Solubility of Organic Hydrochlorides

S. F. KRAMER▲ and G. L. FLYNN

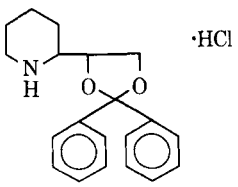
Abstract □ The solubilities of two polysubstituted 1,3-dioxolanes, each containing a 4-(2′-piperidyl) substituent, were examined as a function of pH, temperature, and solvent composition. Mathematical equations describing the total solubility at any arbitrary pH in terms of the independent solubilities of the hydrochloride and free base species and the dissociation constant of the salt were derived and fitted to the data with good result. Alternatively, a method of estimating the true pK′a of a hydrochloride from the solubility profile at a given temperature is indicated. The data show that the shape of the pH profile is more dependent on the solubility of the free base than on that of the hydrochloride. The free base solubility determines the pH range in which the solubility abruptly drops from that of the soluble salt to that of the relatively insoluble free amine. To shift the pH–solubility profile to higher pH values, as may be required in formulation, it is necessary to increase the solubility of the free base. Methods and solvents are indicated whereby this can be accomplished without appreciably affecting the solubility of the hydrochloride form.

Keyphrases □ Hydrochlorides, organic—solubility as a function of pH, temperature, and solvent composition, equations □ Amine hydrochlorides—solubility as a function of pH, temperature, and solvent composition □ Solubility of organic hydrochlorides—pH profiles, temperature dependence, species equilibrium □ pH–solubility profiles—organic hydrochlorides (dexoxadrol and etoxadrol hydrochlorides) □ Temperature effect—solubility of two amine hydrochlorides □ Dexoxadrol hydrochloride—solubility profile □ Etoxadrol hydrochloride—solubility profile

Relationships organizing solubilities of organic compounds are of general pharmaceutical interest because solubility is usually an important factor in the pharmacokinetic profile of a drug, its chemical stability, and, ultimately, its formulation. Present solubility theories mainly apply to nonpharmaceutical solvents and nonpharmaceutical compounds (1, 2). Due to marked deviation from thermodynamic ideality, aqueous solutions and aqueous solubilities have particularly resisted fundamental interpretation and quantification. Somewhat surprisingly, solubility interrelationships between the charged and neutral species of weak organic electrolytes have not been fully characterized, even though these are qualitatively independent of the ideality of a given system. Literature on the subject with respect to amines is primarily concerned with the determination of dissociation constants by solubility methods. For this purpose, Schill (3) published pH–solubility profiles for chloroquine, chlorpromazine, and promazine but only over a very narrow pH range. Even though papers by Green (4), Setnikar (5), and Levy and Rowland (6) do not contain solubility profiles *per se*, they do provide some of the mathematical relationships governing solubilities of amines in aqueous systems. Complete pH–solubility profiles for several pharmaceutical amines were given recently by Friberger and Äberg (7). However, these authors did not characterize total solubility in terms of the solubilities of the independently contributing species.

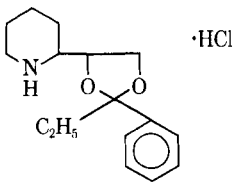
In the present studies, pH–solubility profiles are drawn for two secondary amines, one (I) having analgesic and the other (II) having dissociative anesthetic properties. It will be shown that, while the solubility of the amine hydrochloride generally sets the maximum obtainable concentration for a given amine, the solubility of the free base and the pK′a determine the maximum pH at which formulation as a solution is possible, assuming, of course, that the desired concentration exceeds the free base solubility. Obviously, similar behavior with a reversal of profile is to be expected for organic acids. Therefore, the derived relationships will be useful for delineating solubility profiles of other weak electrolytes as well.

In addition, solubility–temperature dependencies in simple aqueous systems were investigated for II. These



2,2-diphenyl-4-(2′-piperidyl)-1,3-dioxolane hydrochloride[1]

I



2-ethyl-2-phenyl-4-(2′-piperidyl)-1,3-dioxolane hydrochloride[2]

II

[1] The official USAN name for I is dexoxadrol hydrochloride [(+)-2-(2,2-diphenyl-1,3-dioxolan-4-yl)piperidine hydrochloride].

[2] The official USAN name for II is etoxadrol hydrochloride [(+)-2-(2-ethyl-2-phenyl-1,3-dioxolan-4-yl)piperidine hydrochloride].

Table I—Partitioning of II between Chloroform and Water Phases as a Function of pH and at Room Temperature

Buffer	pH	Chloroform–Water Partition Coefficients
0.5 *M* succinate	5.0	5
0.5 *M* succinate	5.5	12
0.5 *M* succinate	6.0	31
0.5 *M* phosphate	6.5	155
0.5 *M* phosphate	7.0	>650
0.5 *M* phosphate	7.5	>650

indicate that the free base solubility is greatly affected by temperature but that the hydrochloride is not. The expected shift in pK′a to lower values as temperature is raised offsets the marked increase in free base solubility, leaving the pH range in which the sharp break in solubility is observed relatively unchanged. As either solvent composition is changed by the addition of miscible organic solvents or the temperature is raised, the solubilities of the free base forms of I and II approach those of their respective hydrochlorides. In these situations, some usual pH profiles are obtained; each has a sharp spike at intermediate pH values due to the sum of the solubilities of both species being significantly greater than the solubility of the hydrochloride alone.

The present studies also indicate a method of pK′a determination using the solubility relationships. Previous investigators in this area (3–6) pointed out the difficulty of measuring pK′a for amines with very insoluble free base species, and they also indicated methods whereby solubility itself can be used to obtain reliable pK′a values. All of these methods have at their foundations the species equilibria discussed in this report.

EXPERIMENTAL

Chemicals—Compounds I and II were obtained as fractionally crystallized materials in a highly purified state. Analyses indicated purity to be >99%. The solvents (dimethylacetamide, polyethylene glycol 300, chloroform, and methylene chloride) were of the purest grade commercially available and were not further processed. Compression distilled water was used exclusively in sample preparation. Buffers were prepared from reagent grade chemicals.

Solubility Determination—Excess quantities of I and II were placed in appropriately sized screw-cap vials and a suitable, predetermined amount of water or mixed solvent was added. The samples were adjusted to the desired pH with either concentrated hydrochloric acid or concentrated sodium hydroxide as necessary and at the temperature of the run. The aluminum-lined caps were screwed down as tightly as possible and further sealed with a wrapping of parafilm. In the case of I, samples were equilibrated at ambient temperature (23°) using a wrist-action shaker for mixing. For II the samples were placed in a calibrated constant-temperature bath held within 0.05° of the temperature of the run. These were mixed by end-over-end rotation. In all cases, samples were equilibrated for more than 2 days.

In certain instances, the solubility of I was also determined by approaching equilibrium from high temperature (supersaturation). In these instances, the vials were heated to 60° for 30 min. prior to placing them on the wrist-action shaker. Regardless of the equilibration method, at the end of the equilibration period the samples were filtered (I through 0.72 micro-Millipore with prefilter and II through glass wool pledgets on the ends of warmed pipets) and appropriately sized aliquots were transferred to tared weighing pans (unbuffered aqueous solutions only) or to separators already containing about an equal amount of 0.5 *M* phosphate buffer of pH 7. To assure complete extraction from the latter solutions with chloroform, chloroform–water partition coefficients were determined for II, the more polar compound, as a function of pH (Table I).

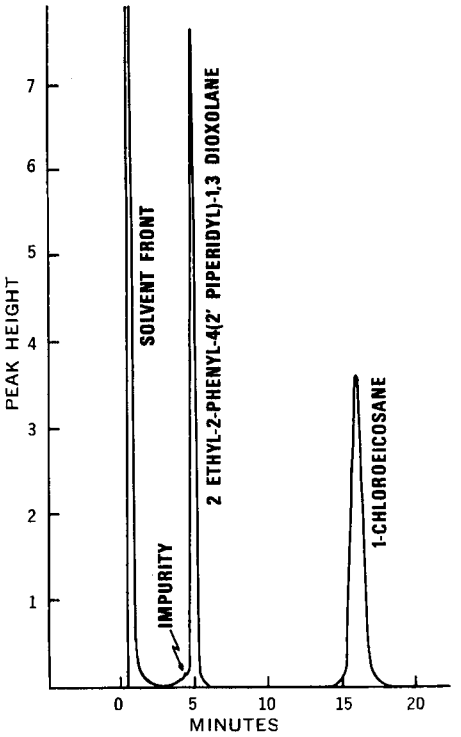


Figure 1—*Typical chromatogram for the assay of II. The peaks from left to right are: the solvent front, an isomeric impurity of II, Compound II, and the internal standard, 1-chloroeicosane. The chromatographic conditions are given in the* Experimental *section. The impurity was not integrated as part of the total area of II because the electronic integrator was set to eliminate its fractional area.*

In the analysis, extraction was accomplished with three portions of chloroform. These were pooled and then brought to dryness on a vacuum evaporator. Where sample weight was the analytical procedure, the samples were air dried in a sheltered area in a hood and weighed periodically until a constant weight was obtained. Weights were recorded to the nearest milligram.

GLC Procedure—The same GLC procedure was found suitable for the analysis of both Compounds I and II (with only minor instrumental adjustments). The exact procedure used for II illustrates the method. One-milliter samples were extracted three times, first with a 10-ml. aliquot of chloroform and then with two 5-ml. portions. After pooling and drying, the residue was taken up in 50 ml. of internal standard solution (1.2 g. 1-chloroeicosane in 1 l. of methylene chloride). The solutions, including appropriately prepared standards, were injected into a gas chromatograph[3] operating as follows: column, 1.2 m. (4 ft.) glass, 3 mm. i.d., containing Diatoport S (80–100 mesh) coated with 5% SE-52; column temperature, 205°; detector temperature, 225°; helium flow rate, 60 ml./min.; hydrogen flow rate, 35 ml./min.; attenuation, as necessary—usually 10 × 6; and sample size, 0.6–1.4 μl.

A typical chromatogram is shown in Fig. 1. Prior to the solubility studies, the assay, including the extraction step, was performed with standard solutions made to contain 0.054, 0.511, and 5.163 mg./ml. The averaged results were 0.049 mg./ml. (12 replicates), 0.525 mg./ml. (11 replicates), and 5.142 mg./ml. (11 replicates), with coefficients of variation of 3.66, 0.92, and 2.12%, respectively. Peak areas were automatically integrated with an integrator[4].

pK′a Determinations—The same solubility method was used with all solutions. The solubility of the free base was determined above pH 10 in each system. Using this value, the pH of the selected sample, and the total drug concentration in that sample, the pK′a was calculated from the usual mass law relationships given in Eqs. 3 and 8. Multiple-point determinations were used, and the individual

[3] Hewlett Packard F&M model 402.
[4] Infotronics model CRS-104.

Table II—Solubility of Compound I at Ambient Temperature (≃23°) as a Function of pH in Purely Aqueous Environs

pH of Solubility Measurement[a]	Experimental Method[b]	Solubility, mg./ml.
5.80	GLC	11.50
5.87	GLC	11.77
6.08	GLC	11.75
6.10	GLC	11.40
6.13	Grav.	11.37
6.40	GLC	12.15
6.68	GLC	12.06
6.68	GLC	11.50
6.80	GLC	11.50
6.86	Grav.	11.00
6.90	Grav.	9.00
6.90	Grav.	8.76
6.91	GLC	6.93
6.97	GLC	7.93
7.23	GLC	2.33
7.55	Grav.	2.01
7.82	Grav.	0.99
8.03	GLC	0.46
8.24	Grav.	0.45
8.48	GLC	0.19
9.74	Grav.	0.084
11.08	GLC	0.061
11.0	UV	0.0695
>10	GLC[c]	0.07

[a] pH of the filtrate after equilibration. [b] GLC = gas–liquid chromatography, UV = spectrophotometric measurement (UV absorption), and Grav. = gravimetric. [c] Independent laboratory.

pK'a values were averaged. In every case the pH chosen was above the break in the solubility–pH profile.

A titration procedure was also utilized with the mixed solvent systems (Compound I only) since the free base became sufficiently soluble to make this analytically convenient. Dilute solutions were titrated with dilute base, the pH being recorded after each incremental base addition. By necessity, very dilute solutions were employed at the lower dimethylacetamide and polyethylene glycol concentrations and it was necessary to subtract the titration curve on pure mixed solvent from that of the solution of I to obtain a satisfactory potentiometric profile for estimation of pK'a.

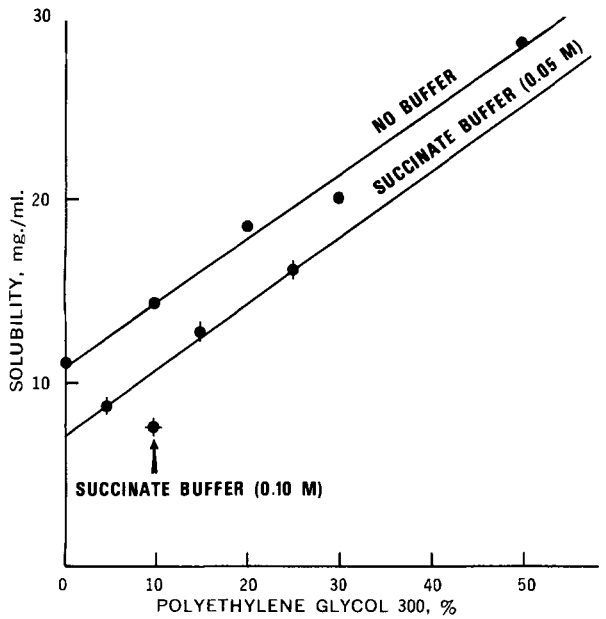


Figure 2—*Solubility of I in the presence of succinate buffers and in water at low pH (pH < 6) and at ambient temperature (≃23°) as a function of percent polyethylene glycol 300 in the system. It will be noted when comparing this plot with Fig. 8 that the hydrochloride solubilities fit equally well on linear and logarithmic axes. This is due to the fact that only a slight increase in solubility occurs as the cosolvent concentration is raised.*

Table III—Solubility of Compound I at Ambient Temperature (≃23°) as a Function of pH in Mixed Solvent Systems

pH in Indicated Solvent[a]	Solubility by GLC Assay, mg./ml.
10% Polyethylene Glycol 300	
5.70	14.0
6.54	14.6
7.40	6.50
20% Polyethylene Glycol 300	
5.52	17.5
6.25	18.5
6.96	18.4
7.40	11.3
30% Polyethylene Glycol 300	
5.90	20.1
6.64	14.4
7.00	20.3
7.47	20.2
7.75	10.0
8.20	3.9
10.10	0.68
11.30	0.60
50% Polyethylene Glycol 300[b]	
4.0 I	28.3
4.5 I	29.2
5.0 I	28.9
5.5 I	29.5
6.0 I	28.14
6.15 II	27.38
6.4 I	29.5
6.83 II	28.14
7.0 I	28.3
7.41 II	28.47
7.75 I	31.6
7.84 II	30.3
7.90 III	27.6
8.20 II	20.8
8.90 II	6.70
12.0 II	3.19
>12.0 IV	2.87
10% Dimethylacetamide	
5.0	19.6
≃11–11.5	0.219
25% Dimethylacetamide	
5.0	34.9
≃11–11.5	0.898

[a] Except for set I at 50% polyethylene glycol 300, the pH was measured after equilibrium. [b] The symbols I and II refer to two entirely independent runs; III indicates a single sample containing 10 mg./ml. tromethamine buffer, and IV indicates a value reported by an outside laboratory.

RESULTS

Solubility and pK'a Determinations on 2,2-Diphenyl-4-(2'-piperidyl)-1,3-dioxolane (I)—The solubilities of I at room temperature (≃23°) in water in solutions of varied pH are tabulated in Table II. These data were determined by several procedures, and a perusal of the table indicates that all methods gave comparable results. With this compound, the free base contribution to solubility at 23° is negligible at and below pH 6.5. Using this value as the cutoff and averaging the remaining results, a hydrochloride solubility of 11.66 mg./ml. is obtained. Similarly, above pH 10 the free base is the only species of significant concentration, the solubility being about 0.067 mg./ml.

The mixed solvent solubilities as a function of pH are presented in Table III. These are broken down by solvent composition. Only the 50% polyethylene glycol system is actually totally characterized, although there are sufficient data at 30% to draw the entire solubility profile. Only select data points were obtained for the 10 and 20% concentrations of polyethylene glycol and 10 and 25% concentrations of dimethylacetamide.

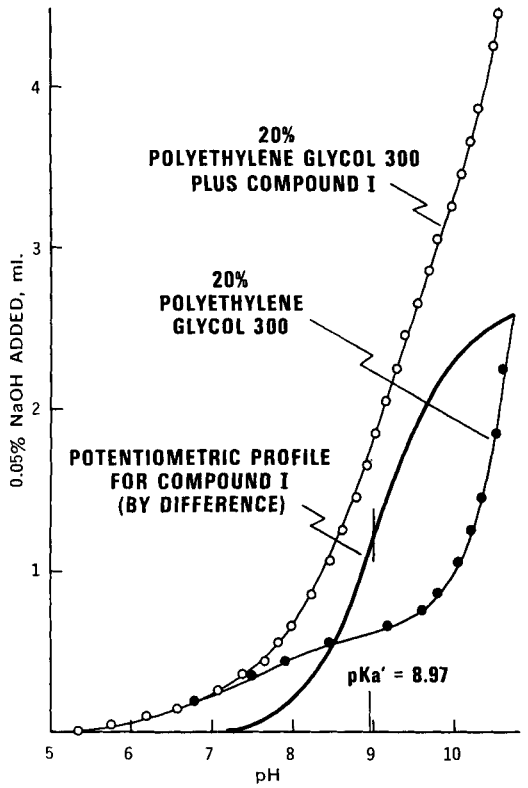


Figure 3—*Titration curve for Compound I in 20% polyethylene glycol 300. The titration was at ambient temperature, using an initial concentration of I of 0.15 mg./ml. The titrant was 0.05% NaOH solution. Note that the titration of solvent alone consumed significant amounts of base and had to be subtracted from the overall profile to get the actual curve for I.*

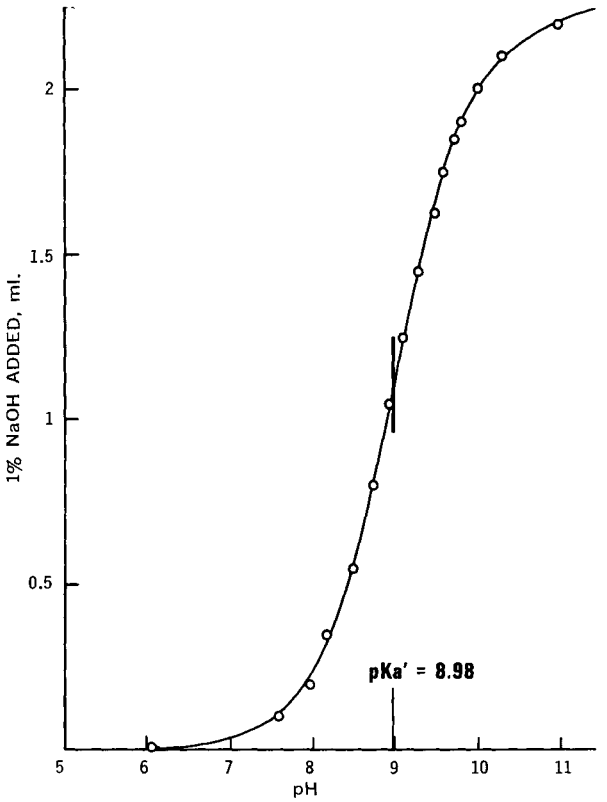


Figure 4—*Titration profile for Compound I in 50% polyethylene glycol 300. In this case the titrant was 1% NaOH and the initial concentration was 3.0 mg./ml. Because of the much higher drug and titrant concentrations employed, no extraordinary accounting of the solvent titration properties was necessary.*

With ultimate formulation in mind, the influence of buffers on the ambient temperature solubility as a function of glycol concentration at pH 6 was also examined. These data were determined using 0.05 and 0.1 *M* succinate buffers and are presented graphically in Fig. 2. The plot indicated that each incremental increase in buffer concentration produces a fixed, incremental decrease in solubility, regardless of the polyethylene glycol concentration. Thus, because the increment is constant, a 0.1 *M* buffer depresses the solubility only 25% in 50% polyethylene glycol but more than halves the solubility in pure water.

To interpret further the solubility data of I and to validate pK'a values determined using solubility relationships, pK'a values were obtained by an independent titrimetric method in those mixed solvent systems where determinations were possible. Figures 3 and 4 are representative titration curves, the first for low percentage polyethylene glycol (20%), where solubility of the free base is analytically marginal, and the second for high percentage polyethylene glycol (50%), where the free base is sufficiently soluble so that standard methods are applicable. In the case of the 20% polyethylene glycol system, the titration had to be performed on the solvent as well as the drug solution and the potentiometric profile was obtained by difference. As in the solubility experiments, all titrations were performed at room temperature. The pK'a values of I obtained in all solvent systems by both of these methods are compiled in Table IV. All were obtained at room temperature (≃23°). The pK'a value for water is an average of four independent calculations at different pH values; the values ranged from 8.7 to 9.0. The pK'a values in polyethylene glycol–water binary solvent mixtures differ little from those obtained in water alone. The same is true for 25% dimethylacetamide. However, values obtained in ethanolic solution are significantly smaller than the aqueous pK'a value.

Solubility Data for 2-Ethyl-2-phenyl-4-(2'-piperidyl)-1,3-dioxolane (II)—The solubilities of II in water at 20° are listed in Table V in the order of increasing pH. Qualitatively these mimic the data seen for I; however, the solubilities of both the hydrochloride and free base are multiples (up to 10 times) of the previously treated analog. The precise ratios cannot be computed because the temperatures of determination differed by about 3°.

Solubilities of II as a function of pH in 0.05 *M* succinate buffer at three temperatures (20, 30, and 40°) are compiled in Table VI. The pH values in both Tables V and VI were determined at the end of sample preparation, prior to certain equilibrium, and at the temperature of the respective solubility experiment. Actual values at equilibrium could have shifted slightly from the pH's recorded, although experience with these systems indicated that such changes are slight, affecting only the second decimal place. For this reason, the values are recorded to the nearest tenth rather than hundredth of a pH. It is immediately noticeable that there is a marked increase in solubility for the free base as temperature is raised. The hydrochloride is not similarly affected. The dependence of the hydrochloride solubility on buffer concentration seen previously with I is either absent in this case or masked within the experimental error.

In Table VII, the pK'a values for II at each temperature are presented. The influence of temperature is seen to be pronounced. The variation mainly reflects changes in pKw as temperature is raised since pK'a = pKw − pKb and pKb for amines is relatively temperature independent.

Table IV—pK'a Values Obtained for Compound I by All Procedures at Room Temperature (≃23°)

Solvent System	Method	pK'a
Water	Solubility estimate	8.85 (8.7–9.0)
20% Polyethylene glycol 300	Titration	8.97
30% Polyethylene glycol 300	Titration	8.98
	Solubility estimate	9.00
40% Polyethylene glycol 300	Titration	9.00
50% Polyethylene glycol 300	Titration	8.98
	Solubility estimate	8.95
25% Dimethylacetamide	Titration	8.85
50% Ethanol	Titration	8.38

Table V—Solubility of Compound II in Distilled Water at 20° as a Function of pH

pH of Solubility Measurement[a]	Solubility, mg./ml.
4.5	49.2
5.25	49.6
6.0	49.5
6.5	48.4
6.75	48.8
7.0	49.3
7.25	49.0
7.5	49.9
7.75	38.9
8.0	4.46
8.5	1.71
9.0	1.02
10.0	1.60
11.0	0.85

[a] In this instance the pH was measured at 20° during sample preparation.

DISCUSSION

General Considerations on Relative Solubilities of Weak Electrolyte Species—The equilibrium for the dissociation of an organic hydrochloride may be expressed by:

$$BH^+ + H_2O \overset{K_a}{\rightleftharpoons} B + H_3O^+ \qquad \text{(Eq. 1)}$$

where BH^+ is the protonated species, B is the free base, and K_a is the effective dissociation constant. Rarely is the concentration of water significantly changed in this equilibrium, and the water molarity is commonly combined with the dissociation constant to give an apparent dissociation constant, K_a'. The mass law expression for this equilibrium is then:

$$K_a' = \frac{[H_3O^+][B]}{[BH^+]} \qquad \text{(Eq. 2)}$$

In logarithmic form, this equation is:

$$pK'a = pH - \log\left(\frac{[B]}{[BH^+]}\right) = pH + \log\left(\frac{[BH^+]}{[B]}\right) \qquad \text{(Eq. 3)}$$

It has been pointed out that these equations are only approximate and must be modified to account for secondary effects of hydronium- or hydroxyl-ion concentration at the extremes of pH or in very dilute solutions (8, 9). However, in the pH range and at the concentrations considered here, these expressions are essentially exact.

Table VI—Temperature and pH Dependency of the Solubility of Compound II in 0.05 *M* Succinate Buffers

Temperature	pH[a]	Solubility, mg./ml.
20°	4.0	49.1
	6.0	54.3
	6.5	55.0
	7.0	52.7
	7.5	29.3[b]
	8.0	9.7[b]
	10.0	0.65
30°	4.0	56.0
	6.0	60.7
	6.5	59.9
	7.0	62.1
	7.5	32.0[b]
	8.0	11.9[b]
	10.0	2.87
40°	5.0	67.4
	6.0	73.5
	6.5	75.7
	7.0	76.8
	7.5	39.4[b]
	8.0	24.1[b]
	10.0	12.2

[a] The pH of the adjusted sample at each given temperature at the end of sample preparation. [b] Used to calculate pK'a.

Table VII—pK'a Values for Compound II at Several Temperatures by the Solubility Method

Solvent	Temperature	pK'a
Water	20°	≃9.25
0.05 *M* succinate buffer	20°	9.14
0.05 *M* succinate buffer	30°	8.50
0.05 *M* succinate buffer	40°	7.92

In general, the relationships drawn in Eqs. 1–3 must be satisfied for all weak electrolytes in equilibrium irrespective of pH and the degree of saturation. At any pH the total concentration of a compound is the sum of the individual concentrations of its respective species; for an amine (primary, secondary, or tertiary) at intermediate pH, this is equal to $[B] + [BH^+]$. In a saturated solution of arbitrary pH, the total concentration is the sum of the solubility of one of the species and the concentration of the other necessary to satisfy the mass law expression. At low pH the following relationship evolves:

$$S_{T,pH<pHmax} = [BH^+]_s + [B] = [BH^+]_s\left(1 + \frac{K_a'}{[H_3O^+]}\right) \qquad \text{(Eq. 4)}$$

where the subscript $pH < pH_{max}$ indicates both that there is a pH of maximum solubility and that this equation is only valid for pH values less than this. The subscript s indicates a saturated species. A similar equation can be formulated for solutions at high pH where the free base solubility is limiting; one obtains:

$$S_{T,pH>pHmax} = [BH^+] + [B]_s = [B]_s\left(1 + \frac{[H_3O^+]}{K_a'}\right) \qquad \text{(Eq. 5)}$$

Each of these equations describes an independent curve which is limited by the solubility of one of the two species. Superposition of these curves produces the pH-solubility profile. The constraint that the solubility of either of the species cannot be exceeded determines which curve is applicable at a given pH.

The pH-solubility profile is nonuniformly continuous at the juncture of the respective solubility curves. This occurs at the precise pH where the species are simultaneously saturated, previously designated as the pH_{max}. At this unique point, Eqs. 4 and 5 are simultaneously satisfied and can be set equal to one another. When these are solved for K_a', a quadratic expression results:

$$\frac{[BH^+]_s}{[H_3O^+]_{max}}(K_a')^2 + ([BH^+]_s - [B]_s)K_a' - [B]_s[H_3O^+]_{max} = 0 \qquad \text{(Eq. 6)}$$

and K_a' is the nontrivial solution of:

$$K_a' = \frac{[B]_s - [BH^+]_s \pm \sqrt{([BH^+]_s - [B]_s)^2 + 4[BH^+]_s[B]_s}}{2([BH^+]_s/[H_3O^+]_{max})} \qquad \text{(Eq. 7)}$$

In practice, this equation is, unfortunately, of limited value because it generally involves small differences in large numbers. It would have utility for species solubility ratios between 0.1 and 10 but then less rigorous methods are available for accurate pK'a determinations. The equation does, however, indicate better than any other the interrelationships between pH_{max} and relative species solubilities for a given pK'a.

Theoretically, either of the two independent species solubility equations can be used at the pH of their intersection, pH_{max}, to calculate pK'a, assuming the individual species solubilities are known. However, from the practical standpoint, only the equation for the relatively insoluble species is of value because the other equation again requires the estimation of small differences in large numbers. Therefore, all methods for determining pK'a values for amines by solubility relationships are dependent on a knowledge of the free base solubility of phenomena occurring above pH_{max}. In systems where micellization is encountered, accurate pK'a determination is dependent on measurement at concentrations less than the CMC. Regardless of the relative solubilities of the re-

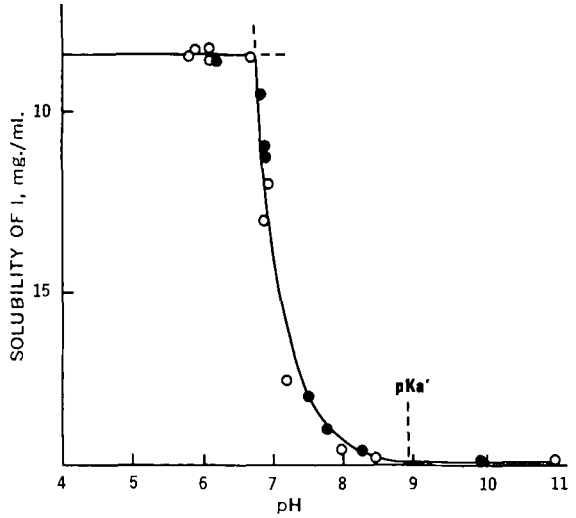


Figure 5—*Solubility of I in water at ambient temperature (≃23°) as a function of pH. All data are in milligrams per milliliter calculated in terms of free base equivalent. The lines drawn through the data are theoretical and were calculated using 0.067 mg./ml. as the free base solubility, 11.5 mg./ml. as the hydrochloride solubility, and 8.85 as the pK'a. Data by both gravimetric (●) and GLC (○) procedures were in good agreement.*

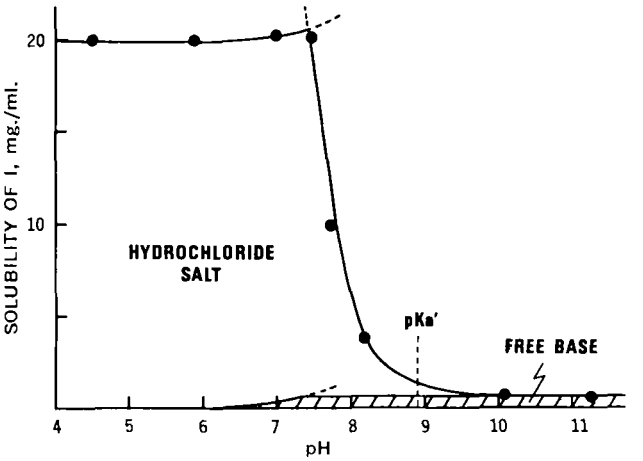


Figure 6—*The pH-solubility profile for I in 30% polyethylene glycol 300 solution at ambient temperature (≃23°). The data are in milligrams per milliliter free base equivalents. The free base is sufficiently soluble so that there is a discernible peak at pH_{max}, the point of intersection of the two curves. The theoretical curves fit to the data were calculated using 20.1 mg./ml. as the hydrochloride solubility, 0.60 mg./ml. as the free base solubility, and 8.98 as the pK'a.*

spective species but in the absence of micelles, *etc.*, the two solubility curves intersect at a sharp angle, making the pH_{max} an easily identifiable reference point. In an uncomplicated system, one can generate the entire solubility profile with but three bits of information: solubility of the hydrochloride salt, solubility of the free base, and the apparent dissociation constant. Apparently, this is not generally recognized. Friberger and Äberg (7), for instance, drew smooth curves through their data, failing to identify the high data points about pH_{max} as the expected solubility trend.

The isolated effects of each of the species solubilities and the pK'a on the shape of the pH-solubility profile are important. Consider the hypothetical case where the pK'a and free base solubility are held constant and the hydrochloride salt solubility varies. As the ratio of the hydrochloride salt solubility to the free base solubility, which is initially assumed to be very much greater than 1, is decreased from 10^n to 10^{n-1}, from 10^{n-1} to 10^{n-2}, *etc.*, and, finally, from 10 to 1, the pH_{max} drifts along the pH axis to higher values, slowly at first but accelerating as a ratio of 1 is approached. Furthermore, as the ratio approaches 10, the fractional solubility of the free base at pH_{max} becomes analytically significant and a noticeable peak is formed in the pH–solubility profile. When the individual species solubilities become equal, the pH_{max} coincides with the pK'a and there is a tent-like peak in the plot, the lines forming the profile intersecting at a point twice the solubility of each species (the sum of the solubilities of both species). At ratios less than 1, a reversal of the trends down to 1 is experienced. Of course, ratios less than 1 are unlikely for amines but, as will be pointed out later, the theory is applicable to other dissociable compounds where the roles of the species are essentially reversed.

If the solubility of the free base of a given amine is very small relative to the hydrochloride, the free base limiting curve of the overall pH–solubility profile cuts deeply into the acidic pH range. In such a circumstance, it may not be possible to prepare a solution of the amine at sufficiently high pH to obtain adequate stability or to ensure physiological compatibility. To get the compound into solution at the desired concentration, it is necessary to raise the solubility of the free base in the system. Roughly, for each 10-fold increase in solubility, there is an upward shift in the profile of about a full pH unit. As discussed shortly, shifts of this size can be accomplished in mixed solvent systems.

While the pK'a does not determine the shape of the pH–solubility profile *per se*, it does fix the location of this profile on the pH coordinate. All other factors being equal, each upward or downward shift in the pK'a is matched exactly by an upward or downward shift in pH_{max}. This can become of importance in several instances. If one chooses a mixed solvent, such as a hydroalcoholic solution, to raise the free base solubility but does not take into account the influence of the solvent on the pK'a, that which is gained in solubility increase can be offset in part by a decrease in pK'a [*i.e.*, the pK'a for I as determined in 50% ethanol (Table IV)]. Additionally, two compounds with identical species solubilities might require very different handling in formulation due to significant differences in pK'a values. It is as important to gauge accurately the pK'a in the systems being considered as to have accurately assessed solubilities.

The difficulty of determination of the pK'a for hydrochlorides with very insoluble free bases is well documented. Several workers developed methods based on solubility relationships. Green (4), for instance, developed a method whereby the pK'a is determined from the *y* intercept of a plot of $[H_3O^+]$ against total solubility. The *x* axis

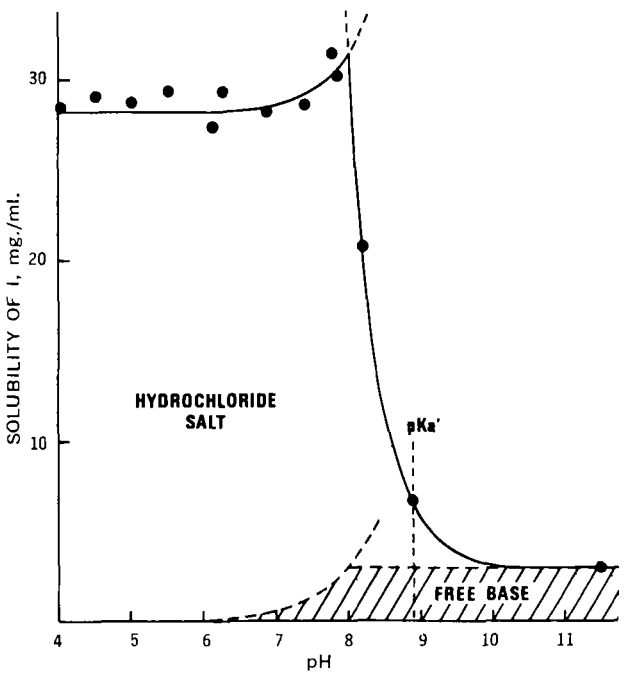


Figure 7—*The pH-solubility profile for I in 50% polyethylene glycol solutions at ambient temperature (≃23°). All data are in milligrams per milliliter free base equivalents. The theoretical curves fit to the data were calculated using 28.3 mg./ml. as the hydrochloride solubility, 3.03 mg./ml. as the free base solubility, and 8.98 as the pK'a.*

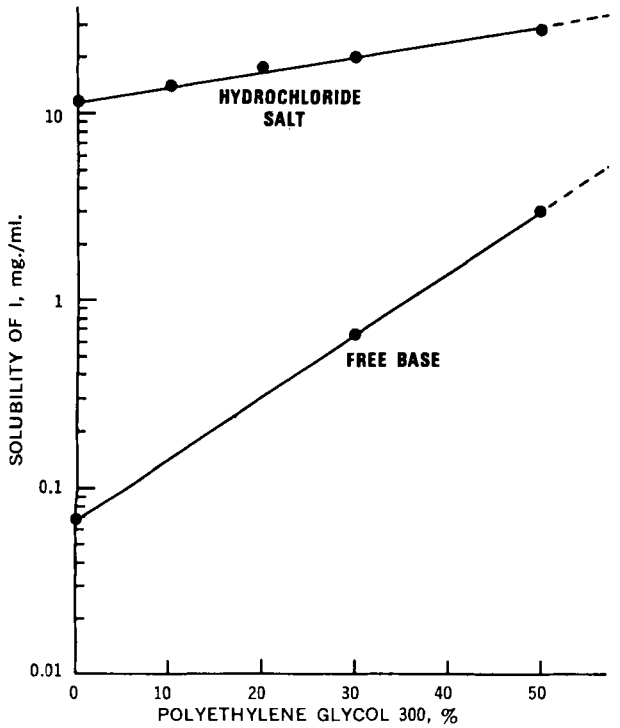


Figure 8—*Logarithm of solubility of each species of I* versus *percent polyethylene glycol 300 in the mixed solvent system. Data are for ambient temperature (≃23°). The free base data unquestionably fit the semilogarithmic relationship. The hydrochloride salt data fit equally well on a linear y coordinate (Fig. 2).*

yields the free base solubility with his method. Setnikar (5) provided an alternative method based upon the Druckrey linear titrant scale. In an excellent paper, Levy and Rowland (6) further developed the theory and provided a method of accurately determining pK'a based upon a knowledge of the free base solubility and the slope of a nonlogarithmic plot of the titration during precipitation. An alternative method of determining pK'a, which is simple and accurate, is to determine the free base solubility first and then to titrate a hydrochloride solution, prepared to contain 10–20 times the free base solubility, to the cloud point (first appearance of precipitation). The pK'a can be obtained from the total concentration at the cloud

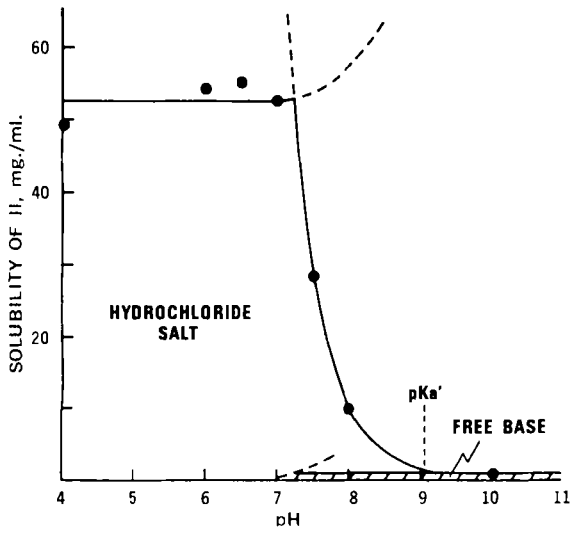


Figure 9—*Solubility of II as a function of pH at 20° in 0.05* M *succinate buffer. The data are in terms of milligrams per milliliter as the free base. The theoretical curves fit to the data were calculated using 52.5 mg./ml. as the hydrochloride solubility, 0.65 mg./ml. as the free base solubility, and 9.15 as the pK'a.*

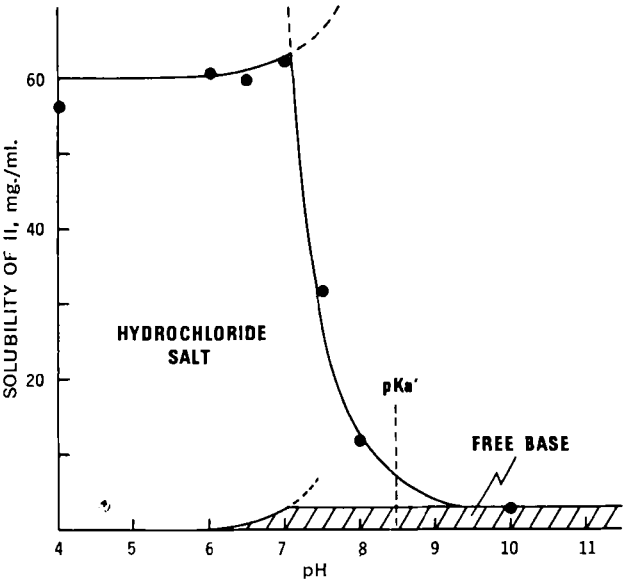


Figure 10—*Solubility of II as a function of pH at 30° in 0.05* M *succinate buffer. The data are in terms of milligrams per milliliter as the free base. The theoretical curves fit to the data were calculated using 60 mg./ml. as the hydrochloride solubility, 2.87 mg./ml. as the free base solubility, and 8.50 as the pK'a.*

point, the free base solubility, and the pH at the cloud point from:

$$pK'a = pH + \log\left[\frac{C_T - [B]_s}{[B]_s}\right] \qquad \text{(Eq. 8)}$$

where C_T is the starting concentration. It is implicit that the endpoint can be detected prior to precipitation of sufficient base to change the total concentration appreciably. In the present studies, a variation on the method was used; pK'a values were calculated from measured equilibrium solubilities at pH values above pH_{max}.

Specific Considerations: Solubilities of I and II—The compounds studied here serve as good models for elucidating species solubility relationships and their interplay. Neither I nor II forms association colloids and, in both cases, the oily amine base appears stable to crystallization, eliminating the complication of two independent free base forms with independent solubilities. However, the hydrolyses of these compounds are acid catalyzed and they are not sufficiently stable to formulate even in moderately acidic solutions (pH 5–6.5). Thus, formulation above pH 6.5 is not only desirable from the standpoint of close physiological pH approximation but also requisite for obtaining adequate shelflife.

The solubility data for I in pure water at ambient temperature are plotted against pH in Fig. 5. The fit to the data is virtually exact. It can be readily seen that formulating I at concentrations above 5 mg./ml. and above pH 6.5 would be precarious due to the proximity of the solubility-limiting line to the pH 6.5 line. There is no room for error in estimation of pH, and drifting of pH in either direction due to interaction of the solution with container components would be problematic. The pH_{max} in this case is at 6.75.

The effect of incorporation of a cosolvent is graphically depicted in Figs. 6 and 7 where the pH–solubility profiles for 30 and 50% polyethylene glycol 300 solutions at room temperature are presented. At both of these solvent compositions, the free base solubility is a significant fraction of the total and there is a discernible peak in the profile at pH_{max}. The free base concentration as a function of pH is indicated by the hash-marked areas below the overall profiles. By difference, the height of the open area represents the hydrochloride species concentration at a given pH. As in the previous case, the lines drawn through the points were calculated from the individual species solubilities and the pK'a. These data are listed for convenience in the figure titles. The following trends with increasing cosolvent volume fraction are evident: (*a*) the hydrochloride salt becomes more soluble, (*b*) the free base becomes more soluble, and (*c*) the pH–solubility profile is shifted markedly to the right. The latter is evidenced in the pH_{max} shifting from 6.75 in the absence of polyethylene glycol to 7.45 and 8.00 at 30 and 50%, respectively. There

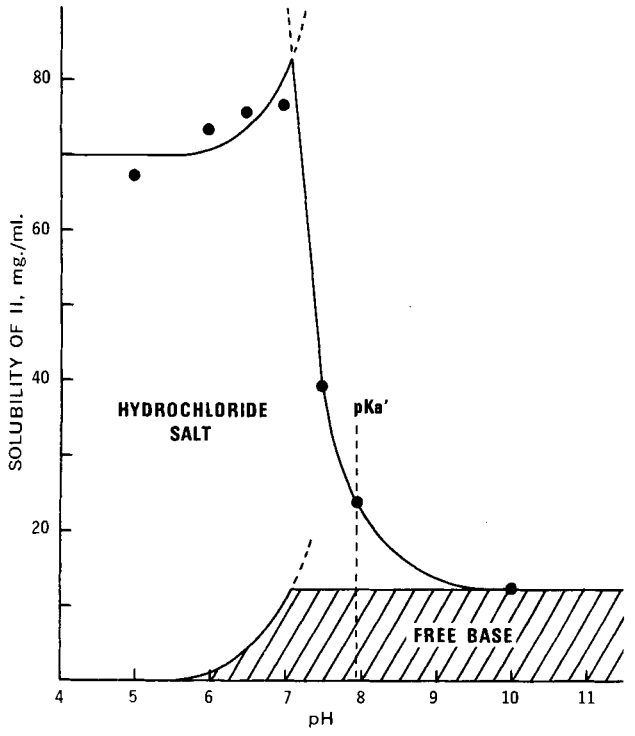


Figure 11—*Solubility of II as a function of pH at 40° in 0.05* M *succinate buffer. All data are in terms of milligrams per milliliter as the free base. The theoretical curves fit to the data were calculated using 70 mg./ml. as the hydrochloride solubility, 12.2 mg./ml. as the free base solubility, and 9.72 as the pK'a.*

is no problem in formulating a solution containing as much as 25 mg./ml. of I at pH 7 in the 50% system.

In Fig. 8, the species solubilities of I are plotted semilogarithmically against glycol concentration. Interestingly, both show a linear dependency between log (solubility) and solvent composition. The hydrochloride data also appear linear on a linear scale (Fig. 2), so the actual dependency for the species is obscured by the smallness of the change in solubility as the polyethylene glycol concentration is increased. The slope for the free base is 0.033 and is 4.15 times as steep as that of the hydrochloride on the logarithmic scale. It is this logarithmic dependency of free base solubility on percent nonaqueous solvent added that makes it possible to effect a significant change in the location of the pH–solubility profile. This is particularly true for this system since the pK'a was essentially invarient over the full solvent range explored (Table IV).

Solubility relationships for II (Tables V and VI) were found to be similar qualitatively to those already discussed. In the case of II, the influence of temperature on the pH–solubility profiles was assessed. In Figs. 9–11, the respective solubility profiles for 0.05 *M* succinate buffer solutions, at 20, 30, and 40°, respectively, are presented. As in the previous cases, these data fit the theoretical lines calculated from the individual species solubilities and the pK'a. Again, there is a good fit to the data. It is readily apparent from these three plots that the hydrochloride salt solubility is only slightly influenced by temperature. On the other hand, the free base solubility is highly dependent on temperature and increases almost 19-fold over the 20° temperature span. Unlike the situation with I in the binary solvent systems, a marked displacement of the pH–solubility profile to higher pH is not commensurate with this large solubility increase because of an expected, offsetting change in the pK'a. It drops from 9.14 at 20° to 7.92 at 40°. That these two influences virtually cancel each other is seen in the fact that the pH_{max} changes by only 0.1 pH unit over this temperature range.

Substitution of one phenyl group in I with an ethyl moiety produces profound changes in the solubilities of both hydrochloride and free base species. The effect is greatest on the free base, which is approximately 10 times more soluble for II than I. The solubility of the hydrochloride of II is only about five times that of I (interpolated graphically at the same temperature). The net effect of the solubility improvement is that solutions of II can be prepared in pure water or dilute buffer to contain up to 50 mg./ml. at ambient temperature and above the minimum pH (pH 6.5) required for adequate stability. Thus, II can be formulated without recourse to mixed solvent systems.

The solubility temperature dependencies of the individual species of II are indicated in Fig. 12. This is a semilogarithmic plot of solubility against reciprocal temperature. The slopes of such plots (or the tangent to a given curve at a given temperature in the case of a nonlinear plot) yield the differential heat of solutions of the respective species. Usually such curves are nonlinear and ΔH_s values are obtained calorimetrically at a given temperature (10). In this case, over this limited temperature span, the plots appear linear and rough estimates of ΔH_s are possible. The value for the hydrochloride is 2.12 kcal./mole; for the free base, it is 15.4 kcal./mole. The relative species temperature dependencies, the free base ≫ hydrochloride, are in accord with the literature (11). However, the magnitudes of the values are unprecedented. Many hydrochlorides have negative heats of solution, with their respective free bases having heats of solution in the 4–8-kcal./mole range (11). The origin of the qualitative disparity between literature ΔH_s values on more traditional amines and their hydrochlorides and the species heats of solution of II is not really known. The highly polar nature of II and the method of measurement (in saturated solution) may be contributive.

Miscellaneous Aspects Relevant to Formulation of Amines—Based on experience to date, neither Compound I nor II has a crystalline form of the free base. While this is not unusual for amines, it also cannot be considered the general case. It is possible that a solution could be prepared subsaturated with respect to the metastable oily free base but supersaturated with respect to the crystalline free base form. Copious free base crystallites might develop. Green (4) cited two instances where this phenomenon was likely operative. The solubility for the free base of perphenazine was dependent on the procedure used; direct measurement in 0.01 *N* NaOH yielded a value about one-third of that found by titration. Furthermore, Green cited a reported solubility for chlorpromazine at 20° which was less than half the one he obtained. Crystalline chlorpromazine base is prepared only with difficulty, making it likely Green's solubility was for the liquid form. Therefore, a good precaution in

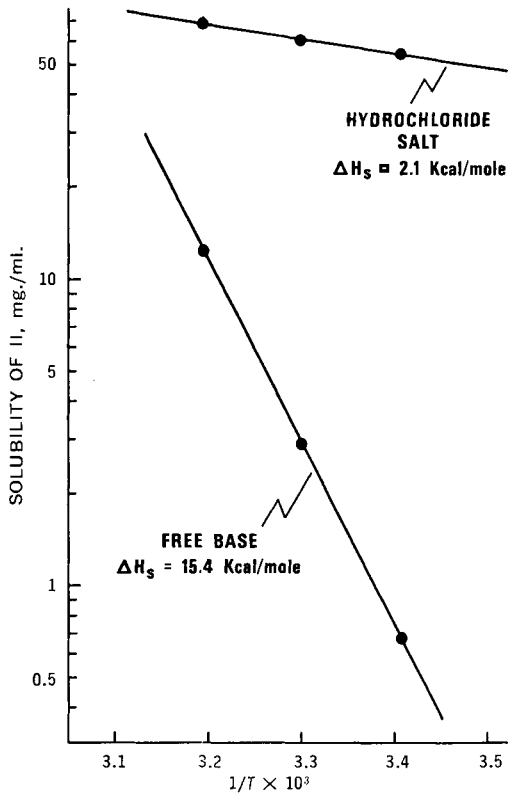


Figure 12—*Plot of the hydrochloride and free base solubilities of II against reciprocal temperature. The slope of the lines yields a crude estimate of the heat of solution,* ΔH_s.

formulating amine salt solutions of concentration greater than the free base solubility is to adjust the pH of the system so that it is at least a half pH unit from the solubility-limiting line.

Because amine solutions are poor self buffers in the pH range where they are totally in solution (as the protonated species), drifting of pH, particularly to more acidic values, is a real formulation problem. In the case of I in unbuffered systems, significant downward pH drifts were experienced in containers in which the solutions had contact with rubber components, particularly in disposable syringes. This was disastrous for this compound in terms of its chemical stability. In the general situation, pH shifts to more alkaline values are also possible. The latter could lead to oiling out of free base in sensitive systems. Both possibilities should be avoided. Thus, a buffer should be considered for each formulation. However, as evidenced in Fig. 2, the selection of a buffer and its concentration must be done with an eye to the buffer's influence on the amine solubility. The data in Fig. 2 are only for succinate buffers; data were also obtained for citrate and acetate buffers, each at a 0.05 *M* concentration, and the effect was qualitatively and quantitatively similar. A 0.05 *M* tromethamine buffer, on the other hand, did not produce as pronounced a depression of the solubility.

The influence of the succinate buffer (Fig. 2) is not interpretable unambiguously with the limited data, but salt formation cannot be ruled out. This raises another point about these systems: salts other than hydrochlorides will have different pH–solubility profiles. One method of improving the solubility picture in the acid pH range is to find a more soluble salt. However, since the dissociation depicted in Eq. 1 will be little affected by the salt anion chosen, solubility in the region of free base control will be negligibly influenced.

Since the dissociations of carboxylic acids and other acidic organic species parallel those discussed here for organic hydrochlorides, it is expected that their pH–solubility profiles can be characterized theoretically using the same treatment. In the special case of carboxylates the roles of the two species will be reversed, the free acid being much less soluble than the corresponding anionic base. Qualitatively, mirror image profiles with respect to the amine hydrochlorides will be obtained and the insolubility of the free acid will determine the minimum pH at which total solution is possible at a given concentration in excess of the free acid solubility. Thus, in carefully characterizing the amine hydrochloride system, the foundations for treatment of other weak electrolytes with respect to relative species solubilities have been laid.

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Inhibition of Platelet Aggregation by Bisulfite–Sulfite

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Abstract □ Blood platelets aggregate in hemostasis and thrombosis. The effect of bisulfite–sulfite, which is frequently added as an antioxidant in injections, on platelet aggregation was examined according to the method of Born and Cross. It was found that the agent (10^{-3}–10^{-2} *M*) inhibited adenosine diphosphate- and collagen-induced aggregation of rabbit platelets in platelet-rich plasma. The platelets that had almost lost their aggregating capacity by treatment with the agent recovered the capacity when they were treated with plasma for the long period. The inhibitory profile of the agent was characteristic, and the agent progressively deaggregated the platelet aggregates. The presence of other blood cells such as erythrocytes had little influence on the effects of the agent on platelets.

Keyphrases □ Platelet aggregation—inhibition by saline bisulfite–sulfite □ Inhibition of platelet aggregation—saline bisulfite–sulfite □ Bisulfite–sulfite—inhibition of platelet aggregation

Bisulfite or sulfite has been frequently added as an antioxidant in injections and used as an antiseptic. Halaby and Mattocks (1) and Wilkins *et al.* (2) demonstrated that the agent possesses great affinity for blood and nonspecific toxicity for tissues. Recent studies on the biopolymers such as nucleic acid (3–6), proteins (7, 8), and lipids (9) showed that the agent readily modified these biopolymers to cause mutation or damage to the functions of the biopolymers. However, little is known about the actions of bisulfite or sulfite on mammalian blood cells.

During this investigation of platelet aggregation, it was found that the functions of platelets were greatly altered by a bisulfite–sulfite mixture. Blood platelets are known to aggregate in hemostasis and thrombosis, and compounds that inhibit platelet aggregation have been extensively investigated (10). The authors now wish to report the strong inhibitory activity of bisulfite–sulfite on platelet aggregation.

MATERIALS AND METHODS

Materials and methods were partly described in the previous papers (11, 12). A mixture of sodium bisulfite and sulfite was ob-