Kinetics of Sulfite-Induced Anaerobic Degradation of Epinephrine

B. R. HAJRATWALA

Abstract □ The rate constant–pH profile of the rate of sulfite-induced disappearance of epinephrine from an aqueous solution under anaerobic conditions was determined at 81° in the 3.63–5.00 pH range at the ionic strength of 0.2. The profile shows a linear relationship with a positive slope. The anaerobic rate shows buffer catalysis above pH 4.4. Metabisulfite was found to be more catalytic than either bisulfite or acetone bisulfite. The concentration of epinephrine used was 5.5×10^{-5} mole/liter.

Keyphrases □ Epinephrine—kinetics of sulfite-induced anaerobic degradation □ Antioxidants—effect on epinephrine degradation, kinetics of sulfite-induced anaerobic degradation □ Degradation—epinephrine, sulfite induced, kinetics □ Stability—epinephrine, kinetics of sulfite-induced degradation □ Sulfites—role in anaerobic degradation of epinephrine, kinetics

Epinephrine degradation in aqueous solutions can occur *via* racemization or oxidation, either in the presence or absence of oxygen. Of various studies (1–5) on epinephrine degradation by racemization, only one (5) was a kinetic study and it was limited to the 0.05–1.40 pH range. The epinephrine concentration ranged from 0.05 to 0.3 mole/liter. The study shows that the racemization of epinephrine in acidic solutions is specific hydrogen-ion catalyzed.

The kinetics of bisulfite-induced degradation of epinephrine were studied (6–10), and the bimolecular kinetics of epinephrine with sulfite ion were established. The study was done in the 4–7 pH range. The kinetics of epinephrine degradation in solution by molecular oxygen are a complex function of epinephrine concentration and oxygen (11).

The degradation kinetics of epinephrine when present in other products, *e.g.*, lidocaine hydrochloride injection (12), have not been published. The purpose of this investigation was to study the anaerobic degradation of epinephrine in aqueous solution over the 3.63–5.00 pH range (this generally covers the range of USP XVIII products containing epinephrine) as a preliminary to stability studies on products containing epinephrine. The concentration of epinephrine used was 0.001% (5.5×10^{-5} mole/liter), the maximum allowed in lidocaine hydrochloride injection. The degradation of epinephrine was induced by sodium metabisulfite.

EXPERIMENTAL

Materials—All materials used were of analytically pure grade. These included epinephrine (the *l*-form), anhydrous sodium acetate, acetic acid, sodium chloride, sodium bisulfite, sodium metabisulfite, and sodium acetone bisulfite.

Standardization of Epinephrine—The epinephrine was standardized against primary standard epinephrine bitartrate USP using a spectrofluorometric method (12). It was found to contain 99.82% epinephrine.

Kinetic Studies—An accurately weighed quantity (approximately 10 mg) of epinephrine was added to a 1-liter amber-colored volumetric flask and dissolved in an appropriate buffer solution containing 0.05% sodium metabisulfite (sodium chloride added to desired ionic strength), which had been preheated to 81°. Type I glass vials (30 ml) were preheated to 81° and filled with approximately 30 ml each of epinephrine solution under anaerobic conditions (using nitrogen). The vials were tightly sealed with rubber stoppers[1] and aluminum seals and were then placed in a constant-temperature circulator bath previously adjusted to 81 ± 0.1°.

The vials were allowed to equilibrate thermally. At appropriate time intervals, vials were removed from the bath and chilled, and their contents were analyzed for epinephrine using the USP XVIII spectrofluorometric method (12). The pH of each sample was measured to ensure the constancy of pH during the entire procedure. The degradation followed first-order kinetics (Fig. 1). The apparent first-order rate constants (Table I) were calculated using re-

Table I—Effect of Acetate Buffer Concentration on Degradation of Epinephrine at 81° and Ionic Strength 0.2

pH	Buffer Concentration, mole/liter	$k \times 10^3$, hr^{-1}	$t_{1/2}$, hr
3.63	0.02	2.98	233
	0.03	2.78	250
	0.07	2.89	241
4.15	0.02	3.96	175
	0.03	4.13	168
	0.07	3.86	179
4.62	0.01	4.83	144
	0.03	5.01	138
	0.07	4.94	140
	0.10	4.90	141
5.00	0.03	6.08	114
	0.07	6.35	109
	0.10	6.70	103

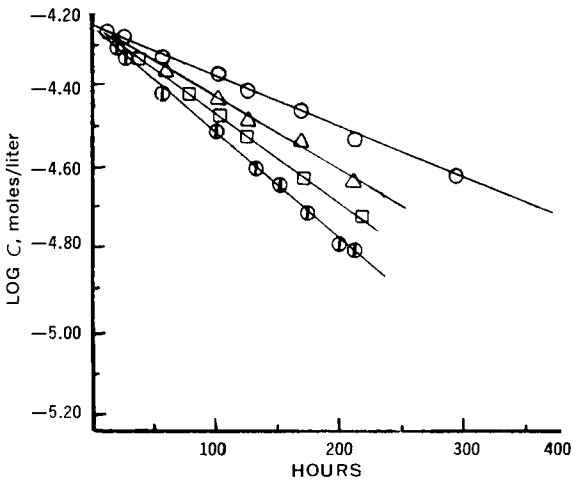

Figure 1—*Plot showing the overall first-order character of anaerobic degradation of epinephrine at various pH values in 0.03* M *acetate buffer at 81°. Key:* ○, *pH 3.63;* △, *pH 4.15;* □, *pH 4.62; and* ◐, *pH 5.00.*

[1] 1109 compound rubber stoppers, West Co., Phoenixville, PA 19460

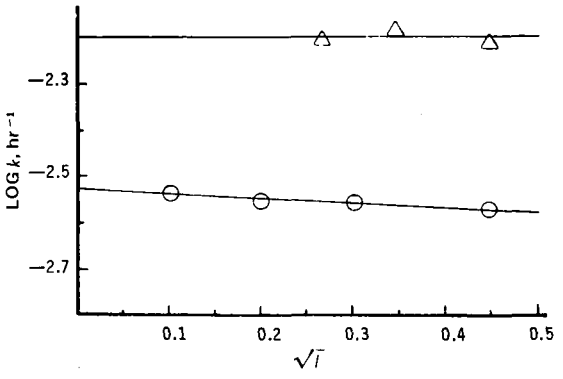

Figure 2—*Effect of ionic strength* (I) *on the degradation of epinephrine at 81° in 0.1* M *acetate buffers at various pH values. Key:* ○, *pH 3.63; and* △, *pH 5.00.*

gression analysis and 5–10 points. Correlation coefficients were between −0.918 and −0.999.

Effect of Ionic Strength—The effect of ionic strength was investigated at pH 3.63 and 5.00, using 0.1 *M* acetate buffers adjusted to ionic strengths of 0.01, 0.04, 0.07, 0.09, 0.12, and 0.20 by addition of sodium chloride (Fig. 2). The concentration of metabisulfite used was 0.05%.

Effect of Various Antioxidants—The antioxidants used were sodium bisulfite, sodium metabisulfite, and sodium acetone bisulfite in concentrations of 2.63×10^{-3} mole/liter in 0.03 *M* acetate buffer at pH 4.63 and ionic strength of 0.02 (Table II).

Degradation in Acetate Buffers—The degradation of epinephrine was studied in buffer containing varying concentrations of acetate at pH 3.63, 4.15, 4.62, and 5.00 at constant ionic strength of 0.2. Figure 3 shows the relationship between buffer concentration and the rate of degradation of epinephrine. The concentration of metabisulfite used was 0.05%.

pH–Rate Constant Profile—Table III shows the effect of hydrogen-ion concentration. The first-order rate constants (k_0) were obtained by extrapolation from Fig. 3 at zero buffer concentration. The pH–rate constant profile is shown in Fig. 4.

RESULTS AND DISCUSSION

At pH 5.00, the rate constant is independent of ionic strength. This indicates either the reaction of positive or negative ions with a neutral molecule or a unimolecular reaction of a positive, negative, or neutral molecule. At pH 3.63, the rate constant decreases with increasing ionic strength with a slope much less than 1 (approximately 0.1). A negative slope of less than 1 suggests that the reaction of ions of unlike sign is not dominating and that other reactions are occurring at this pH.

Table II shows that metabisulfite-induced degradation is greater than that for bisulfite or acetone bisulfite at pH 4.63. Riegelman and Fischer (13) showed that boric acid, which chelates with the catechol nucleus of epinephrine, protects it from inactivation by sulfite (6). Unfortunately, boric acid is toxic and cannot be used in injectables. Hence, sulfites continue to be used as antioxidants in marketed epinephrine-containing products (14), although they are far from ideal.

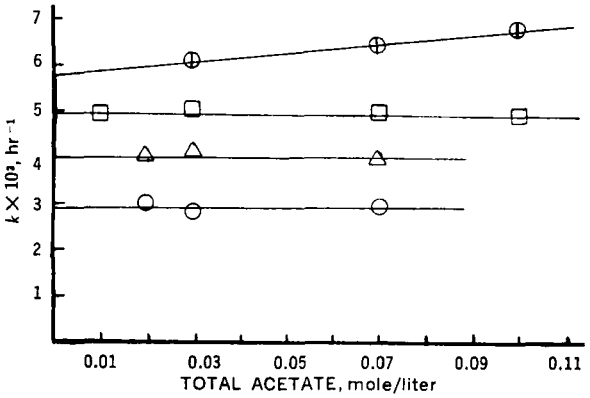

Figure 3—*Effect of acetate concentration on the rate of degradation of epinephrine at 81° at various pH values and ionic strength of 0.2. Key:* ○, *pH 3.63;* △, *pH 4.15;* □, *pH 4.62; and* ⊕, *pH 5.00.*

Table II—Effect of Various Antioxidants (2.63×10^{-3} mole/liter) on Anaerobic Degradation of Epinephrine in 0.03 *M* Acetate Buffer, pH 4.73, at 81° and Ionic Strength 0.02

Antioxidant	$k \times 10^3$, hr^{-1}	$t_{1/2}$, hr
Sodium bisulfite	3.81	182
Sodium acetone bisulfite	3.97	175
Sodium metabisulfite	4.92	141

The effect of total acetate concentration on the rate of degradation of epinephrine is shown in Fig. 3. The total acetate concentration is:

$$[Ac]_T = [HOAc] + [OAc^-] \qquad \text{(Eq. 1)}$$

where [HOAc] is undissociated acetic acid and [OAc^-] is dissociated acetic acid. The rate constants shown in Table I were obtained from log concentration *versus* time plots at each buffer concentration.

Only at pH 5.00 does increasing total acetate concentration increase the rate of degradation of epinephrine. Using the Harned and Owen equation (15), the pKa for acetic acid was calculated as 4.88 at 81°. The epinephrine pKa is 8.55 (16). Since this value is far removed from the pH range used in this study, the temperature effect on pKa of epinephrine was ignored.

In the pH range studied, epinephrine exists almost entirely as the positively charged (EpH^+) species. At pH 4.62 and below, neither the HOAc nor the OAc^- species is catalytic to the EpH^+ species. At pH 5.00, acetate (probably OAc^-) appears to be catalytic to the EpH^+ species.

The pH–rate constant profile is a valuable way of presenting kinetic data for reactions that are pH sensitive, *e.g.*, degradation of epinephrine. The pH–rate constant relationship of Table III can be expressed by the linear equation:

$$k_0(hr^{-1}) = 2.10 \times 10^{-3}pH - 4.76 \times 10^{-3} \qquad \text{(Eq. 2)}$$

with a standard error of 3.7×10^{-5}.

In considering the effect of pH on the degradation of epinephrine, the following reactions are taken into account:

$$Ep \xrightarrow{k_1} \text{products} \qquad \text{(Reaction 1)}$$

$$Ep + H^+ \xrightarrow{k_2} \text{products} \qquad \text{(Reaction 2)}$$

$$Ep + OH^- \xrightarrow{k_3} \text{products} \qquad \text{(Reaction 3)}$$

$$EpH^+ \xrightarrow{k_4} \text{products} \qquad \text{(Reaction 4)}$$

$$EpH^+ + H^+ \xrightarrow{k_5} \text{products} \qquad \text{(Reaction 5)}$$

$$EpH^+ + OH^- \xrightarrow{k_6} \text{products} \qquad \text{(Reaction 6)}$$

where Ep is unprotonated base and EpH^+ is protonated base, and:

$$Ep + H_2A \xrightarrow{k_7} \text{products} \qquad \text{(Reaction 7)}$$

$$Ep + HA^- \xrightarrow{k_8} \text{products} \qquad \text{(Reaction 8)}$$

$$Ep + A^{-2} \xrightarrow{k_9} \text{products} \qquad \text{(Reaction 9)}$$

$$EpH^+ + H_2A \xrightarrow{k_{10}} \text{products} \qquad \text{(Reaction 10)}$$

$$EpH^+ + HA^- \xrightarrow{k_{11}} \text{products} \qquad \text{(Reaction 11)}$$

$$EpH^+ + A^{-2} \xrightarrow{k_{12}} \text{products} \qquad \text{(Reaction 12)}$$

Table III—Catalytic Effect of Hydrogen Ion on Degradation of Epinephrine at 81° and Ionic Strength 0.2[a]

pH	$k_0 \times 10^3$, hr^{-1}	$k_{calc} \times 10^3$, hr^{-1}
3.63	2.85	2.867
4.15	4.00	3.960
4.62	4.92	4.947
5.00	5.75	5.746

[a] The k_0 values were obtained by extrapolation from Fig. 3; the k_{calc} values were obtained using Eq. 2.

where H_2A, HA, and A^{-2} are undissociated, monodissociated, and didissociated species of sulfurous acid, respectively. Kinetically, Reactions 1 and 6, 2 and 4, 7 and 11, and 8 and 12 are equivalent and cannot be distinguished from each other. Thus, Reactions 1, 2, 3, 5, 7, 8, 9, and 10 are considered. The overall velocity of the reaction should be equal to the sum of the rates of these reactions:

$$-\frac{d(Ep)_T}{dt} = k_1(Ep) + k_2(Ep)(H^+) + k_3(Ep)(OH^-) + k_5(EpH^+)(H^+) + k_7(Ep)(H_2A) + k_8(Ep)(HA^-) + k_9(Ep)(A^{-2}) + k_{10}(EpH^+)(H_2A) \quad \text{(Eq. 3)}$$

Because of the overall first-order character of the reaction:

$$-\frac{d(Ep)_T}{dt} = k_{obs}(Ep)_T \quad \text{(Eq. 4)}$$

combining Eq. 4 and:

$$K_a^e = \frac{(H^+)(Ep)}{(EpH^+)} \quad \text{(Eq. 5)}$$

$$K_{a_1}^s = \frac{(H^+)(HA^-)}{(H_2A)} \quad \text{(Eq. 6)}$$

$$K_{a_2}^s = \frac{(H^+)(A^{-2})}{(HA^-)} \quad \text{(Eq. 7)}$$

and substituting in Eq. 3 gives:

$$k_{obs} = \frac{k_1K_a^e}{(K_a^e + H^+)} + \frac{k_2K_a^e(H^+)}{(K_a^e + H^+)} + \frac{k_3K_a^eK_w/(H^+)}{(K_a^e + H^+)} + \frac{k_5(H^+)^2}{(K_a^e + H^+)} + \frac{k_7K_a^eA_T(H^+)^2}{(K_a^e + H^+)[(H^+)^2 + K_{a_1}^sH^+ + K_{a_1}^sK_{a_2}^s]} + \frac{k_8K_a^eK_{a_1}^sA_T(H^+)}{(K_a^e + H^+)[(H^+)^2 + K_{a_1}^sH^+ + K_{a_1}^sK_{a_2}^s]} + \frac{k_9K_a^eK_{a_1}^sK_{a_2}^sA_T}{(K_a^e + H^+)[(H^+)^2 + K_{a_1}^sH^+ + K_{a_1}^sK_{a_2}^s]} + \frac{k_{10}(H^+)^3A_T}{(K_a^e + H^+)[(H^+)^2 + K_{a_1}^sH^+ + K_{a_1}^sK_{a_2}^s]} \quad \text{(Eq. 8)}$$

where $A_T = (H_2A) + (HA^-) + (A^{-2})$, $(Ep)_T = (EpH^+) + (Ep)$, and K_w = autoprotolysis constant.

For the pH range studied, $H^+ \gg K_a^e$ and $K_{a_2}^s \ll H^+ \ll K_{a_1}^s$ [$K_{a_1}^s = 1.54 \times 10^{-2}$ and $K_{a_2}^s = 1.02 \times 10^{-7}$ (19)]. Thus, Eq. 8 reduces to:

$$k_{obs} = a + b(H^+) + c/(H^+) + d/(H^+)^2 \quad \text{(Eq. 9)}$$

where $a = k_2K_a^e + (k_7K_aA_T/K_{a_1}^s)$, $b = k_5 + (k_{10}A_T/K_{a_1}^s)$, $c = k_1K_a^e + k_8K_a^eA_T$, and $d = k_3K_a^eK_w + k_9K_a^eK_{a_1}^sA_T$. However, Eq. 9 does not fit the experimental data.

There are three possible reasons for the apparent failure of the proposed mechanism to fit the experimental results:

1. The mechanism is more complex than the one proposed, probably involving free radicals.

2. The proposed mechanism is incorrect and the various assumptions made in proposing the set of 12 reactions are incorrect. However, this is not very likely; the complex degradation of morphine and ascorbic acid was successfully described on the basis of similar principles (18, 19).

3. The mechanism may hold good, but over the narrow pH range the polynomial (Eq. 9) has an approximately linear segment.

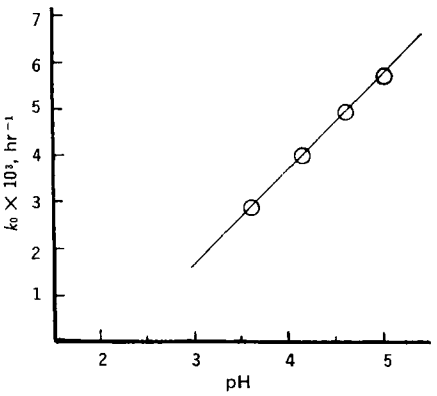

Figure 4—*The pH–rate constant profile for sulfite-induced epinephrine degradation in aqueous solution at 81° and ionic strength of 0.2.*

SUMMARY AND CONCLUSION

The pH dependence of sulfite-induced anaerobic degradation of epinephrine was determined at 81° over the 3.63–5.00 pH range at the ionic strength of 0.2. The rate of disappearance of epinephrine was obtained by determining the concentration of unreacted epinephrine *versus* time using the USP XVIII spectrofluorometric method. An apparent first-order rate of degradation was observed.

The primary salt effect was almost zero at pH 3.63 and 5.00. Acetate ions, undissociated and monodissociated, are not catalytic to protonated epinephrine at pH 4.62 and below. At pH 5.00, monodissociated acetate ions appear to be catalytic to protonated epinephrine species. The pH–rate constant profile shows a linear relationship in the pH regions studied (Eq. 2).

The pH–rate constant profile is not readily explained on a mechanism based on a set of 12 reactions (among species present).

The effects of various antioxidants show that metabisulfite is more catalytic to epinephrine degradation than bisulfite or acetone bisulfite.

REFERENCES

(1) H. Berry and G. B. West, *Quart. J. Pharm. Pharmacol.*, **17**, 242(1944).

(2) H. Hellberg, *J. Pharm. Pharmacol.*, **7**, 191(1955).

(3) L. A. Haddock, *Quart. J. Pharm. Pharmacol.*, **6**, 496(1933).

(4) G. Florence and G. Shapira, *Arch. Phys. Biol., Suppl.*, **17**, 94(1951).

(5) L. C. Schroeter and T. Higuchi, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 426(1958).

(6) L. C. Schroeter, T. Higuchi, and E. E. Schuler, *ibid.*, **47**, 723(1958).

(7) T. Higuchi and L. C. Schroeter, *ibid.*, **48**, 535(1959).

(8) *Ibid.*, **49**, 331(1960).

(9) T. Higuchi and L. C. Schroeter, *J. Amer. Chem. Soc.*, **82**, 1904(1960).

(10) L. C. Schroeter and T. Higuchi, *J. Pharm. Sci.*, **51**, 888(1962).

(11) T. D. Sokoloski and T. Higuchi, *ibid.*, **51**, 172(1962).

(12) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 366.

(13) S. Riegelman and E. Z. Fischer, *J. Pharm. Sci.*, **51**, 206(1962).

(14) B. R. Hajratwala and B. K. Lee, paper presented at the American Society of Hospital Pharmacists 28th annual meeting, San Francisco, Calif., Apr. 1971.

(15) H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolytic Solutions," Reinhold, New York, N.Y., 1958, pp. 665, 667, 758.

(16) M. M. Tuckerman, J. R. Mayer, and F. C. Nachod, *J. Amer. Chem. Soc.*, **81**, 92(1959).
(17) "Handbook of Chemistry and Physics," 52nd ed., Chemical Rubber Co., Cleveland, Ohio, 1971–72, p. D-121.
(18) S. M. Blaug and B. R. Hajratwala, *J. Pharm. Sci.*, **61**, 556(1972).
(19) S. Y. Yeh and J. L. Lach, *ibid.*, **50**, 35(1961).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 3, 1974, from *Research and Development Department, Invenex Pharmaceuticals, San Francisco, CA 94124*

Accepted for publication July 17, 1974.

Present address: Department of Pharmacy, University of Otago, Dunedin, New Zealand.

Solubility of Nonelectrolytes in Polar Solvents III: Alkyl *p*-Aminobenzoates in Polar and Mixed Solvents

S. H. YALKOWSKY ˣ, G. L. AMIDON *, G. ZOGRAFI *, and G. L. FLYNN ‡

Abstract □ The relative solubilities of *n*-alkyl *p*-aminobenzoates in water, propylene glycol–water mixtures, propylene glycol, and several other pharmaceutically important solvents can be predicted on the basis of a theoretical equation. This equation relates the activity coefficient of the hydrophobic portion of the molecule to the product of its surface area and its interfacial tension [free energy per unit area of a hydrocarbon (tetradecane) against the polar or semipolar solvent of interest]. The assumptions, conclusions, and applicability of the theoretical relationship are compared to those of the Scatchard–Hildebrand approach.

Keyphrases □ *p*-Aminobenzoates, alkyl—solubility in polar and mixed solvents, equation developed for predicting solubility □ Alkyl *p*-aminobenzoates—solubility in polar and mixed solvents, equation developed for predicting solubility □ Solubility—alkyl *p*-aminobenzoates in polar and mixed solvents, equation developed for predicting solubility, compared to Scatchard–Hildebrand approach □ Solvents, polar—solubility of nonelectrolytes (alkyl *p*-aminobenzoates)

From a pharmaceutical point of view, the most important physical-chemical property of a substance is its aqueous solubility. In addition to designating the maximum concentration (blood level) attainable for a drug, aqueous solubility is a dominant factor in partitioning and adsorption onto biological surfaces. Solubility in water-miscible polar solvents and in mixed aqueous solvents is also of great potential utility in the design of parenteral, topical, and liquid vehicles for drugs.

The ability to predict the effects of even simple structural modifications or vehicle modifications on solubility can be of great value in the design of improved drugs and drug delivery systems. Theoretical descriptions of solubility have mainly been restricted to either nonpolar solutes in nonpolar solvents (1–4) or to salts and other highly polar solutes in water (5) and are thus not directly applicable to either aqueous (or polar) solvents of pharmaceutical interest. Several empirical correlations between structure and aqueous solubility have been published (6–8) but have not received wide acceptance.

Recently, the authors (9, 10) applied an "interfacial" model to the solubilities of aliphatic alcohols and hydrocarbons in water. This model equates the combined attractive and repulsive forces between the hydrocarbon portion of the molecule and water with the product of the molecular surface area and the free energy per unit area (the latter being related to the curvature corrected hydrocarbon–water interfacial tension). It has been used successfully for primary, secondary, tertiary, linear, branched, and cyclic alcohols and hydrocarbons (10) and also for other liquid series[1]. It is also applicable to series whose members are crystalline provided that the ideal solubility (determined from thermal data) is taken into account.

THEORETICAL

In an ideal solution, the solute–solute and solvent–solvent interactions are equivalent to the solute–solvent interactions, and there is no change in heat or volume on mixing. Thus, the only thermodynamic factor affecting solubility is the entropy of mixing, which results in infinite miscibility or a mole fractional solute solubility (X_2) of unity. This is frequently written as:

$$-\log (X_2)^{\text{ideal}} = 0 \qquad \text{(Eq. 1)}$$

If the solute is a solid, the crystal lattice energy opposes the solution process. The magnitude of this effect on solubility is approximately:

$$-\log (X_2)^{\text{ideal}} = \frac{\Delta H_f}{2.303R} \frac{(T_f - T)}{T_f T} \qquad \text{(Eq. 2)}$$

where ΔH_f is the molar heat of fusion of the crystal having an absolute melting point of T_f, R is the gas constant, and T is the absolute temperature. At the melting point, where the solute becomes a liquid, $(T_f - T)$ vanishes and Eq. 2 becomes Eq. 1.

Virtually all pharmaceutically important solutes have aqueous and polar solvent solubilities well below their ideal values. For these solutes, the deviation from ideality is described by an activity coefficient (ac) defined so that:

$$-\log X_2^{\text{exp}} = \log X_2^{\text{ideal}} + \log (ac) \qquad \text{(Eq. 3)}$$

The activity coefficient reflects the sum of: *(a)* the work required to remove a solute molecule from its surrounding of other solute molecules, W_{22}; *(b)* the work required to create a cavity in the sol-

[1] S. H. Yalkowsky and G. L. Amidon, unpublished observations.