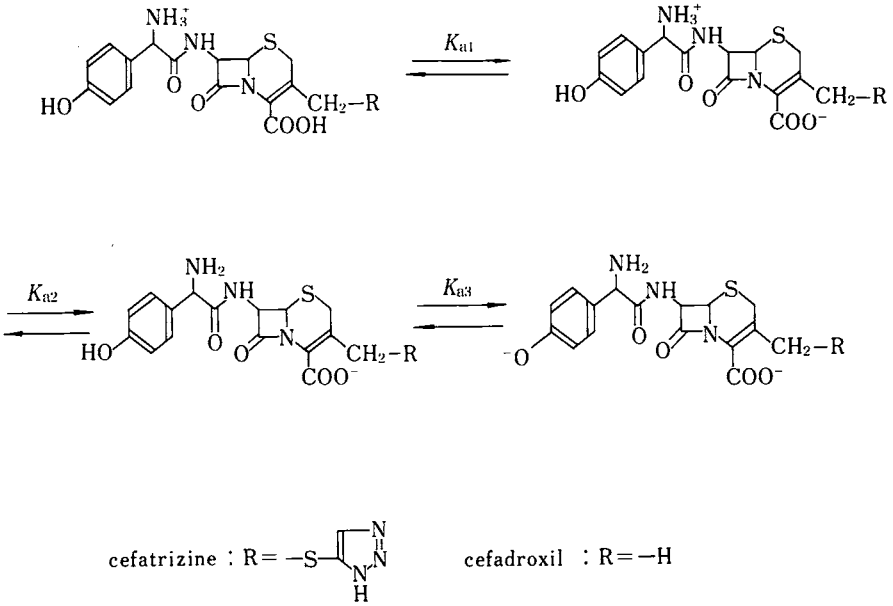
Cefatrizine is a new orally absorbed aminocephalosporin antibiotic with a broad spectrum of antibacterial activity.[4] Chemically, cefatrizine is 7-[D-α-amino-α-(4-hydroxyphenyl)acetamido]-3-([(1*H*-1,2,3-triazol-5-yl)-thio]methyl}-3-cephem-4-carboxylic acid. It differs from cefadroxil, which is another orally effective aminocephalosporin, in the replacement of a 3-methyl group with a thio-triazole moiety (see Chart 1).

The aqueous stability kinetics and the degradation mechanisms of cefadroxil have been reported.[1] The present paper describes the degradation kinetics of cefatrizine, and the comparative stability, solubility and dissolution rates of cefatrizine and cefadroxil.

Experimental

Materials——Cefatrizine propylene glycolate (832 μg/mg) and cefadroxil monohydrate (947 μg/ml) were used as supplied by Banyu Pharmaceutical Co. (Tokyo) and Bristol Myers Co. (Tokyo), respectively. Buffers and all other chemicals were of reagent grade from Wako Junyaku Kogyo Co. (Osaka) and were used without further purification.

Procedures——Degradation Kinetics: The kinetic study of cefatrizine was carried out at 35 ± 0.1 °C and an ionic strength of 0.5 in the same way as that for cefadroxil,[1] unless otherwise stated. The degradation was initiated by dissolving an accurately weighed amount of cefatrizine in an appropriate buffer solution or 0.5 M potassium chloride solution preheated to the desired temperature to make a final concentration of 5×10^{-3} M. When the pH of the solution was maintained by means of a pH-stat (TTT2 titrator and ABU12b autoburet, Radiometer, Copenhagen, Denmark) during the kinetic run, 0.5 M KCl solution containing 1×10^{-4} M disodium edetate was used in order to avoid possible heavy metal-catalyzed degradation. Samples were withdrawn at suitable time intervals, cooled in an ice



cefatrizine : R= cefadroxil : R=—H

Chart 1

TABLE I. Dissociation Constants[a] of Cefatrizine and Cefadroxil

Cephalosporin	Temp. (°C)	pK_{a_1} [b]	pK_{a_2} [b]	pK_{a_3} [c]
Cefatrizine	35	2.60±0.02	7.00±0.16	9.54±0.06
	37	2.62±0.02	6.99±0.08	9.42±0.03
Cefadroxil	35[d]	2.64±0.03	7.30±0.03	9.69±0.06
	37	2.70±0.03	7.22±0.03	9.62±0.03

a) Determined at an ionic strength of 0.5. Each value represents the mean±S.D. of three determinations.
b) Determined by potentiometric method.
c) Determined by spectrophotometric method.
d) From ref. 1.

bath, and diluted with a 0.2 M acetate buffer of pH 4.0 to prevent possible degradation during the analysis.

The concentration of residual cefatrizine was analyzed by a high-performance liquid chromatographic (HPLC) method. In addition to the HPLC determination of the overall rate of cefatrizine degradation, the decrease in the concentration of the primary amino group in cefatrizine during the degradation was followed by a colorimetric assay as used in the cefadroxil degradation study.[1]

Determination of Ionization Constants: The dissociation equilibria of cefatrizine and cefadroxil are shown in Chart 1. The apparent pK_{a_1} and pK_{a_2} values for the dissociation of the carboxylic acid and α-ammonium moieties of both antibiotics were determined by the potentiometric titration of 0.01 M antibiotic aqueous solution at an ionic strength of 0.5 and at 35 or 37 °C. The values of pK_{a_3} for the phenolic groups were determined spectrophotometrically as described previously for cefadroxil.[1] The calculation for cefatrizine was made from the change in absorbance at 255 nm measured with a model UV-200 double-beam recording spectrophotometer (Shimadzu Seisakusho, Kyoto). These results are summarized in Table I.

Determination of Solubility and Dissolution Rates: The experimental procedures were described in detail previously.[5] Cefatrizine and cefadroxil were assayed by the HPLC method used for their degradation kinetics. The experimental conditions were chosen on the basis of the present and previous kinetic data[1] so that the degradation of the antibiotics was within 5%. Accordingly, experiments on the temperature effects on solubilities and dissolution rates were conducted in 0.5 M KCl. Since the solution pH was <pH 5, there was no significant degradation of either antibiotic below 60 °C.

Analytical Procedures——HPLC: The liquid chromatograph (TRIROTOR-II, Japan Spectroscopic Co., Tokyo) was equipped with a ultraviolet (UV) detector (UVIDEC-100-III, Japan Spectroscopic Co.) set at 254 nm and a 4.0 × 300-mm stainless steel column prepacked with octadecyl-silane chemically bonded on totally porous silica gel (μBondapak C_{18}, Waters Associates, Milford, Mass., U.S.A.). The mobile phase was an acetonitrile–0.01 M ammonium acetate mixture; the contents of acetonitrile were 7 and 2% for cefatrizine and cefadroxil, respectively. The chromatography was performed at ambient temperature, and the samples were eluted at a flow rate of 1 or 2 ml/min to obtain appropriate retention times. Peak heights were used to quantitate the amount of each antibiotic. Standard curves of the peak height of an antibiotic *versus* concentration showed a linear response in the concentration range of 10—100 μg/ml. The sample peak heights were converted to concentrations by comparison with a standard curve which was obtained daily.

Colorimetric Assay: The procedure used for cefadroxil[1] was adopted for cefatrizine degradation.

Data Analysis——The non-linear least squares treatments of the kinetic data were run on a FACOM-170F digital computer at the Data Processing Center, Kanazawa University.

Results

Degradation Kinetics of Cefatrizine

Reaction Order and Observed Rate Constants——The kinetics of cefatrizine degradation was studied by the HPLC method, by following the disappearance of the intact antibiotic as a function of time. Constant pH was maintained by using an appropriate buffer system in the kinetic runs with a half-life > 1 d or by using a pH-stat in the relatively fast degradations. The results obtained indicate that in aqueous solutions, the degradation of 5 mM cefatrizine follows pseudo first-order kinetics with respect to cefatrizine under constant pH, temperature, and ionic strength conditions. Figure 1 shows typical plots of several experimental runs at

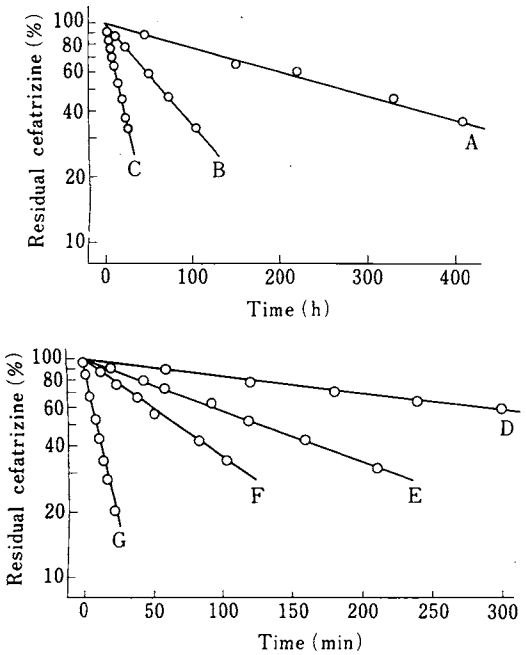


Fig. 1. Apparent First-Order Plots for the Degradation of 5×10^{-3} M Cefatrizine at Various pH Values, 35 °C, and an Ionic Strength of 0.5, Determined by HPLC Assay

A, pH 2.51 (0.15 M citrate buffer); B, pH 4.57 (0.2 M acetate buffer); C, pH 6.42 (0.112 M citrate buffer); D, pH 8.00 (pH-stat); E, pH 6.68 (0.2 M phosphate buffer); F, pH 10.5 (pH-stat); G, pH 11.5 (pH-stat).

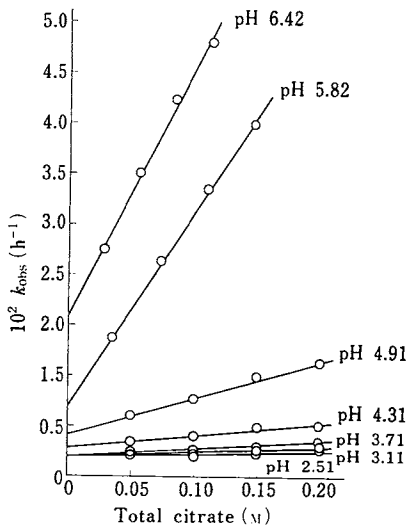


Fig. 2. Plots of Pseudo-First-Order Rate Constant *versus* Total Citrate Buffer Concentration for Cefatrizine Degradation at Various pH Values, 35 °C, and an Ionic Strength of 0.5

The lines were generated from Eq. (2) by using the rate constants given in the text.

TABLE II. Effects of Buffer Concentration and pH on the Pseudo-First-Order Rate Constants for Cefatrizine Degradation at 35 °C and an Ionic Strength of 0.5

pH (Buffer)	$10^2 k_{obs}$, h^{-1}				$10^2 k_{pH}$, h^{-1}
	0.05 M	0.10 M	0.15 M	0.20 M	
2.51 (Citrate)	0.224	0.236	0.255	0.277	0.20
3.11 (Citrate)	0.225	0.255	0.269	0.291	0.20
3.71 (Citrate)	0.246	0.263	0.304	0.353	0.20
4.31 (Citrate)	0.341	0.408	0.488	0.517	0.29
4.91 (Citrate)	0.599	0.780	0.992	1.13	0.42
5.82 (Citrate)	1.38 (0.037 M)	2.14 (0.073 M)	2.86 (0.110 M)	3.50 (0.146 M)	0.69
6.42 (Citrate)	2.24 (0.028 M)	3.01 (0.056 M)	3.73 (0.084 M)	4.30 (0.112 M)	1.60
4.25 (Acetate)	0.321	0.480	0.609	0.665	0.23
4.57 (Acetate)	0.461	0.714	0.793	1.05	0.29
5.27 (Acetate)	0.764	1.08	1.42	1.64	0.48
6.14 (Phosphate)	6.30	9.42	13.7	17.1	2.45
6.68 (Phosphate)	12.1	21.5	28.1	33.1	6.30
7.20 (Phosphate)	19.0	30.0	38.4	43.2 (0.18 M)	10.5
7.00 (pH-stat)	—	—	—	—	5.76
7.00 (pH-stat)	—	—	—	—	7.55 (37 °C)
7.00 (pH-stat)	—	—	—	—	19.5 (44 °C)
7.00 (pH-stat)	—	—	—	—	32.4 (48 °C)
7.00 (pH-stat)	—	—	—	—	82.4 (58 °C)
8.00 (pH-stat)	—	—	—	—	11.1
9.00 (pH-stat)	—	—	—	—	15.5
10.00 (pH-stat)	—	—	—	—	27.1
10.50 (pH-stat)	—	—	—	—	62.2
11.00 (pH-stat)	—	—	—	—	193
11.50 (pH-stat)	—	—	—	—	210 (30 °C)
11.50 (pH-stat)	—	—	—	—	425
11.50 (pH-stat)	—	—	—	—	470 (37 °C)
11.50 (pH-stat)	—	—	—	—	687 (40 °C)
11.50 (pH-stat)	—	—	—	—	1410 (45 °C)
12.00 (pH-stat)	—	—	—	—	1175

35 °C and ionic strength 0.5, at selected pH values. Table II lists the observed first-order rate constants, k_{obs}, determined from the slopes of semilogarithmic plots of the cefatrizine residual percent *versus* time, and the buffer systems.

General Acid-Base Catalysis——The catalytic effect of the buffer species in the citrate, acetate, and phosphate buffers used in these kinetic studies were determined at constant pH, temperature, and ionic strength in solutions containing 5 mM cefatrizine; only the total buffer concentration was varied. These experiments were carried out at several pH values within the effective range for each buffer employed.

Figure 2 illustrates the buffer effect of various citrate species on the degradation of cefatrizine in the pH range between 2.51 and 6.42. The observed rate constants are the summation of the specific rate constant for the buffer species and the pH-dependent rate constant at zero buffer concentration. Therefore, the observed rate constant, k_{obs}, may be written as:

$$k_{obs} = k_{pH} + k_{H_3A}[H_3A] + k_{H_2A^-}[H_2A^-] + k_{HA^{2-}}[HA^{2-}] + k_{A^{3-}}[A^{3-}] \quad (1)$$

where k_{pH} represents the rate constant at zero buffer concentration; other k values are the second-order rate constants with respect to citrate buffer species; $[H_3A]$, $[H_2A^-]$, $[HA^{2-}]$, and

$[A^{3-}]$ are the concentrations of undissociated citric acid, dihydrogen citrate ion, monohydrogen citrate ion, and citrate ion, respectively. By expressing each concentration in terms of total citrate concentration $[Cit]_T$, hydrogen ion activity, and the three dissociation constants, K_1^{Cit}, K_2^{Cit}, and K_3^{Cit} of citric acid, Eq. (2) can be obtained.

$$k_{obs}=k_{pH}+[Cit]_T\frac{k_{H_3A}(a_{H^+})^3+k_{H_2A^-}K_1^{Cit}(a_{H^+})^2+k_{HA^{2-}}K_1^{Cit}K_2^{Cit}a_{H^+}+k_{A^{3-}}K_1^{Cit}K_2^{Cit}K_3^{Cit}}{(a_{H^+})^3+K_1^{Cit}(a_{H^+})^2+K_1^{Cit}K_2^{Cit}a_{H^+}+K_1^{Cit}K_2^{Cit}K_3^{Cit}} \quad (2)$$

In this case as well as in the case of cefadroxil degradation,[1] no attempt was made to incorporate the catalytic constants with respect to the protonated and zwitterionic species of cefatrizine because of difficulty in differentiation of their reactivities. Taking the dissociation constants[6] of citric acid as $pK_1^{Cit}=3.08$, $pK_2^{Cit}=4.75$, and $pK_3^{Cit}=5.40$, the citrate catalytic constants at 35 °C and ionic strength of 0.5 were determined from Eq. (2) by using the non-linear least-squares computer program, NONLIN[7] to be $k_{H_3A}=4.23\times10^{-3}\,M^{-1}\,h^{-1}$, $k_{H_2A^-}=9.81\times10^{-2}\,M^{-1}\,h^{-1}$, $k_{HA^{2-}}=7.88\times10^{-2}\,M^{-1}\,h^{-1}$, and $k_{A^{3-}}=0.264\,M^{-1}\,h^{-1}$. The solid lines in Fig. 2 represent the best fits of the observed points.

The catalytic effects of acetate buffers (pH 4.25—5.27) and phosphate buffers (pH 6.14—7.20) on the rate of degradation of cefatrizine are shown in Figs. 3 and 4, respectively. Treatment of the kinetic data in a manner analogous to that used in the interpretation of citrate buffer catalysis as described previously for cefadroxil degradation[1] afforded Eqs. (3) and (4), respectively:

$$k_{obs}=k_{pH}+[Ac]_T\frac{k_{HAc}a_{H^+}+k_{Ac^-}K^{HAc}}{a_{H^+}+K^{HAc}} \quad (3)$$

$$k_{obs}=k_{pH}+[Ph]_T\frac{k_{H_2PO_4^-}a_{H^+}+k_{HPO_4^{2-}}K_2^{Ph}}{a_{H^+}+K_2^{Ph}} \quad (4)$$

where $[Ac]_T$ and $[Ph]_T$ represent the total acetate and phosphate concentrations, respectively; K^{HAc} and K_2^{Ph} represent the dissociation constants for acetic acid and dihydrogen phosphate ion, respectively. By employing $pK^{HAc}=4.58$[8] and $pK_2^{Ph}=6.59$[1] at 35 °C and $\mu=0.5$, the

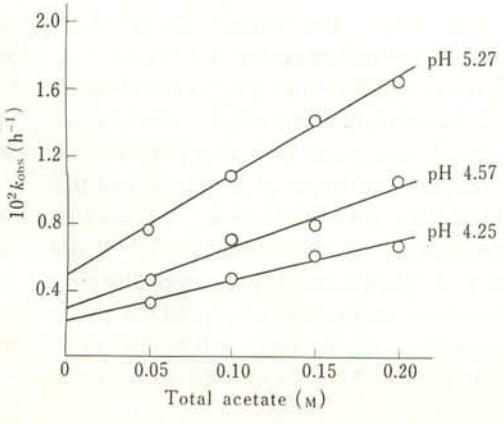


Fig. 3. Plots of Pseudo-First-Order Rate Constant *versus* Total Acetate Buffer Concentration for Cefatrizine Degradation at Various pH Values, 35 °C, and an Ionic Strength of 0.5

The lines were generated from Eq. (3) by using the rate constants given in the text.

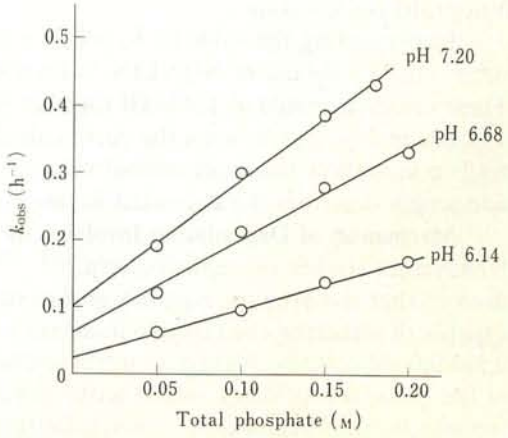


Fig. 4. Plots of Pseudo-First-Order Rate Constant *versus* Total Phosphate Buffer Concentration for Cefatrizine Degradation at Various pH Values, 35 °C, and an Ionic Strength of 0.5

The lines were generated from Eq. (4) by using the rate constants given in the text.

following catalytic rate constants were obtained using NONLIN;[7] $k_{HAc}=1.36\times10^{-3}\,M^{-1}\,h^{-1}$, $k_{Ac^-}=7.16\times10^{-2}\,M^{-1}\,h^{-1}$, $k_{H_2PO_4^-}=0.211\,M^{-1}\,h^{-1}$, and $k_{HPO_4^{2-}}=2.28\,M^{-1}\,h^{-1}$. The solid lines in Figs. 3 and 4 represent the best fits to the observed points.

pH-Rate Profile——The pH dependence of the overall first-order rate constant, k_{pH}, of cefatrizine degradation at 35 °C and $\mu=0.5$ is shown in Fig. 5. The rate constants used in construction of the graph were obtained from the intercepts of the plots of k_{obs} *versus* total buffer concentrations at various pH values (Figs. 2—4). The results obtained by using a pH-stat were incorporated (Table II).

In the pH range studied, the amphoteric antibiotic cafatrizine exists in four different ionic forms; as a cation (AH_2^+), a zwitterion ($AH^\pm$), a mono-anion (A^-), and a di-anion (A^{2-}), the apparent pK_a values of AH_2^+, $AH^\pm$, and A^{2-} being 2.60, 7.00, and 9.54, respectively at 35 °C and $\mu=0.5$ (Chart 1).

For the sake of comparison, pH-rate profiles for cefadroxil,[1] cephaloglycin,[9] cephalexin,[9] and cephradine[9] obtained under the same kinetic conditions are redrawn in Fig. 5. The pH-dependence curve of k_{pH} for cefatrizine shows the same shape as those for all other aminocephalosporins except that in acidic medium for cephaloglycin. Since there is no break near pK_{a_1} or pK_{a_3} in the log k_{pH}-pH profile of cefatrizine, the dissociations of the 4-carboxylic acid and the phenol moieties apparently have no effect on the degradation rate, as also observed for cefadroxil.[1] The apparent inflections at pH around the pK_{a_2} values in the pH-rate profiles of all aminocephalosporins indicate that the dissociation equilibria of the side-chain α-ammonium groups do influence the degradation rates.

The total shape of the log k_{pH}-pH profile of cefatrizine can be interpreted by the following equation which also successfully described those of ther aminocephalosporins:[1,9]

$$k_{pH}=k_o+k_b\left(\frac{K_{a_2}}{a_{H^+}+K_{a_2}}\right)+k_{OH}\frac{K_w}{a_{H^+}} \tag{5}$$

where k_o represents the first-order rate constant for the water-catalyzed degradation of cefatrizine; k_b represents the first-order rate constant for the spontaneous (or water-catalyzed) degradation of the mono-anionic and di-anionic species of cefatrizine; k_{OH} represents the second-order rate constant for the base-assisted degradation of all species; K_w is the autoprotolytic constant.

Incorporating the value of $K_w=2.09\times10^{-14}$ at 35 °C,[10] the various rate constants were determined by the use of NONLIN[7] with weighing based on the reciprocal of each k_{pH} value. These values are listed in Table III together with those for the other aminocephalosporins.[1,9] The line in Fig. 5 represents the curve calculated by substituting these k values into Eq. (5) while points show the experimental values. The good agreement indicates that this equation adequately describes the degradation rate constants of cefatrizine as a function of pH.

Mechanism of Degradation Involving the Side-Chain α-Amino Group——It became clear from recent studies on cephaloglycin,[9,11-13] cephalexin,[9,13-15] cephradine,[9,13,16] and cefadroxil[1] that the primary α-amino groups in the C-7 side-chain of aminocephalosporins are capable of attacking the reactive β-lactam moieties intramolecularly to yield the piperazine-2,5-dione derivatives. If such an intramolecular reaction occurs, there will be a consumption of the side-chain primary amino group during the degradation, as confirmed previously for cephaloglycin,[13] cephalexin,[13] and cefadroxil.[1]

The decrease in the concentration of the primary amino groups in cefatrizine solution during the degradation was followed by means of the trinitrobenzene-sulfonic acid assay. Figure 6 shows a plot of the disappearance of amino groups in 0.15 M phosphate buffer at pH 7.20, 35 °C, and $\mu=0.5$. The inserted plot indicates that the loss of amino groups follows first-order kinetics. The apparent rate constant was in reasonably good agreement with the first-order rate constant for the total disappearance followed by the HPLC method for the same

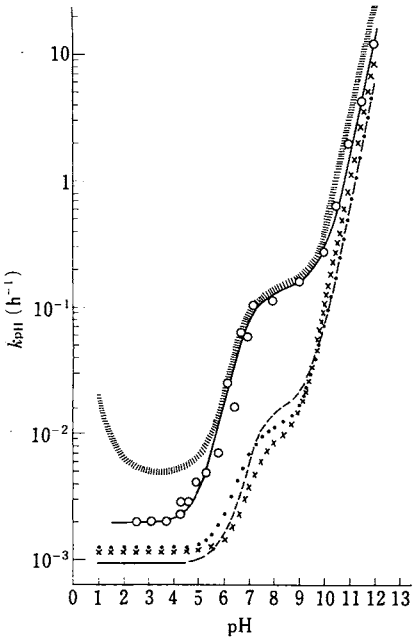


Fig. 5. $\log k_{pH}$-pH Profiles for the Degradation of Cefatrizine at 35 °C and an Ionic Strength of 0.5

The solid line represents the curve calculated from Eq. (5) and the constants listed in Tables I and III; the points are the experimental values. The other lines refer to the $\log k_{pH}$-pH profiles for cefadroxil (----), cephalexin (× × ×), cephradine (●●●), and cephaloglycin (wwww) at 35 °C and an ionic strength of 0.5 (from refs. 1 and 9).

TABLE III. Rate Constants[a] for Degradation of Aminocephalosporins at 35 °C and an Ionic Strength of 0.5

Cephalosporin	$10^3 k_0$ h^{-1}	$10^2 k_b$ h^{-1}	$10^{-2} k_{OH}$ $M^{-1} h^{-1}$
Cefatrizine	1.96	14.4	5.84
Cefadroxil[b]	0.941	1.61	2.54
Cephalexin[c]	1.15	1.01	2.64
Cephradine[c]	1.10	0.740	3.98
Cephaloglycin[c]	5.00	13.5	13.1

a) Defined in Eq. (5). *b*) From ref. 1. *c*) From ref. 9.

sample, as shown in the figure. This result leads to the conclusion that the degradation of cefatrizine proceeding at neutral pH is dominated by the intramolecular nucleophilic attack of the unprotonated side-chain amino group on the β-lactam carbonyl moiety, probably to yield the piperazine-2,5-dione product.

Effect of Temperature——The temperature dependence of the degradation of cefatrizine was studied in non-buffered solution at pH 7.00 and 11.50 and at $\mu=0.5$ using a pH-stat technique. The Arrhenius plots are shown in Fig. 7. From these data, the apparent activation energies at pH 7.00 and 11.50 were determined to be 22.6 and 24.4 kcal/mol, respectively.

In aqueous solution at pH 7.00 and 35 °C, cefatrizine was degraded *via* an intramolecularly catalyzed reaction (k_b-reaction) to the extent of 96% and *via* a water-catalyzed β-lactam opening reaction (k_0-reaction) to the extent of 4%. Therefore, the activation energy (22.6 kcal/mol) determined at this pH mainly reflects the intramolecular reaction initiated by the attack of the α-amino group on the β-lactam moiety.

At pH 11.50, the hydroxide ion-assisted degradation proceeded exclusively, the contribution of the k_b-reaction being only 0.1%. The apparent activation energy of 24.4 kcal/mol at this alkaline pH may include the heat of ionization of water, 13.1 kcal/mol.[10] The net

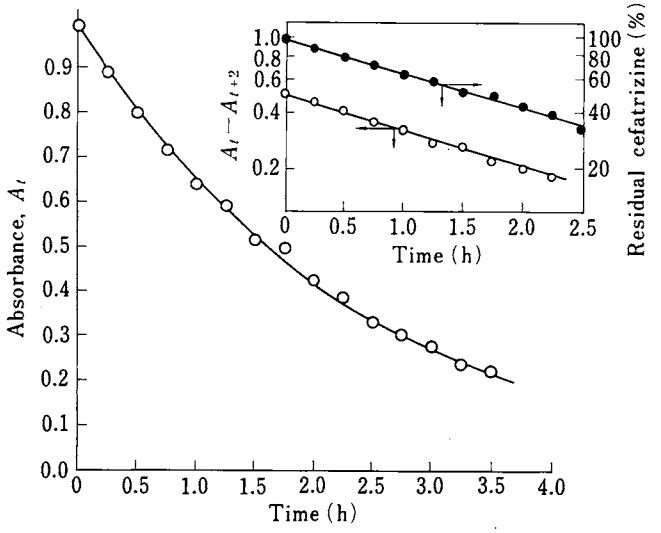


Fig. 6. Time Courses for Primary Amino Group Disappearance during 1×10^{-3} M Cefatrizine Degradation in 0.15 M Phosphate Buffer (pH 7.20) at 35 °C and an Ionic Strength of 0.5

Absorbance refers to the absorbance produced by subjecting equal aliquots of the reaction solution to the trinitrobenzenesulfonic acid assay. The insert shows the first-order plots of the data (○) obtained by Guggenheim treatment (2 h intervals) in comparison with the HPLC assay (●) of the same sample.

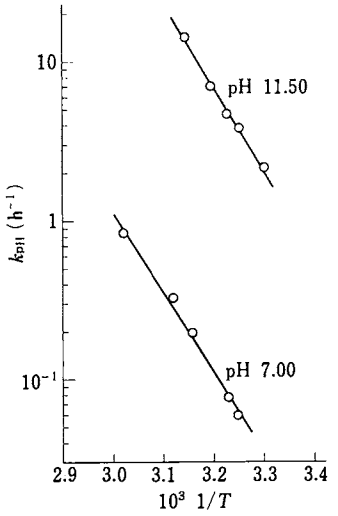


Fig. 7. Arrhenius Plots of the Apparent First-Order Rate Constants, k_{pH}, for Cefatrizine Degradation at pH 7.00 and 11.50, and an Ionic Strength of 0.5.

The pH values were maintained by the use of a pH-stat.

activation enthalpy, $\Delta H^{\neq}$, of the hydroxide ion-assisted degradation of cefatrizine was calculated to be 10.7 kcal/mol at 35 °C.

Solubility-pH Profile

The solubilities of cefatrizine propylene glycolate and cefadroxil monohydrate were determined at 37 °C and $\mu = 0.5$, and the data are listed in Table IV.

The relationship between the solubility and pH resembles the profiles reported for other aminocephalosporins[17] and also aminopenicillins[5]. The total solubility, C_T, can be expressed as a function of the solution pH:

$$C_T = C_o\left(1 + \frac{a_{H^+}}{K_{a_1}} + \frac{K_{a_2}}{a_{H^+}}\right) \quad (6)$$

TABLE IV. Intrisic Solubility, Heat of Solution, and Dissociation Constants of Aminocephalosporins at 37 °C and an Ionic Strength of 0.5

Cephalosporin	Molecular weight	Solubility $10^2\ C_0$ M (mg/ml)	Heat of solution ΔH_{sol} kcal/mol	Dissociation constants	
				pK_{a_1}	pK_{a_2}
Cefatrizine propylene glycolate	538.6	4.60 (24.8)	5.25	2.62	6.99
Cefadroxil monohydrate	381.4	4.59 (17.5)	1.35	2.70	7.22
Cephalexin monohydrate[a)]	365.4	4.72 (17.2)	1.39	2.67	6.96
Cephradine monohydrate[a)]	367.4	7.08 (26.0)	1.58	2.63	7.35
Cephaloglycin dihydrate[a)]	441.4	3.36 (14.8)	1.87	2.03	6.89

a) From ref. 17.

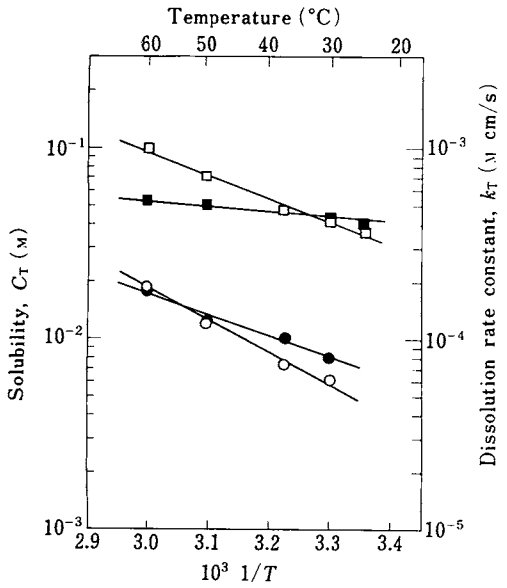


Fig. 8. Plots of Solubilities (Squares, Left Scale) and Dissolution Rate Constants at 228 rpm (Circles, Right Scale) of Cefatrizine Propylene Glycolate (Open Symbols) and Cefadroxil Monohydrate (Closed Symbols) in 0.5 M KCl *versus* the Reciprocal of Absolute Temperature

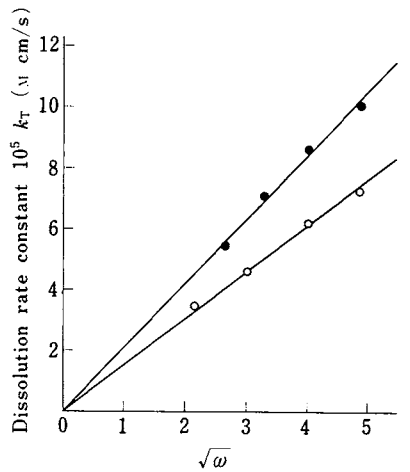


Fig. 9. Effect of Stirring Speed, $\sqrt{\omega}$, on the Dissolution Rate Constants, k_T, of Cefatrizine Propylene Glycolate (○) and Cefadroxil Monohydrate (●) in 0.5 M KCl at 37 °C

The minimum solubility (C_0) estimated from the solubility at the isoelectric point, $pI = 1/2(pK_{a_1} + pK_{a_2})$, and the dissociation constants under the experimental conditions used (37 °C and $\mu = 0.5$) are listed in Table II. The theoretical curves generated from Eq. (6) fitted reasonably well with the experimental points for both cefatrizine and cefadroxil (not shown).

Both aminocephalosporins exhibited very similar pH-dependent solubilities with the minimum solubility of 4.6×10^{-2} M. For comparison, the C_0 values of several aminocephalosporins determined under the same conditions[17] are also listed in Table IV; the solubility-pH profile of cephalexin monohydrate is very similar to those of cefatrizine propylene glycolate and cefadroxil monohydrate.

As shown in Fig. 8, the apparent equilibrium solubilities observed over the temperature range of 25—60 °C, when plotted in the classical van't Hoff fashion, gave a good linear relationship. The values of the heat of solution, ΔH_{sol}, for cefatrizine and cefadroxil were

TABLE V. Theoretical Equations[a] for the Dissolution Rate Constants at 228 rpm and Diffusion Coefficients of Aminocephalosporins at 37 °C and an Ionic Strength of 0.5

Cephalosporin	Theoretical equation,[b] k_T (theor) M cm²/s	Diffusion coefficient 10^6 D, cm²/s
Cefatrizine propylene glycolate	$1.18\times10^{-2}(a_{H^+})_h+7.45\times10^{-5}+1.59\times10^{-2}(a_{OH^-})_h$	
Cefadroxil monohydrate	$1.22\times10^{-2}(a_{H^+})_h+9.73\times10^{-5}+1.36\times10^{-2}(a_{OH^-})_h$	
Cephalexin monohydrate[c]	$1.22\times10^{-2}(a_{H^+})_h+9.43\times10^{-5}+1.40\times10^{-2}(a_{OH^-})_h$	
Cephradine monohydrate[c]	$1.35\times10^{-2}(a_{H^+})_h+12.4\times10^{-5}+1.50\times10^{-2}(a_{OH^-})_h$	
Cephaloglycin dihydrate[c]	$0.43\times10^{-2}(a_{H^+})_h+5.19\times10^{-5}+1.59\times10^{-2}(a_{OH^-})_h$	

a) Equation (9).
b) Calculated from Eqs. (9) and (10) with the parameters listed in Table IV and those cited in the text.
c) From ref. 17.

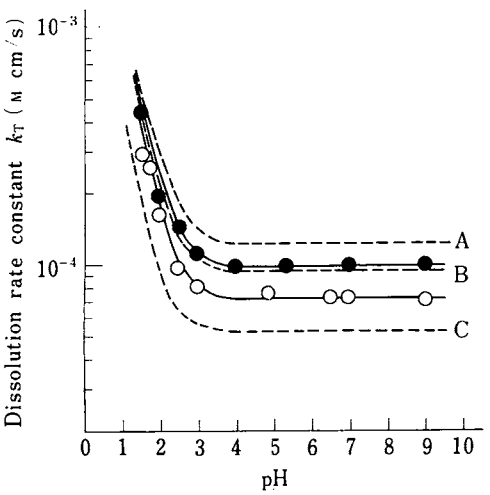


Fig. 10. $\log k_T$-pH Profiles for the Dissolution of Cefatrizine Propylene Glycolate (○) and Cefadroxil Monohydrate (●) at 228 rpm, 37 °C, and an Ionic Strength of 0.5

The points are experimental values, and the solid curves were generated from the theoretical equations listed in Table V. The dotted lines are the theoretical curves (from ref. 17, see Table V) for the dissolution rate constants of cephradine monohydrate (A), cephalexin monohydrate (B), and cephaloglycin dihydrate (C) at 228 rpm and an ionic strength of 0.5.

calculated from the slopes in Fig. 8 to be 5.25 and 1.35 kcal/mol, respectively. The difference in solvated forms between the two antibiotics may be responsible for this difference in the ΔH_{sol} values.

Dissolution Rate-pH Profile

The dissolution rate of drug solid in aqueous solution can be described by Eq. (7) under the sink condition:[5]

$$\left(\frac{dC}{dt}\right)_0=\frac{S}{V}k_T \tag{7}$$

where C represents the concentration of the dissolved drug at time t in the medium, S represents the area of the solid surface exposed to the solvent, V represents the volume of the dissolution medium, and k_T represents the dissolution rate constant in units of M cm s^{-1}.

When the dissolution of both antibiotics from the rotating disk is rate-limited by diffusion, the dissolution rate constants at the isoelectric pH values can be predicted theoretically[18] as:

$$k_T=0.620D^{2/3}\nu^{-1/6}\omega^{1/2}C_0 \tag{8}$$

where D is the diffusion coefficient of drug, ν is the kinematic viscosity of the dissolution

medium, and ω is the angular velocity of rotation. The observed dissolution rate constants, k_T, measured in 0.5 M KCl at four different rotating speeds are plotted against $\sqrt{\omega}$ in Fig. 9, and give a reasonably straight line, apparently obeying Eq. (8). The D-values of the two antibiotics could be calculated from the slopes employing $\nu = 6.99 \times 10^{-3}\,\mathrm{cm^2\,s^{-1}}$ [19] and are listed in Table V with the corresponding C_o values.

Dissolution rate constants, k_T, were determined as a function of the bulk solution pH at 37 °C and $\mu = 0.5$ (Fig. 10). For comparison with previous studies on other aminopenicillins[5] and aminocephalosporins,[17] the rotating speed was fixed at 228 rpm. The dissolution rate constants increased linearly with decreasing pH in the acidic medium below pH 3. Above this pH, the rate constants were independent of pH for both cephalosporins used in this study, as observed for the other amino-β-lactam antibiotics.

The complicated pH-dependent dissolution kinetics of these antibiotics can be interpreted theoretically by Eq. (9) which was derived previously:[5]

$$k_T = \frac{1}{h}\left[D_{H^+}\frac{D_{A^+}C_o}{D_{H^+}K_{a_1} + D_{A^+}C_o}(a_{H^+})_h + D_{A^\pm}C_o + D_{OH^-}\frac{D_{A^-}K_{a_2}C_o}{D_{OH^-}K_w + D_{A^-}K_{a_2}C_o}(a_{OH^-})_h\right] \quad (9)$$

where subscripts A^+, $A^\pm$, and A^- refer to the cationic, zwitterionic and anionic (both mono- and di-anions) species of cefatrizine or cefadroxil, respectively; D is the diffusion coefficient; $(a_{H^+})_h$ and $(a_{OH^-})_h$ are the hydrogen-ion and hydroxide-ion activities, respectively in the bulk solution; and h is the diffusion layer thickness calculated from Eq. (10):[18]

$$h = 1.612 D^{1/3} \nu^{1/6} \omega^{-1/2} \quad (10)$$

Assuming $D_{A^+} = D_{A^\pm} = D_{A^-}$ and employing $D_{H^+} = 4.12 \times 10^{-5}\,\mathrm{cm^2/s}$,[20] $D_{OH^-} = 3.43 \times 10^{-5}\,\mathrm{cm^2/s}$,[21,22] $K_w = 2.38 \times 10^{-14}$,[10] and the pertinent parameters determined in this study (Tables IV and V), the theoretical dissolution rate constants, k_T (theor) under the present experimental conditions can be derived from Eqs. (9) and (10), and are listed in Table V. The theoretical curves generated from these equations exhibit reasonably good agreement with the experimental points for both antibiotics.

Figure 10 also includes the theoretical curves for other aminocephalosporins transcribed from the previous report,[17] showing similar dissolution kinetic behavior with respect to the change in pH of the bulk dissolution medium. Among aminocephalosporins, it is clear that cephradine monohydrate, which has the highest solubility (see Table IV), gives the highest dissolution rate, and that cephaloglycin dihydrate (with the lowest solubility) exhibits the lowest dissolution rate, over the pH range below 9. Both cefatrizine propylene glycolate and cefadroxil monohydrate were found to have moderate solubilities and dissolution rates.

Figure 8 shows plots of the logarithm of the apparent dissolution rate constants of cefatrizine and cefadroxil in 0.5 M KCl at 228 rpm against the reciprocal of the absolute temperature. The values of heat of dissolution, ΔH_{dis}, calculated from the slopes of these lines were 7.54 and 4.99 kcal/mol, respectively. By subtracting the heat of solubility, ΔH_{sol}, from that of dissolution, the intrinsic energy concerned with the diffusion of the antibiotics and viscosity of the solution can be obtained. The calculated energies, $\Delta H_{dis} - \Delta H_{sol}$, were 2.29 and 3.64 kcal/mol for cefatrizine propylene glycolate and cefadroxil monohydrate, respectively. The energies are very similar to those for other aminocephalosporins determined under the same conditions, and are within the range of 3—5 kcal/mol considered reasonable for a diffusion process.[17]

Discussion

The degradation rates of several aminocephalosporins in the neutral and basic regions apparently depend on the nature of substituents at the 3-position (see Fig. 5). Cefatrizine was

highly reactive, like cephaloglycin, while the derivatives with a 3-methyl group, cephalexin, cephradine, and cefadroxil, are the most resistant to degradation. A comparison of the specific rate constants associated with the reactions of cefatrizine and cephaloglycin with the corresponding constants for the latter three antibiotics (Table III) shows that the β-lactam moiety of the first two antibiotics is about twofold more susceptible to attack of hydroxide-ion and about 10 times more susceptible to intramolecular attack by the C-7 side chain amino group. The difference in reactivity between the first two antibiotics and the latter three antibiotics may be ascribed to the difference in the long-range inductive effect on the electrophilicity of the β-lactam carbonyl carbon atom toward nucleophiles such as hydroxide ion and amines and/or the effectiveness as a leaving group of the 3-methylene moiety (R in Chart 1) which may lower the energy of the transition state, as suggested previously for other cephalosporin degradations.[9,13]

In acidic medium below pH 4, cefatrizine is reasonably stable with a half-life of 14 d at 35 °C and $\mu=0.5$, Among aminocephalosporins such as cephaloglycin, cephalexin, cephradine, cefadroxil, and cefatrizine, the stabilities of which have been investigated in our laboratory,[1,9] cephaloglycin is cosidered to be the most acid-unstable, and the other analogues exhibit similar acid-stability (see Fig. 5). Instability of cephaloglycin is due to the ester hydrolysis of the C-3 side chain rather than cleavage of the β-lactam moiety.[9] However, cephaloglycin has ten times greater acid-stability than ampicillin,[23] an aminopenicillin. It seems safe to say from our present and previous studies on the stability of β-lactam antibiotics that aminocephalosporins are more acid-stable than aminopenicillins. The destruction of aminocephalosporins during a gastric residence time of 30 min at pH 1.3 is almost negligible, whereas about 10% of aminopenicillins is degraded under these conditions.[5,23]

From our quantitative studies on the solubility and dissolution rates of five aminocephalosporins,[17] it is suggested that the dissolution of these antibiotics cannot be a rate-limiting step in their absorption process when they are ingested with sufficient water, as concluded previously[5] for aminopenicillins.

However, for cefatrizine its instability at the intestinal and/or body pH is suspected to give a reduced urinary recovery, as previously mentioned for cephaloglycin.[9] At the physiological temperature of 37 °C, the half-lives of cefatrizine are estimated to be 9.2 and 6.6 h in non-buffered solution at pH 7.0 and 7.4, respectively. Since the reported plasma half-life of cefatrizine is 1.4—1.7 h after oral administration in man,[24-26] the maximum extent of the chemical destruction of cefatrizine in the body is calculated to be about 20% based on the assumption of first-order kinetics. This result suggests that, if the degradation in body fluids proceeds in the same fashion as in aqueous solution at physiological pH and temperature, the recovery of cefatrizine in urine can never exceed 80%, even though rapid and complete absorption after the oral dose is established. Gaver and Deeb reported that the half-lives of cefatrizine in human plasma were 3—4 d at 7 °C and 15—16 h at room temperature,[26] whereas the half-lives of the chemical degradation extrapolated from the present kinetic study are 12 d and 24 h, respectively. Comparison of these data indicates that cefatrizine is 2—3 times more unstable in human plasma than in non-buffered aqueous solution under the same conditions. As suggested previously, some complex reaction other than chemical degradation may occur simultaneously in the plasma.

Widely varying recoveries of 35—82% in urine have been reported after the oral administration of cefatrizine. Because of the chemical instability of cefatrizine, the differences among the reported urine recoveries may be ascribed to variations in the preparation and handling of oral dosing solutions and in the time and temperature of storage and/or handling of urine samples before and during analysis. Cefatrizine is degraded when stored in a solution of pH 7.0, the half-lives of the β-lactam degradation at 25 and 7 °C being predicted to be 1.6 and 16 d, respectively, in good agreement with the reported values of 2—3 and 15 d at pH 6.8

in urine.[26] The present kinetic study suggests that adjusting the urine pH to below 4 markedly improves the stability of cefatrizine. Gaver and Deeb[26] demonstrated that, in urine acidified to pH 4, more than 95% of the original activitiy was still present after 4 d at room temperature and afrer 7 d at 7 °C, in accordance with our calculation.

On the other hand, 80—95% of cefadroxil was excreted in urine after the ingestion of a single 500 mg tablet.[27-29] Cefadroxil is ten times more stable under the physiological pH and temperature conditions than cefatrizine, suggesting negligible chemical degradation in the body. High recovery in urine is also found for cephalexin and cefradine, indicating complete absorption and no significant chemical and enzymatic degradations in the body.

Acknowledgement The authors wish to express their appreciation to Miss Yang Whie-Fang for her skillful technical assistance. They are also grateful to Banyu Pharmaceutical Co. and Bristol Myers Co. for the gifts of aminocephalosporins used in this study.

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