minor contribution of renal clearance to the overall erythromycin elimination and also to the compensating influences of increasing distribution volumes with decreasing elimination rate constants, both of which are involved in the calculation of serum clearance.

DISCUSSION

The changes in erythromycin elimination characteristics associated with the varying renal function observed support previous contentions that clearance of this antibiotic from the circulation is generally not influenced markedly by renal impairment (3, 4). The regression of β *versus* creatinine clearance was described by the linear expression $y = 0.21 + 0.001x$ ($r = +0.74$, $p < 0.05$), which is numerically similar to the expression reported previously (4); the linear regression of k_{el} *versus* creatinine clearance was described by $y = 0.49 + 0.002x$ ($r = +0.36$, $p > 0.05$).

The large distribution volumes (Table IV) are consistent with reported extensive tissue penetration of erythromycin (7–10). The apparent increase in distribution with decreasing renal function was possibly due to the displacement of this highly protein-bound drug from serum proteins in renal failure, resulting in a larger proportion of drug distributing into extravascular tissues. This phenomenon was demonstrated previously for a number of highly protein-bound drugs both *in vivo* (11, 12) and *in vitro* (13). Application of this concept to erythromycin needs to be verified since protein binding was not measured in the present study.

Although extensive tissue distribution by erythromycin is confirmed by the present data, the degree of tissue penetration is probably considerably underestimated. Drug bound to circulating proteins is not immediately available for extravascular distribution and, provided the drug is not bound to other tissues, the drug concentration in extravascular fluids tends to equilibrate with the concentration of free drug in serum. If the distribution volumes are calculated on this basis, and circulating drug is assumed to be approximately 90% protein bound (14), then calculated volumes are approximately 10 times greater than those given in Table IV. Since erythromycin is sequestered by certain tissues (7) and since there is no information on tissue binding of erythromycin, its true distribution volume cannot be calculated with any accuracy.

Although the calculated distribution volumes in Table IV may be underestimates of true values, they are reasonably consistent among normal individuals (Subjects 1–3) and may thus provide a basis for calculating absolute absorption efficiencies from oral doses of erythromycin or its salts.

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Physicochemical Properties of Amphoteric β-Lactam Antibiotics I: Stability, Solubility, and Dissolution Behavior of Amino Penicillins as a Function of pH

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Received August 22, 1977, from the *Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan.* Accepted for publication November 16, 1977.

Abstract □ The degradation rate, solubility, and dissolution rate of amino penicillins, amoxicillin, ampicillin, epicillin, and cyclacillin, were determined quantitatively as a function of pH. In the pH range studied, 0.30–10.50, the degradation of amoxicillin and epicillin followed pseudo-first-order kinetics to give the same type of pH–rate profiles as those of ampicillin and cyclacillin. Cyclacillin anhydrate was the most soluble, followed in order by ampicillin anhydrate, ampicillin trihydrate, amoxicillin trihydrate, and epicillin anhydrate. These pH–solubility profiles showed U-shaped curves. The dissolution rate constants from the rotating disk were analyzed by the simultaneous chemical reaction and diffusion models. Their relative bioavailability after a single oral administration was assessed from their physicochemical properties determined *in vitro*.

Keyphrases □ Penicillins, various amino—stability, solubility, and dissolution rates, effect of pH □ Stability—various amino penicillins, effect of pH □ Solubility—various amino penicillins, effect of pH □ Dissolution rates—various amino penicillins, effect of pH □ Antibacterials—various amino penicillins, stability, solubility, and dissolution rates, effect of pH

Amphoteric penicillins like ampicillin, amoxicillin, cyclacillin, and epicillin exhibit a broad spectrum of antibacterial activity, are very acid stable, and are used orally (1). After a single equal oral dose, the peak serum concentration was four times higher for cyclacillin (2) and two times higher for amoxicillin (3–6) than for ampicillin; the peak for epicillin was significantly lower (6).

The physicochemical properties and GI membrane permeabilities of these amphoteric penicillins may cause significant differences in their serum concentrations. An

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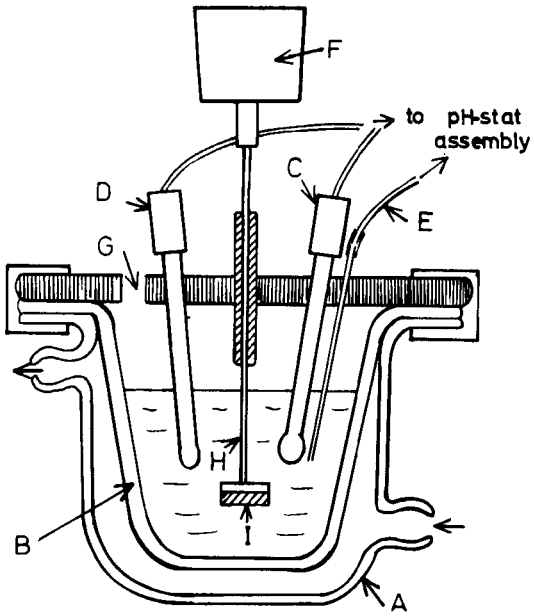


Figure 1—*Apparatus for determination of dissolution rates as a function of solution pH. Key: A, thermostated jacket; B, dissolution vessel; C, glass electrode; D, calomel electrode; E, titration buret; F, speed-controllable synchronous motor; G, sampling hole; H, rotating shaft assembly; and I, sample disk.*

enhanced bioavailability of anhydrous ampicillin compared with the trihydrated product was claimed to be due to its greater solubility and dissolution rate in distilled water (7). Hou and Poole (8) suggested that the rapid attainment of a higher serum level of cyclacillin than ampicillin is related to the difference in their solubilities. A reduction of dose water volume from 250 to 25 ml markedly decreased the serum amoxicillin level, but the ampicillin level was affected only to a small extent (9). This difference was explained by the difference in their water solubility (9).

The literature concerning the comparative absorption properties of the anhydrate and trihydrate forms of ampicillin is conflicting. Recently, Hill *et al.* (10) suggested that ampicillin absorption is not dissolution rate limiting, this suggestion being inconsistent with the previous claim of Poole *et al.* (7).

The objectives of this study were to compare quantitatively the stability, solubility, and dissolution behavior of several amphoteric penicillins as a function of solution pH and to assess the role of physicochemical properties affecting their relative bioavailabilities. The degradation kinetics (11, 12) and pH-dependent solubility (8) of some β-lactam antibiotics were studied extensively previously, but not those of amoxicillin and epicillin. The kinetics of the dissolution of all amino penicillins in the whole pH region have not been studied.

EXPERIMENTAL

Materials—Ampicillin sodium[1] (955 μg/mg), ampicillin anhydrate[1] (1015 μg/mg), ampicillin trihydrate[2] (pure powder), amoxicillin trihydrate[3] (850 μg/mg), cyclacillin anhydrate[1] (999 μg/mg), epicillin sodium[2] (pure powder), and epicillin anhydrate[2] (pure powder) were used as supplied.

[1] Takeda Chemical Industries, Osaka, Japan.
[2] Sankyo Co., Tokyo, Japan.
[3] Fujisawa Pharmaceutical Co., Osaka, Japan.

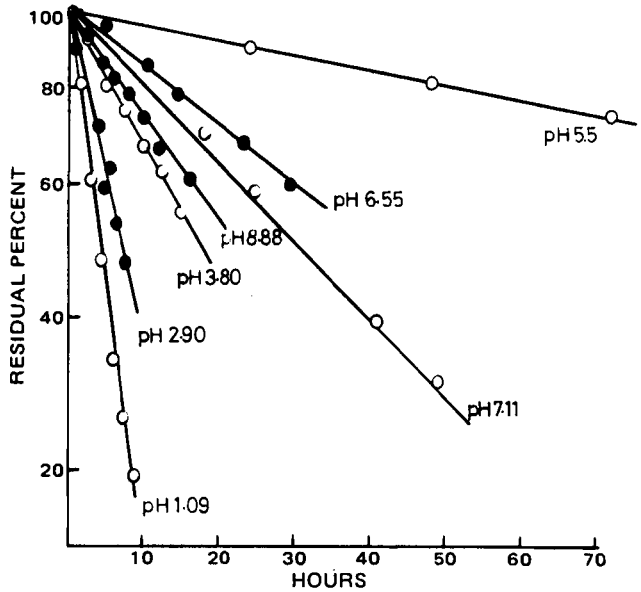


Figure 2—*First-order plots for the degradation of amoxicillin* (○) *and epicillin* (●) *in the buffers of various pH values at 35° and μ = 0.5.*

All other chemicals were reagent grade and were used without further purification.

Procedures—*pKa Determination*—The apparent pKa values of the amphoteric penicillins were determined by potentiometric titration (13) at an ionic strength of 0.5 at 35 and 37°. Twenty milliliters of 1.0×10^{-2} *M* penicillin, adjusted with potassium chloride to a constant ionic strength of 0.5, was titrated[4] with 0.2 *N* HCl (μ = 0.5 with potassium chloride) or 0.2 *N* KOH (μ = 0.5 with potassium chloride). The apparent pKa values of the amphoteric penicillins were computed, from the pH change[5] and the volume of the titrant added, by the equation of Glasstone and Hammel (14).

The values obtained from at least three experiments are listed in Tables I and II, where pK_1 and pK_2 refer to the dissociation of the 3-carboxylic acid and α-amino groups, respectively. The value of pK_3 for the phenolic group of amoxicillin was determined spectrophotometrically (13). The calculation was made from the change in absorbance[6] at 245 nm for 7.50×10^{-5} *M* amoxicillin, adjusted to 0.5 μ with potassium chloride, at various pH values and 37° to give $pK_3 = 9.55 \pm 0.04$.

Solubility—The procedure employed was essentially the same as that described by Hou and Poole (8). Excess penicillin powder was added to a glass-stoppered flask, followed by addition of 0.5 *M* KCl aqueous solution adjusted to the appropriate pH with standard hydrochloric acid or potassium hydroxide solution. The flask was placed in a constant-temperature bath at 37° and mechanically shaken for 2 hr. A sample was taken through a 0.45-μm membrane filter[7], the pH value was measured, and the sample was assayed after appropriate dilution, if necessary, with distilled water.

Amino penicillins were determined by iodometric titration (11) and by UV spectrophotometric measurement at 260 nm for ampicillin and amoxicillin and at 250 nm for epicillin. The penicillin concentration was calculated from respective calibration curves obtained earlier.

Determination of Degradation Rates—The kinetic studies of amoxicillin and epicillin were carried out in the same way as a similar study on ampicillin (11). Degradation was initiated by the addition of a known weight of amoxicillin or epicillin sodium to a buffer solution of 0.5 μ (with potassium chloride), which was preheated to 35°, to make a final concentration of 5×10^{-3} *M*. When the half-life was more than 1 day, the solution was sealed in a 5-ml ampul.

The residual concentration was analyzed by the iodometric titration method described for ampicillin (11). When the solution pH was maintained by means of a pH-stat[8] during the kinetic study, 0.5 *M* KCl

[4] Radiometer ABU12b autoburet, Copenhagen, Denmark.
[5] Radiometer PHM26 pH meter, Copenhagen, Danmark.
[6] Shimadzu UV200S double-beam recording spectrophotometer, Kyoto, Japan.
[7] Sartorius membrane filter GmbH, Göttingen, Germany.
[8] Radiometer pH stat-titrimeter assembly consisting of TTT11 titrator, PHM26 pH meter, and ABU12b autoburet, Copenhagen, Denmark.

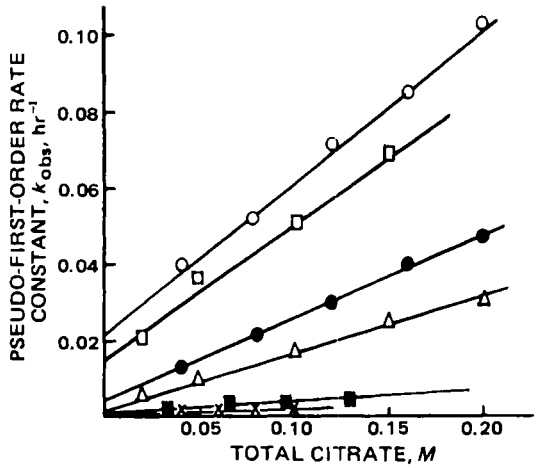


Figure 3—*Plots of the pseudo-first-order rate constant* versus *total citrate buffer concentrations for the degradation of amoxicillin at 35° and μ = 0.5. Key;* ○, *pH 2.80;* □, *pH 3.13;* ●, *pH 3.80;* △, *pH 4.23;* ■, *pH 5.50; and* ×, *pH 6.80.*

aqueous solution containing 1×10^{-4} *M* edetate disodium was used. Edetate disodium was added to avoid possible heavy metal-catalyzed degradation.

The pseudo-first-order rate constants, k_{obs}, were obtained by the least-squares method.

Determination of Dissolution Rates—The procedure for following the dissolution from a rotating disk was similar to that of Nogami *et al.* (15), except for the pH control technique. Figure 1 is a schematic representation of the rotating-disk dissolution apparatus. It consists of four main parts: the outside water-jacketed beaker maintained at 37 ± 0.05°, a stainless steel rotating shaft, a controllable synchronous motor, and pH electrodes. The tip of the titrant delivery tube is connected to the pH-stat assembly.

Disks of 200 mg and 1.0-cm diameter were prepared by compression of the crystals at about 120 kg/cm² in a compression punch-die assembly. When the samples were compressed, phase conversion was not observed from their IR spectra. The disk was stuck to the rotating shaft with an adhesive.

The solutions were sampled periodically, followed by the immediate addition of the same volume of 0.5 *M* KCl, preheated to 37°. The samples were analyzed by the iodometric method and/or by UV spectrophotometry. The penicillin concentration dissolved at each sampling time was corrected for dilution according to:

$$C = C_n^{app} + \sum_{n=1}^{n-1} \frac{V_n^s}{V_T} C_n^{app} \qquad \text{(Eq. 1)}$$

where C_n^{app} and C represent apparent and true concentrations dissolved at the nth sampling time, respectively; and V_T and V_n^s are the volumes of bulk solution and of sampling, respectively.

In most cases, the dissolution medium was recirculated by a peristaltic pump through a 1-cm path length flowcell in a double-beam UV spectrophotometer[6], and the increase in absorbance at 260 nm for ampicillin and amoxicillin and at 250 nm for cyclacillin and epicillin was recorded to give a continuous plot of the amount dissolved *versus* time.

Determination of Diffusion Coefficient—The method was that employed by Goldberg and Higuchi (16), except for the use of a cellulose membrane[9] instead of a silver membrane and two 50-ml conical flasks instead of two 150-ml flasks. The 0.5 *M* KCl containing a penicillin (1×10^{-2} *M*) was placed in one compartment (A) up to the stopcock, while almost the same volume of 0.5 *M* KCl was added to the other compartment (B). The entire cell was then immersed in a constant-temperature water bath.

The solution in Compartment B was stirred with a magnetic stirrer and recirculated for the continuous UV determination by the same procedure used for the dissolution study. The potassium chloride solution was treated to remove air prior to the experiments.

The cell constant was determined at 30 and 37° using 0.5 *M* KCl. The diffusion coefficient of sulfonamide was determined by this apparatus to be 1.28×10^{-5} cm²/sec, in good agreement with the reported value of 1.29×10^{-5} cm²/sec (17).

[9] Visking Co.

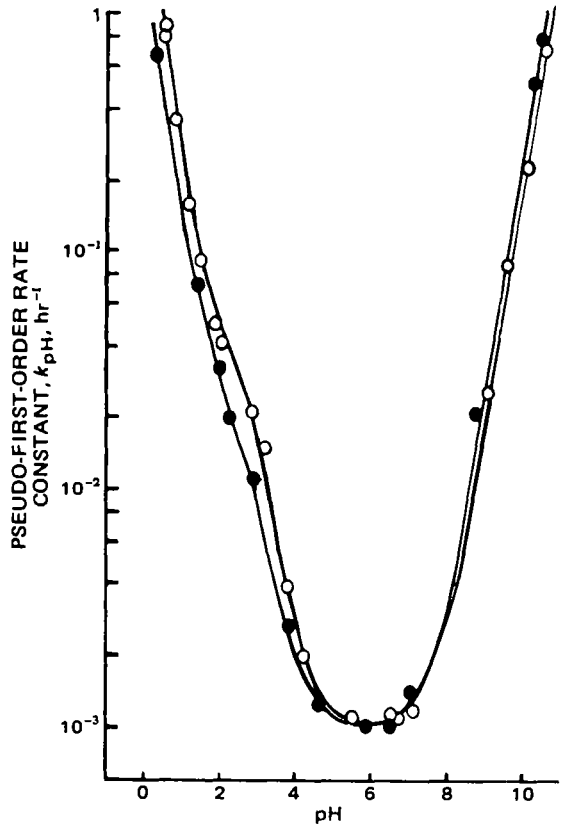


Figure 4—*Log k_{pH}–pH profiles for the degradation of amoxicillin* (○) *and epicillin* (●) *at 35° and μ = 0.5. The points are experimental values. The solid curves were generated from Eq. 2 and parameters listed in Table I.*

RESULTS

Degradation Rate–pH Profiles—The kinetics and mechanism of the degradation of amphoteric penicillins such as ampicillin (11) and cyclacillin (12) were reported previously. At constant pH and with excess buffer, the degradation of amoxicillin and epicillin at a low concentration of 5×10^{-3} *M* followed first-order kinetics (Fig. 2). As observed for ampicillin and cyclacillin, β-lactam cleavage of amoxicillin and epicillin was subjected to general acid–base catalyses. Typical plots of the citrate buffer catalytic effect on the rate of amoxicillin are shown in Fig. 3; a straight line was obtained at every pH. Extrapolation of such plots to zero-buffer concentration provides, as the intercepts, the values of pseudo-first-order rate constants, k_{pH}, corresponding to the non-buffer-catalyzed degradation of β-lactams. The k_{pH} values also were determined by the use of a pH-stat.

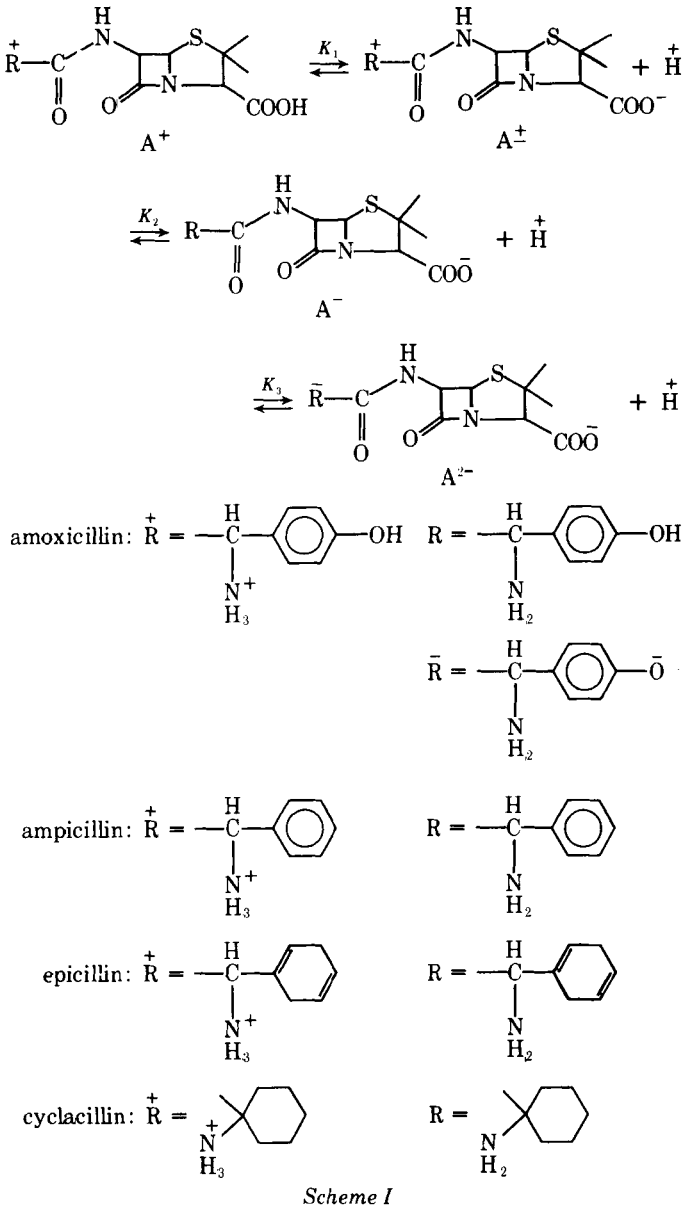
The pH dependence curves of k_{pH} of amoxicillin and epicillin are illustrated in Fig. 4, showing the same shape as those for ampicillin (11) and cyclacillin (12). Since there is no break near pK_3 of the log k_{pH}–pH profile of amoxicillin, the dissociation of the phenolic moiety apparently has no effect, as expected, on the β-lactam cleavage.

The rate expression given in Eq. 2 holds for amoxicillin and epicillin, based on the previous claim (12, 18) that the reactive species of penicillins

Table I—Rate Constants[a] for the Degradation of Amino Penicillins and Dissociation Constants at 35° and μ = 0.5

Amino Penicillin	k_H, M^{-1} hr^{-1}	$10^2 k_0$, hr^{-1}	$10^3 k_0'$, hr^{-1}	$10^{-3} k_{OH}$, M^{-1} hr^{-1}	pK_1[b]	pK_2[b]	pK_3[c]
Amoxicillin	1.68	4.19	1.00	1.16	2.63	7.16	9.55
Ampicillin[d]	1.82	5.56	0.750	2.57	—	—	—
Epicillin	1.35	2.08	0.969	1.23	2.77	7.17	—
Cyclacillin[e]	4.61	4.33	2.49	1.10	—	—	—

[a] Rate constants are defined by Eq. 2 and obtained by the least squares analysis. The K_w value used for the calculation was 2.09×10^{-14} at 35° and μ = 0.5 (26). [b] Determined by the potentiometric method. [c] Determined by the UV spectrophotometric method at 37° and μ = 0.5. [d] Values were recalculated according to Eq. 2 for the data reported by Hou and Poole (11). [e] Values were recalculated according to Eq. 2 for the data reported by Yamana *et al.* (12).



Scheme I

for acid-catalyzed and spontaneous degradations is one with the undissociated 3-carboxylic acid moiety:

$$k_{pH} = (k_H a_{H^+} + k_0)f_{A^+} + (k_0' + k_{OH'}K_w/a_{H^+})(f_{A^\pm} + f_{A^-}) \quad \text{(Eq. 2)}$$

where k_H and k_0 are the second-order rate constant for hydrogen-ion-catalyzed degradation and the first-order rate constant for spontaneous degradation of the cationic species, respectively; k_0' and $k_{OH'}$ are the first- and second-order rate constants for the respective spontaneous and hydroxide-ion-catalyzed degradations of species other than the cationic one, respectively; f_{A^+}, $f_{A^\pm}$, and f_{A^-} represent the mole fractions of the cationic, zwitterionic, and anionic species of amphoteric penicillins, respectively (Scheme I); and K_w is the autoprotolytic constant.

Table I lists these rate constants calculated from the least-squares analysis of the present data for amoxicillin and epicillin, together with the reestimated parameters according to the present kinetic expression of Eq. 2 for ampicillin (11) and cyclacillin (12).

Solubility–pH Profile—The pH–solubility behavior of ampicillin and cyclacillin was studied previously in aqueous buffer solution at 25° and μ = 0.5 (8). For a quantitative comparison of the relative solubility of amphoteric penicillins, the pH–apparent solubility profiles were determined under physiological temperature conditions for these two penicillins and also for amoxicillin and epicillin. The results (Fig. 5) indicate U-shaped pH–solubility curves, with the minimum solubility at the pH near the isoelectric point as observed previously (8).

If the intrinsic solubility of the amphoteric penicillins is due to the solubility, C_0, of the electrically neutral zwitterion ($A^\pm$ in Scheme I), the

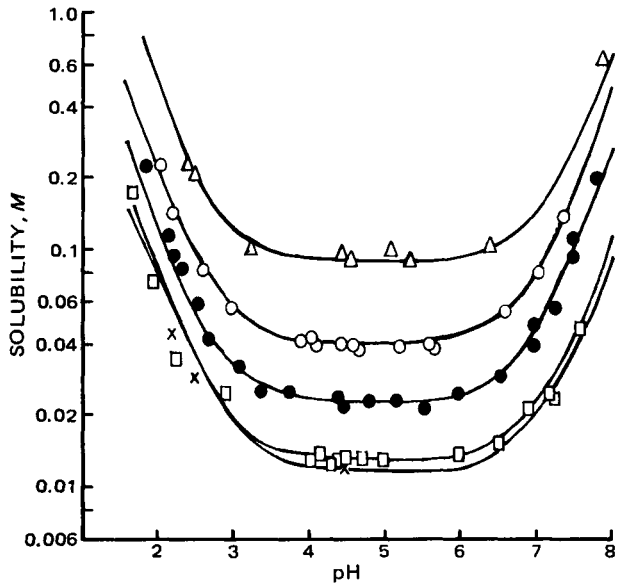


Figure 5—*Solubility–pH profiles of amino penicillins at 37° and μ = 0.5. The points are experimental values. The solid curves were generated from Eq. 3 and parameters listed in Table II. Key; △, cyclacillin anhydrate; ○, ampicillin anhydrate; ●, ampicillin trihydrate; □, amoxicillin trihydrate; and ×, epicillin anhydrate.*

total solubility, C_T, as a function of the solution pH can be expressed as:

$$C_T = C_0\left(\frac{a_{H^+}}{K_1} + 1 + \frac{K_2}{a_{H^+}}\right) \quad \text{(Eq. 3)}$$

The C_0 values estimated from the solubility at the isoelectric point and the dissociation constants under the experimental conditions (37° and μ = 0.5) are listed in Table II. The values of C_0 for ampicillin anhydrate, its trihydrate, and cyclacillin were in good agreement with those in distilled water (10, 19, 20) (Table II). The theoretical curves generated from Eq. 3 fit reasonably the experimental points for all amino penicillins (Fig. 5).

Over the entire pH range investigated, cyclacillin anhydrate was the most soluble and epicillin anhydrate exhibited the lowest solubility, with a sevenfold difference between them. Amoxicillin trihydrate was one-half as soluble as ampicillin trihydrate, which provided an inverse relationship in their comparative serum levels after oral administration (3–6).

The apparent equilibrium solubilities of amoxicillin were determined at 20–50°. Classical van't Hoff plots gave a reasonably good linear relationship (Fig. 6). The value of the heat of solution was calculated from the slope to be 6.2 kcal/mole, being comparable with the 5.4 kcal/mole value reported for ampicillin trihydrate (19).

Dissolution Rates—*Kinetics*—Figure 7 shows typical plots of the concentration, C, of penicillins dissolved *versus* time, t. In all cases, these plots were reasonably linear at an early stage of dissolution, indicating that dissolution follows zero-order kinetics:

$$\left(\frac{dC}{dt}\right)_0 = \frac{S}{V}k_T \quad \text{(Eq. 4)}$$

where S is the area of the solid surface exposed to the solvent, V is the volume of the bulk solution, and k_T is the dissolution rate constant. As shown in Fig. 8, the relationship between the dissolution rate for the unit

Table II—Intrinsic Solubilities and Dissociation Constants of Amino Penicillins at 37° and μ = 0.5

Amino Penicillin	Molecular Weight	Solubility[a], $10^2 C_0$, M	Dissociation Constant pK_1[b]	pK_2[b]	pK_3[c]
Amoxicillin trihydrate	419.4	1.30	2.67	7.11	9.55
Ampicillin anhydrate	349.4	3.97	2.67	6.95	—
Ampicillin trihydrate	403.4	2.23	—	—	—
Epicillin anhydrate	351.4	1.20	2.77[d]	7.17[d]	—
Cyclacillin anhydrate	341.4	8.90	2.64	7.18	—

[a] Defined by Eq. 3. [b] Determined by the potentiometric method. [c] Determined by the UV spectrophotometric method. [d] Determined at 35°.

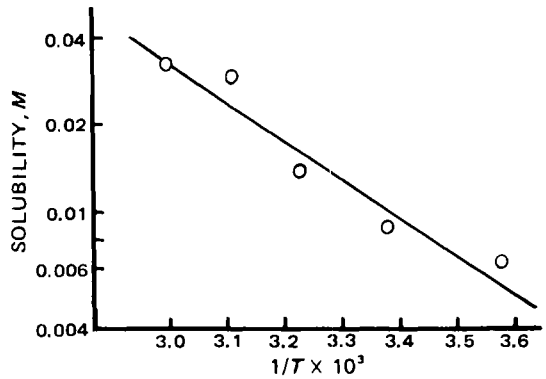


Figure 6—*Plots of the solubility of amoxicillin trihydrate in 0.5 M KCl versus a reciprocal of absolute temperature.*

surface area, $(dC/dt)_0/S$, obtained from the slope of these plots and a reciprocal of V from 20 to 200 ml provided straight lines through the origin in accordance with Eq. 4. With a constant speed of rotation, disk surface area, volume of dissolution medium, and temperature, analyses both by the sampling method and the direct flowcell method gave consistent dissolution rates that were not influenced significantly by the change in the dissolution vessel (Fig. 7).

When the dissolution rate constants, k_T, calculated according to Eq. 4, in 0.5 M KCl were plotted against the square root of the speed of rotation, a good linear relationship was obtained for all antibiotics (Fig. 9). If these dissolution processes from the rotating disk follow the rate-limiting diffusion, the dissolution rate constants can be predicted theoretically as (21):

$$k_T = 0.620D^{2/3}\nu^{-1/6}\omega^{1/2}C_0 \qquad \text{(Eq. 5)}$$

where D is the diffusion coefficient of a drug, ν is the kinematic viscosity of the dissolution medium, and ω is the angular velocity of rotation. With $\nu = 6.99 \times 10^{-3}$ cm²/sec (15) and the values of C_0 listed in Table II, the diffusion coefficients of the amino penicillins in 0.5 M KCl can be calculated from the respective slopes in Fig. 9 according to Eq. 5. The D values thus evaluated (Table III) were in fair agreement with those determined from the diffusion cell method (see *Experimental*). The present value for ampicillin was close to D (25°) = 4.58×10^{-6} cm/sec reported by Padfield and Kellaway (22).

These results strongly indicate that the dissolution of amphoteric penicillins into neutral pH solution is essentially controlled by diffusion of their zwitterionic molecules saturated near the surface of the disk.

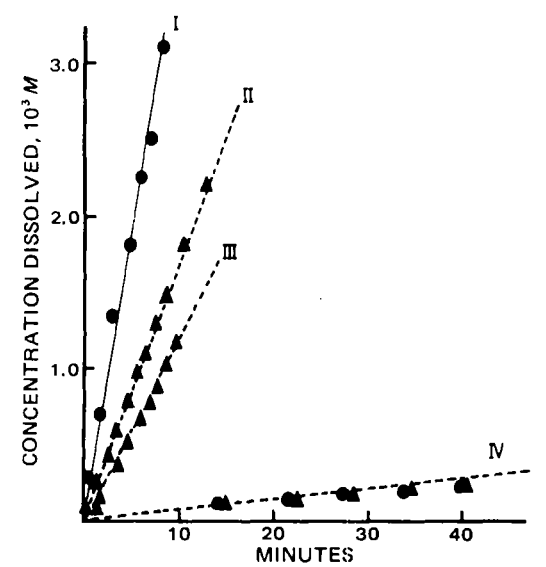


Figure 7—*Dissolution of amino penicillins into 0.5 M KCl at 228 rpm and 37° using the rotating-disk method. Key: cyclacillin anhydrate, I; ampicillin anhydrate, II; ampicillin trihydrate, III; amoxicillin trihydrate, IV; ●, analyzed by iodometry; and ▲, analyzed by UV spectrophotometry. Dotted lines represent the results from the continuous UV determination by means of the flowcell.*

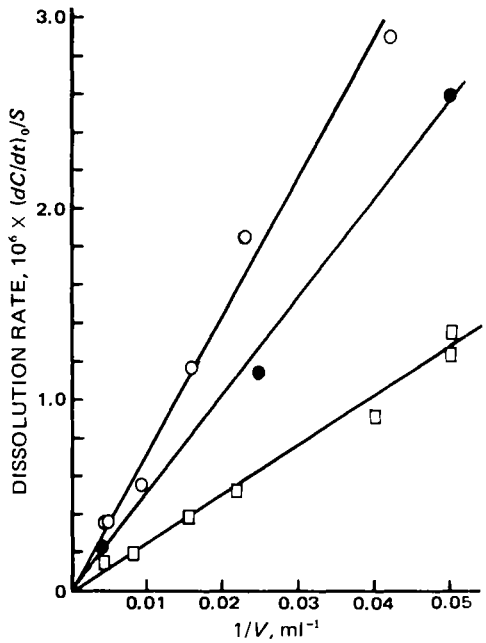


Figure 8—*Plots of the dissolution rate of amoxicillin trihydrate (□), ampicillin trihydrate (●), and ampicillin anhydrate (○) from the unit surface area, $(dC/dt)_0/S$, versus the reciprocal of the volume of the dissolution medium at 228 rpm, 37°, and $\mu = 0.5$.*

Theoretical Consideration of pH-Dependent Dissolution Rate—Dissolution rate constants, k_T, were determined as a function of the bulk solution pH at 228 rpm, 37°, and $\mu = 0.5$ (Fig. 10). The dissolution rate constants increased linearly in an acidic medium below pH 3. Beyond this pH, the rate constants were invariable for every penicillin. These phenomena are consistent with the results obtained for ampicillin by Hill *et al.* (10), who reported that the intrinsic dissolution rates were 10 times

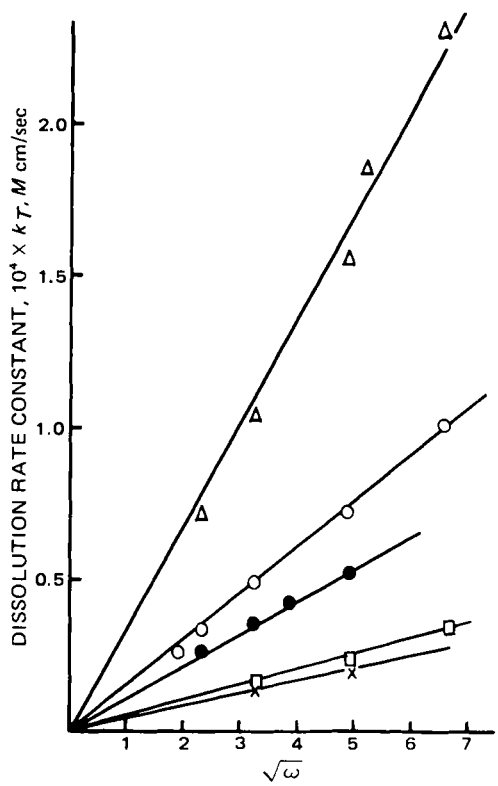


Figure 9—*Effect of stirring speed, $\sqrt{\omega}$, on the dissolution rate of amino penicillins in 0.5 M KCl at 37°. Key: △, cyclacillin anhydrate; ○, ampicillin anhydrate; ●, ampicillin trihydrate; □, amoxicillin trihydrate; and ×, epicillin anhydrate.*

Table III—Theoretical Equations [a] for the Dissolution Rate Constants at 228 rpm and Diffusion Coefficients of Amino Penicillins at 37° and $\mu = 0.5$

Amino Penicillin	Theoretical Equations[b], k_T (theor.), M cm/sec	Diffusion Coefficients, $10^6 \times D$, cm²/sec: Dissolution Rate[c]	Diffusion Cell[d]
Amoxicillin trihydrate	$7.03 \times 10^{-3}(a_{H^+})_h + 2.58 \times 10^{-5} + 1.41 \times 10^{-2}(a_{OH^-})_h$	4.84	3.71
Ampicillin anhydrate	$1.16 \times 10^{-2}(a_{H^+})_h + 7.29 \times 10^{-5} + 1.46 \times 10^{-2}(a_{OH^-})_h$	4.31	4.57
Ampicillin trihydrate	$1.03 \times 10^{-2}(a_{H^+})_h + 5.22 \times 10^{-5} + 1.29 \times 10^{-2}(a_{OH^-})_h$	6.20	4.60
Epicillin anhydrate	$7.05 \times 10^{-3}(a_{H^+})_h + 1.90 \times 10^{-5} + 1.58 \times 10^{-2}(a_{OH^-})_h$	3.45	4.60
Cyclacillin anhydrate	$1.41 \times 10^{-2}(a_{H^+})_h + 1.66 \times 10^{-4} + 1.45 \times 10^{-2}(a_{OH^-})_h$	4.41	4.54

[a] Equation 6. [b] Calculated from Eqs. 6 and 7 with the parameters listed in Tables II and III and those cited in the text. [c] Determined in 0.5 M KCl and used for the calculation of Eqs. 6 and 7. [d] Determined in 0.5 M KCl by means of the diffusion cell apparatus described in the text.

faster in 0.053 N HCl than in distilled water. The rank order of dissolution rates among penicillins was similar to that of their solubilities, but there was a clear difference in the shapes between these two pH profiles.

The complicated and pH-dependent dissolution behavior of amphoteric penicillins can be interpreted by the diffusion layer theory, predicted in terms of: (*a*) drug transfer from a nondisintegrated disk to an aqueous diffusion layer saturated with the drug, (*b*) transfer of the drug from the diffusion layer to the bulk medium, and (*c*) back-diffusion of hydrogen and hydroxide ions and rapid dissociation equilibrium. Scheme II describes qualitatively the steady-state situation of these dissolution processes. The theory leads to Eq. 6 for the steady-state dissolution rate constants of amphoteric penicillins under the sink conditions (see *Appendix*):

$$k_T = \frac{1}{h}\left[D_{H^+}\frac{D_{A^+}C_0}{D_{H^+}K_1 + D_{A^+}C_0}(a_{H^+})_h + D_{A^\pm}C_0 + D_{OH^-}\frac{D_{A^-}K_2C_0}{D_{OH^-}K_w + D_{A^-}K_2C_0}(a_{OH^-})_h \right] \quad \text{(Eq. 6)}$$

where h is the diffusion layer thickness, D's are the diffusion coefficients of species indicated by the subscripts, and $(a_{A^+})_h$ and $(a_{OH^-})_h$ are the hydrogen-ion and hydroxide-ion activities, respectively, in the bulk solution. The effective diffusion boundary layer thickness, h, may be written as (21):

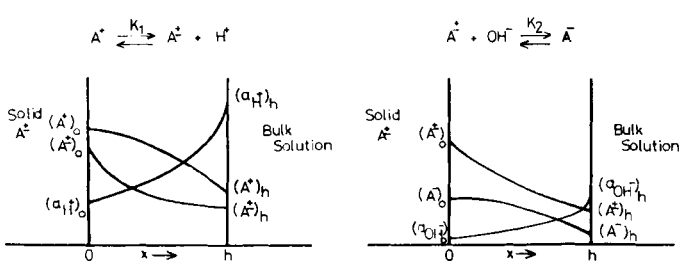
$$h = 1.612 D^{1/3} \nu^{1/6} \omega^{-1/2} \quad \text{(Eq. 7)}$$

By assuming $D_{A^+} = D_{A^\pm} = D_{A^-}$ and employing $D_{H^+} = 4.12 \times 10^{-5}$ cm²/sec (23), $D_{OH^-} = 3.43 \times 10^{-5}$ cm²/sec (24, 25), $K_w = 2.38 \times 10^{-14}$ (26), and the parameters determined in this work (Tables II and III), the theoretical dissolution rate constants, k_T (theor.), for the present experimental conditions of 228 rpm, 37°, and $\mu = 0.5$ can be derived from Eqs. 6 and 7 (Table III). Theoretical curves generated from these equations are illustrated in Fig. 10, indicating a reasonably good agreement with the experimental points for every amino penicillin.

Effect of Ionic Strength, Surfactant, and Temperature—The dissolution rates of amino penicillins were determined in the presence of various amounts of potassium chloride. Figure 11 shows that ionic strength had almost no effect on the dissolution rates of the trihydrate forms of both amoxicillin and ampicillin but that an increase in ionic strength markedly decreased the dissolution rate of ampicillin anhydrate.

The addition of 0.04–0.05 M of surfactants increased the solubility of amino penicillins in most cases and tended to increase the dissolution rate as a result of solubilization (Table IV).

The temperature dependence of the dissolution rate constant, k_T, was investigated for amoxicillin trihydrate. The Arrhenius plot is shown in Fig. 12. The activation energy calculated from the slope was 9.67 kcal/mole. This value is comparable with the 9.09-kcal/mole value determined by Wadke and Reier (27) for ampicillin trihydrate, both energies being reasonable for diffusion-controlled dissolution (27) with the respective heats of solution of 6.2 and 5.4 kcal/mole.



Scheme II—Concentration profiles during dissolution of amino penicillins into acidic medium (left) and alkaline medium (right).

DISCUSSION

The instability of penicillin in acidic solution is well known because of the intramolecular attack of the side-chain amide on the β-lactam moiety. Introduction of an electron-withdrawing group may be expected to reduce such a reaction rate. Since the protonated α-amino group in amoxicillin, ampicillin, epicillin, and cyclacillin plays a powerful electron-attracting role, these amino penicillins are markedly stable to acids, being almost independent of the structure attached to the amino group. The half-lives of the amino penicillins studied were essentially identical, being 15–20 hr at pH 2, 35°, and $\mu = 0.5$; the half life of penicillin G was only 7 min under similar conditions (28). The present results indicate that the extent of the destruction of the β-lactam group of aminopenicillins in the stomach environment is less significant and does not affect their comparative serum levels after oral administration.

The present kinetic analysis of the dissolution of amino penicillins as a function of solution pH supports the mechanism that the transport of amino penicillins from the solid surface to the bulk solution is essentially followed by the simultaneous diffusion and dissociation–equilibrium reaction of the zwitterionic species. The rank order of the dissolution rates of five types of amino penicillins was parallel, over the whole pH region, to that of their solubilities, as expected from the dissolution theory. The serum concentration after a single oral dose with sufficient water decreased in the order of cyclacillin anhydrate > amoxicillin trihydrate > ampicillin anhydrate ≧ ampicillin trihydrate ≧ epicillin anhydrate (2, 6); this pattern did not conform to the solubility order of cyclacillin anhydrate > ampicillin anhydrate > ampicillin trihydrate > amoxicillin trihydrate > epicillin anhydrate.

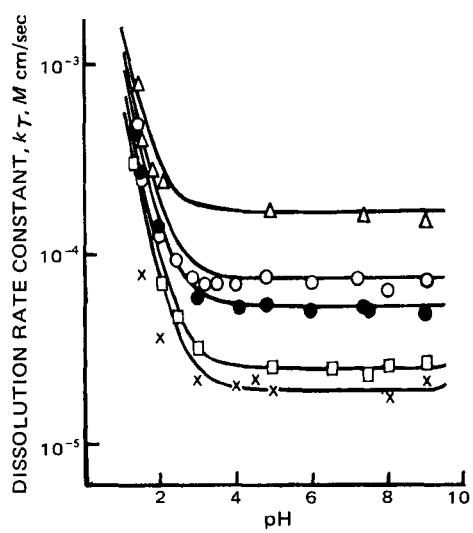


Figure 10—*Log* k_T–*pH profiles for the dissolution of amino penicillins at 228 rpm, 37°, and* $\mu = 0.5$. *The points are experimental values. The solid curves were generated from the theoretical equations listed in Table III. Key:* △, *cyclacillin anhydrate;* ○, *ampicillin anhydrate;* ●, *ampicillin trihydrate;* □, *amoxicillin trihydrate; and* ×, *epicillin anhydrate.*

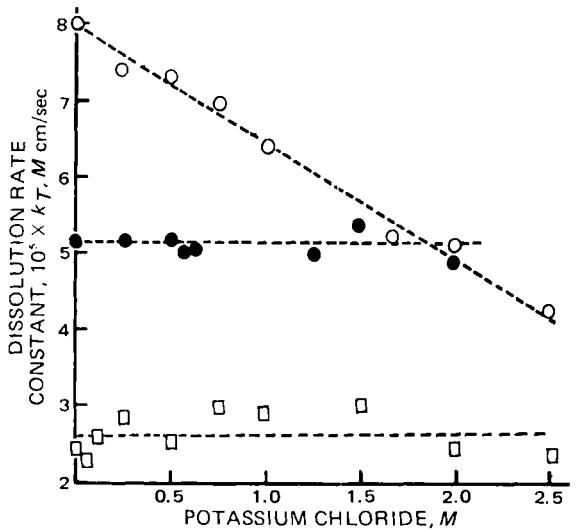


Figure 11—*Effect of ionic strength on the dissolution rate of ampicillin anhydrate* (○), *ampicillin trihydrate* (●), *and amoxicillin trihydrate* (□).

Above pH 3, the dissolution rates from the rotating disks were independent of the pH of the bulk solution; below this pH, the rates markedly increased with the increased acidity of the solution as a result of back-diffusion of the hydrogen ion toward the solid surface. Accordingly, the dissolution of the solids is expected to be fairly rapid under the gastric pH 1–3 conditions. For example, the time for 500 mg of a solid amino penicillin with a 100-cm^2 surface area to dissolve completely in 250 ml of water can be assessed from the present kinetic results, and it is only about 0.5 min at pH 1 and about 3–20 min at pH 3 under the GI agitation condition close to 55 rpm (29).

The amino penicillins, once dissolved, cannot be precipitated at any part of the GI tract, even considering the effect of ionic strength and surfactants such as bile acid. Naturally, administration of amino penicillins with a small amount of water may reduce bioavailability. Welling *et al.* (9) demonstrated that serum ampicillin levels were only slightly affected by fluid volumes, while amoxicillin levels were significantly reduced when 500 mg of each antibiotic was given with 25 ml of water compared to 250 ml of water. This marked effect on amoxicillin level may be related to the 1.7-fold difference in water solubility between ampicillin trihydrate and amoxicillin trihydrate.

The present results on the stability, solubility, and dissolution behavior of amino penicillins suggest that the dissolution of all types of amino penicillins cannot be a rate-determining step of their absorption when they were ingested with sufficient water, so their comparative serum levels may result from their comparative GI membrane permeability. The

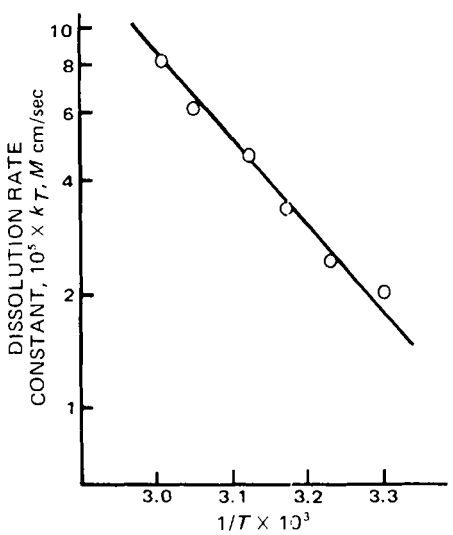


Figure 12—*Plots of the dissolution rate constants at 228 rpm of amoxicillin trihydrate in 0.5* M *KCl* versus *a reciprocal of absolute temperature.*

Table IV—Surfactant Effect on the Dissolution Rate Constants at 228 rpm and Solubilities of Amino Penicillins at 37°

Amino Penicillin	0.04 M Sodium Lauryl Sulfate: Dissolution Rate, $10^5 k_T$, M cm/sec	0.04 M Sodium Lauryl Sulfate: Solubility, $10^2 C_0$, M	0.04 M Cholic Acid: Dissolution Rate, $10^5 k_T$, M cm/sec	0.04 M Cholic Acid: Solubility, $10^2 C_0$, M
Amoxicillin trihydrate	2.67	1.6	--	--
Ampicillin anhydrate	9.63[a]	7.8[a]	9.00	4.40
Ampicillin trihydrate	6.59	3.5	5.44	2.63
Epicillin anhydrate	3.97	2.0	—	—

[a] Sodium lauryl sulfate (0.05 M).

difference in the bioavailability between two different hydrated forms of ampicillin, as observed previously (7), probably results from pharmaceutical formulation factors rather than their physicochemical properties such as solubility and dissolution rate. The conclusion for ampicillin is in agreement with the claim of Hill *et al.* (10) and with the recent *in vivo* observation that there is no significant difference in bioavailability between anhydrous ampicillin and its trihydrate form (10, 30).

APPENDIX: DERIVATION OF EQ. 6

In the acidic region, for the case of $A^{\pm}$ (solid) dissolving in the presence of a_{H^+}, the important equilibrium is:

$$K_1 = \frac{(A^{\pm})a_{H^+}}{(A^+)} \qquad \text{(Eq. A1)}$$

The simultaneous chemical reaction and diffusion model (31) gives the following appropriate differential equation:

$$D_{A^{\pm}}\frac{d^2(A^{\pm})}{dx^2} = -D_{A^+}\frac{d^2(A^+)}{dx^2} = D_{H^+}\frac{d^2a_{H^+}}{dx^2} \qquad \text{(Eq. A2)}$$

The integration of Eq. A2 yields:

$$D_{A^{\pm}}\frac{d(A^{\pm})}{dx} + C_1 = -D_{A^+}\frac{d(A^+)}{dx} + C_2 = D_{H^+}\frac{da_{H^+}}{dx} \qquad \text{(Eq. A3)}$$

where C_1 and C_2 are constants.

By applying proper material balance at $x = h$, $C_2 = 0$ and C_1 becomes:

$$C_1 = \left(D_{H^+}\frac{da_{H^+}}{dx}\right)_{x=h} - \left[D_{A^{\pm}}\frac{d(A^{\pm})}{dx}\right]_{x=h} \qquad \text{(Eq. A4}a\text{)}$$

$$C_1 = \left[D_{A^{\pm}}\frac{d(A^{\pm})}{dx}\right]_{x=0} \qquad \text{(Eq. A4}b\text{)}$$

Therefore, C_1 is the dissolution rate of $A^{\pm}$.

To obtain C_1, further integration of Eq. A3 from $x = h$ to $x = 0$ yields:

$$C_1 = \frac{1}{h}[D_{H^+}(a_{H^+})_h - D_{H^+}(a_{H^+})_0 + D_{A^{\pm}}(A^{\pm})_0 - D_{A^{\pm}}(A^{\pm})_h] \qquad \text{(Eq. A5)}$$

and also, by applying Eq. A1, yields:

$$(a_{H^+})_0 = \frac{D_{H^+}(a_{H^+})_h K_1 + D_{A^+}(A^+)_h K_1}{D_{A^+}(A^{\pm})_0 + D_{H^+}K_1} \qquad \text{(Eq. A6)}$$

Taking the boundary conditions $(A^+)_h = 0$, $(A^{\pm})_h = 0$, and $(A^{\pm})_0 = C_0$, C_1 is reduced to:

$$C_1 = \frac{1}{h}\left[D_{H^+}\frac{D_{A^+}C_0}{D_{H^+}K_1 + D_{A^+}C_0}(a_{H^+})_h + D_{A^{\pm}}C_0\right] \qquad \text{(Eq. A7)}$$

In the basic region, the important equilibrium is:

$$K_2 = \frac{(A^-)K_w}{(A^{\pm})a_{OH^-}} \qquad \text{(Eq. A8)}$$

Similar treatment of the dissolution of zwitterion solid into bulk basic solution gives:

$$C_1 = \frac{1}{h}\left[D_{A^{\pm}}C_0 + D_{OH^-}\frac{D_{A^-}K_2C_0}{D_{OH^-}K_w + D_{A^-}K_2C_0}(a_{OH^-})_h\right] \qquad \text{(Eq. A9)}$$

From Eqs. A7 and A9, the overall rate expression of Eq. 6 can be obtained.

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ACKNOWLEDGMENTS

Presented in part at the 97th annual meeting of the Pharmaceutical Society of Japan, Tokyo, Japan, April 1977.

The authors acknowledge the gifts of amino penicillins from Takeda Chemical Industries and Sankyo Co. They also thank Mr. W.-Z. Hwang and Miss H. Yamazaki for technical assistance.

Determination of Salicylic Acid and Aspirin in Multicomponent Tablets by Liquid Chromatography on a Nonionic Resin

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Received September 26, 1977, from the *Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2.* Accepted for publication November 18, 1977.

Abstract □ Aspirin and free salicylic acid were determined in combinations containing caffeine, phenacetin, salicylamide, and acetaminophen by liquid chromatography on poly(methyl methacrylate) resin. Precision and accuracy were ±0.6% for aspirin and ±2% for salicylic acid, the latter at levels corresponding to 0.02% of the aspirin content.

Keyphrases □ Aspirin—liquid chromatographic analysis in multicomponent tablets □ Salicylic acid—liquid chromatographic analysis in multicomponent tablets □ Liquid chromatography—analyses, aspirin and salicylic acid in multicomponent tablets □ Analgesics—aspirin and salicylic acid, liquid chromatographic analyses in multicomponent tablets

Of the methods reported for the determination of aspirin and salicylic acid in pharmaceutical preparations, relatively few permit their simultaneous determination. Partition chromatography combined with spectrophotometric measurement has been used for free salicylic acid determination in aspirin products (1–3). Direct spectrophotometry (4) and fluorometric methods (5, 6) also have been used for free salicylic acid determination.

The simultaneous determination of aspirin and salicylic acid has been performed by UV spectrophotometry (7), but this method and methods based on IR spectrophotometry (8) and nonaqueous titration (9) all lack sensitivity for the low levels of salicylic acid present in freshly prepared aspirin tablets. The simultaneous determination of aspirin and salicylic acid based on fluorometric or colorimetric quantitation has been performed on a continuous-flow automated analyzer (10).

GLC separation of aspirin and salicylic acid was achieved on high molecular weight polyethylene glycol containing isophthalic acid and supported on glass microbeads (11). Salicylic acid eluted after aspirin and showed considerable tailing, precluding use of the method for free salicylic acid in tablets. The presence of active

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