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Extended Hildebrand Solubility Approach: *p*-Hydroxybenzoic Acid in Mixtures of Dioxane and Water

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Abstract □ The extended Hildebrand solubility approach was used to reproduce the solubilities of *p*-hydroxybenzoic acid in a dioxane–water system. The solubility parameter of *p*-hydroxybenzoic acid was determined and found to be ~15 $(cal/cm^3)^{1/2}$. Residual plots (scattergrams) were used in conjunction with R^2, F, and standard deviation values to determine whether a quadratic, cubic, quartic, or higher degree polynomial was required in the calculations. The earlier iteration method for back-calculations of solubilities was replaced by the more reliable root-finder method[1]. The solubility profile of *p*-hydroxybenzoic acid in dioxane–water mixtures did not follow a log linear relationship even in the ranges where the solubility parameters of the water–cosolvent mixture might be expected to produce a straight-line function, as observed in other studies.

Keyphrases □ Dioxane—extended Hildebrand solubility approach, *p*-hydroxybenzoic acid, water □ *p*-Hydroxybenzoic acid—extended Hildebrand solubility approach, water □ Hildebrand solubility approach, extended—*p*-hydroxybenzoic acid in mixtures of dioxane and water □ Solubility parameters—*p*-hydroxybenzoic acid in mixtures of dioxane and water

The theory of solutions is one of the most challenging and least understood branches of physical chemistry. The Hildebrand–Scatchard theory of regular solution (1–3) is the pioneer approach in this field. In recent years the solubility parameter concept has been extended to the practical realm of industrial and commercial paints, inks, polymers, plastics, insecticides, and pharmaceuticals. Recently, the Hildebrand–Scatchard equation has been successfully modified and the solubilities of methylxanthines estimated in binary solvents within an error of ≤10% values approximating those of experimentally determined solubilities (4, 5). The original Hildebrand–Scatchard equation has been discussed in a number of papers (4–6) in this series and need not be repeated here.

[1] International Mathematical and Statistical Library.

BACKGROUND

The Hildebrand–Scatchard approach may be used to estimate solubility only for relatively nonpolar drugs in nonpolar solvents according to regular solution theory (1). The solubility parameter, δ, is the square root of cohesive energy density (1), with subscripts 1 and 2 for solvent and solute, respectively. In pharmaceutical solutions, the geometric mean (1) of δ_1^2 and δ_2^2, that is, $\delta_1\delta_2 = (\delta_1^2\delta_2^2)^{1/2}$, is too restrictive and ordinarily provides a poor fit to experimental data in irregular solutions. Instead, $\delta_1\delta_2$ can be replaced by W, which is allowed to take on values as required to yield correct mole fraction solubilities, X_2:

$$-\log X_2 = -\log X_2^i + A(\delta_1^2 + \delta_2^2 - 2W) \qquad \text{(Eq. 1)}$$

where

$$\log X_2^i \cong \frac{\Delta H_m^f}{2.303R}\left(\frac{1}{T_m} - \frac{1}{T}\right) \qquad \text{(Eq. 2)}$$

and

$$A = \frac{V_2\phi_1^2}{2.303RT} \qquad \text{(Eq. 3)}$$

ΔH_m^f is the heat of fusion of the solute and R is the gas constant. T_m is the melting point of the solute, T is the temperature of interest, and both are in °K. The volume fraction of the solvent (ϕ_1) is expressed as:

$$\phi = \frac{V_1(1 - X_2)}{V_1(1 - X_2) + V_2X_2} \qquad \text{(Eq. 4)}$$

V_1 and V_2 are the molar volumes of solvent and supercooled solute, respectively. For drugs in binary solvent mixtures, it has been found (4–6) that the W values may be regressed in a power series on the solvent solubility parameters:

$$W = C_0 + C_1\delta_1 + C_2\delta_1^2 + \ldots\ldots + C_n\delta_1^n \qquad \text{(Eq. 5)}$$

One obtains a reasonable estimate, W_{calc}, by this procedure. W_{calc} is then substituted in Eq. 1 for W to obtain mole fraction solubilities in polar binary solvents which are usually within <10% of the experimental results.

According to well-known thermodynamic principles, the relationship of mole fraction solubility to ideal mole fraction solubility and activity coefficient may be expressed as:

$$-\log X_2 = -\log X_2^i + \log \alpha_2 \qquad \text{(Eq. 6)}$$

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Table I—Solubility of *p*-Hydroxybenzoic Acid in Dioxane–Water Mixtures at 25 [a]

Volume Fraction of Dioxane, f	Solubility parameter, δ_1	Molar Volume, V_1	Mole Fraction Solubility, X_2	Solubility, S_2, moles/liter	A [b]	K [c]	δ_{app} [d]	Equation 18 $\log \alpha_2/A$ (obs)	Equation 18 $\log \alpha_2/A$ (calc)	Equation 18 $X_{2\,calc}$	Equation 18 Residual	Equation 18 Percent Error
0.0	23.45	18.08	0.00060	0.0335	0.0687	1.070	23.42	15.96	16.02	0.00059	0.00001	1.7
0.20	20.76	21.22	0.0072	0.330	0.0649	1.047	20.59	0.25	−0.01	0.0075	−0.0003	−4.2
0.45	18.07	25.88	0.0302	1.06	0.0558	1.034	17.79	−10.87	−10.50	0.0291	0.0011	3.6
0.50	16.73	29.20	0.0478	1.45	0.0512	1.034	16.53	−15.75	−15.59	0.0472	0.0006	1.3
0.55	16.06	31.25	0.0585	1.64	0.0490	1.038	15.94	−18.23	−18.13	0.0581	0.0004	0.7
0.60	15.39	33.65	0.0710	1.83	0.0469	1.044	15.37	−20.86	−20.58	0.0699	0.0011	1.5
0.65	14.71	36.33	0.0820	1.95	0.0456	1.052	14.82	−22.84	−22.90	0.0823	−0.0003	−0.4
0.70	14.04	39.64	0.0939	2.05	0.0445	1.061	14.29	−24.70	−24.88	0.0947	−0.0008	−0.9
0.80	12.70	48.32	0.115	2.10	0.0439	1.087	13.23	−27.05	−27.24	0.116	−0.001	−0.9
0.85	12.03	54.27	0.121	2.02	0.0450	1.102	12.67	−28.93	−27.22	0.123	−0.002	−1.7
0.90	11.33	61.84	0.127	1.89	0.0463	1.122	12.05	−26.60	−25.98	0.123	0.004	3.1
0.95	10.68	71.84	0.122	1.62	0.0494	1.141	11.42	−24.59	−23.44	0.114	0.008	6.6
1.00	10.01	85.71	0.0844	0.968	0.0570	1.152	10.52	−18.47	−19.09	0.0890	−0.0046	−5.5

[a] V_2 = 94.35 cm³/mole, δ_2 = 15.3 (cal/cm³)$^{1/2}$, $X_2{}^i$ = 0.00747, log $X_2{}^i$ = −2.1267. [b] Eqs. 3 and 4. [c] $K = W/\delta_1\delta_2$, a proportionality factor (13). [d] Eq. 15.

where α_2 is the activity coefficient of the solute in the solution. Equation 1 may be written, in light of Eq. 6, as:

$$\log\left(\frac{X_2{}^i}{X_2}\right) = \log \alpha_2 = A(\delta_1{}^2 + \delta_2{}^2 - 2W) \qquad \text{(Eq. 7)}$$

and

$$(\log \alpha_2)/A = \delta_1{}^2 + \delta_2{}^2 - 2W \qquad \text{(Eq. 8)}$$

Log α_2/A may be regressed directly against δ_1, bypassing W, and obviating the need for δ_2 as described in earlier work (6). The estimated solubility, $X_{2\,calc}$, is identical to that obtained with W_{calc} except for rounding-off errors. The entire procedure, referred to as the Extended Hildebrand Solubility Approach (4), is conducted by a computer program, the regression subroutine of which is found in SPSS (7). The program allows a stepwise addition of independent variables, analysis of variance, and examination of the residuals, $X_2 - X_{2\,calc}$, by means of scatter plots. These plots are useful in indicating when the proper degree of the polynomial has been reached.

EXPERIMENTAL

Materials—*p*-Hydroxybenzoic acid[2] was recrystallized from aqueous ethanol (8) then dried at 105° overnight. The heat of fusion, 7510 cal/mole, of *p*-hydroxybenzoic acid was determined by differential scanning calorimetry[3]. The melting point obtained from this method is 485.9°K which is consistent with the value from a hot-stage method[4]. A pure grade of dioxane[5] was used as received. Since *p*-hydroxybenzoic acid is a weak acid with pK_a equal to 4.54 ($K_a = 2.9 \times 10^{-5}$) at 25° (9), the pH of water affects its solubility. Furthermore, the dissociation constant, K_a, decreases in organic aqueous mixtures (9, 10). To ensure that the *p*-hydroxybenzoic acid was in undissociated form, the pH of the water was adjusted to 2.0 using hydrochloric acid. The pH 2 hydrochloric acid–aqueous solution was used alone or mixed with dioxane as the binary solvent for this study.

Solubility Analysis—The solubility of *p*-hydroxybenzoic acid was determined in binary solvent mixtures of dioxane and water. A suitable amount of dioxane, water, or binary solvent was introduced into screw-capped vials containing an excess amount of solute. After being sealed with several turns of electrical tape, the vials were submerged in water at 25 ± 0.1° and were shaken at 100 cpm for 24 hr in a constant-temperature bath[6]. Preliminary studies showed that this time period was sufficient to ensure saturation at 25°.

After equilibrium had been attained, each vial was removed and analyzed. The solutions were transferred to a syringe and filtered using a filter[7] of pore size <1 μm. After suitable dilution, the solutions were assayed using a spectrophotometer[8] set at the wavelength of maximum absorption of the solute. The solubility of the solute was determined at least six times for each solvent, and the average value was taken. The experimental variation in solubility was <3% in replicate samples and was consistent with a titrimetric assay method used with a number of samples. The densities of the saturated solutions were determined with calibrated pycnometers at 25° and the variation was <0.03% for six determinations.

Stability of Solute—The stability of the solute was tested by subjecting the saturated solutions to TLC[9] using 2-propanol–ammonia–water (8:1:1) (11) as a developing system, then observing the developed TLC plate under a long wavelength UV-lamp[10]. There was no evidence of decomposition within 36 hr.

Ideal Solubility—The ideal solubility of *p*-hydroxybenzoic acid may be calculated from the heat of fusion of the solid and the melting temperature:

$$\log X_2{}^i \cong \frac{\Delta H_m{}^f}{2.303R}\left(\frac{1}{T_m} - \frac{1}{T}\right) \qquad \text{(Eq. 9)}$$

$$\log X_2{}^i = \frac{7510}{(2.303)(1.987)}\left(\frac{1}{485.9} - \frac{1}{298.15}\right)$$

giving:

$$\log X_2{}^i = -2.127 \qquad X_2 = 0.00747$$

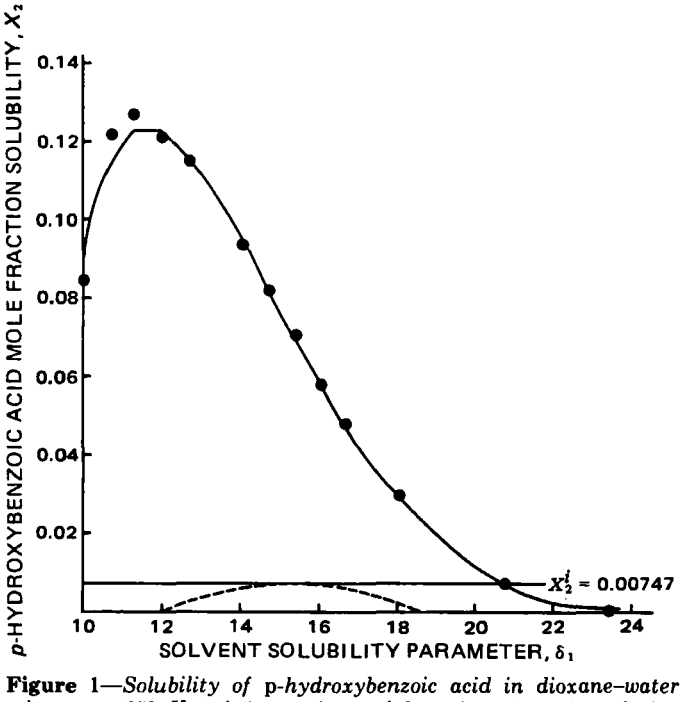
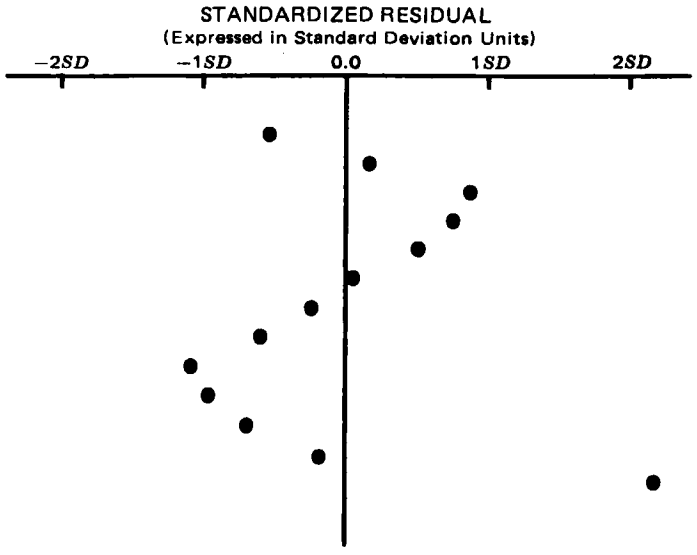


Figure 1—*Solubility of* p-*hydroxybenzoic acid in dioxane–water mixtures at 25°. Key: (●) experimental data; (- - - -) regular solution curve; (——) back calculation using regression equation, Eq. 18.*

[2] Matheson Coleman Bell, Norwood, OH 45212.
[3] Perkin-Elmer DSC Model 1 B, Norwalk, Conn.
[4] Digital Melting Point Analyzer Model 335, Fisher Scientific Co., Fair Lawn, NJ 07410.
[5] Mallinckrodt Chemical Works, St. Louis, MO 63160.
[6] Blue-M Electric Co., Blue Island, Ill.
[7] Filter Paper, Glass Fiber, Whatman Grade FG/F.
[8] Beckman Model 25 Spectrophotometer.
[9] Polygram SILG/UV 254, Brinkman Instruments, Inc., Westbury, NY 11590.
[10] Chromato-VUE Cabinet Model CC-20, Ultra-Violet Products, Inc., San Gabriel, Calif.



No.	log α / A (calc)	Residual	SD units
1	17.2444	-1.2875	-0.56
2	-0.1179	0.3633	0.16
3	-12.8684	2.0037	0.87
4	-17.4991	1.7514	0.76
5	-19.3853	1.1607	0.50
6	-20.9854	0.1226	0.05
7	-22.3169	-0.5243	-0.23
8	-23.3405	-1.3574	-0.59
9	-24.5295	-2.5171	-1.09
10	-24.6949	-2.2385	-0.97
11	-24.5620	-2.0389	-0.89
12	-24.1590	-0.4257	-0.19
13	-23.4618	4.9874	2.17

Figure 2—*Plot of residuals obtained from quadratic regression equation, Eq. 16, for p-hydroxybenzoic acid in dioxane–water mixtures at 25°. (Log α_2)/A_{calc} is plotted along the vertical axis and residuals along the horizontal axis as described in this report. The residual in Table I is the difference between the observed x_2 and x_{2calc}, whereas the residuals in Figs. 2, 3, and 4 are [(logα_2)/A(obs)] − [(logα_2)/ A(calc)].*

RESULTS AND DISCUSSION

The solubility of *p*-hydroxybenzoic acid in dioxane–water at 25° is found in Table I, and the solubility profile is shown in Fig. 1 where X_2 is plotted against δ_1.

Estimating the Solubility Parameter of *p*-Hydroxybenzoic Acid from its Solubility Profile—It has been shown (12) that the solubility parameter of a solid can be estimated from the point of maximum solubility in a binary solvent, such as ethyl acetate and ethanol. When a solvent mixture is found that yields a peak in the solubility profile for a regular solution, δ_1 is assumed to equal δ_2.

In an irregular solution, these relations do not obtain exactly as in a regular solution, and the geometric mean, $W_{12} = (\delta_1^2\delta_2^2)^{1/2}$, seldom can be assumed to apply. W_{12} and $\delta_1\delta_2$ may be related by introducing a proportionality factor, K (13)[11]:

$$W_{12} = K\delta_1\delta_2 \qquad \text{(Eq. 10)}$$

Equation 8 then becomes:

$$\frac{\log \alpha_2}{A} = \delta_1^2 + \delta_2^2 - 2K\delta_1\delta_2 \qquad \text{(Eq. 11)}$$

The partial derivative of (log α_2)/A is taken with respect to δ_1 and the result is set equal to zero to obtain the value of δ_2 at the peak in the solubility profile:

$$\left(\frac{\partial(\log \alpha_2/A)}{\partial(\delta_1)}\right)_{\delta_2} = 2\delta_1 - 2K\delta_2 = 0 \qquad \text{(Eq. 12)}$$

and

$$\delta_1 = K\delta_2 \qquad \text{(Eq. 13)}$$

Thus, in irregular solutions, δ_1 is not equal to δ_2 at the maximum in the solubility profile, but is equal to $K\delta_2$ (13). As can be seen in Table I and Fig. 1, the maximum solubility of *p*-hydroxybenzoic acid in dioxane–water is at a solvent solubility parameter of 11.33 (*i.e.*, dioxane–water 90:10%, v/v) and K = 1.122. Therefore, the solubility parameter of *p*-hydroxybenzoic acid is 10.10 as obtained from:

$$\delta_2 = \frac{\delta_1}{K} = \frac{11.33}{1.122} = 10.10 \text{ (cal/cm}^3)^{1/2} \qquad \text{(Eq. 14)}$$

Solubility parameters of solid drugs can also be obtained by a group contribution method developed by Fedors (15). The δ value of *p*-hydroxybenzoic acid obtained from Fedors' method[12] is 15.3. A method for obtaining the solubility parameter of a drug molecule using internal pressure values and accurately measured solution densities was also previously suggested (16).

It has been shown that the solubility parameter of the solute obtained from the peak of the solubility profile may vary depending on the mixed solvent used (6). If the cosolvent has the tendency to form a complex with the solute, the maximum solubility may be shifted to the δ_1 value of a mixed solvent which contains a high percentage of this solvating cosolvent. Dioxane is a Lewis base and *p*-hydroxybenzoic acid a Lewis acid; these molecules have a tendency to form a complex, which may account for the shift of maximum solubility to the lower δ_1 value of the dioxane–water mixture. It is not necessary to know δ_2 to calculate the solubility in mixed solvents, for it has been found (4–6) that the estimated solubility can be obtained from a regression of (log α_2)/A against δ_1, obviating the need of δ_2. The back calculation of *p*-hydroxybenzoic acid solubility in dioxane–water is discussed later.

Effect of Solute on the Solubility Parameter of the Solution—The amount of solute in a saturated solution may alter the solubility parameter of the reference solvent (17) when in high concentration, since:

$$\delta_{app} = \Sigma\delta_i\phi_i \qquad \text{(Eq. 15)}$$

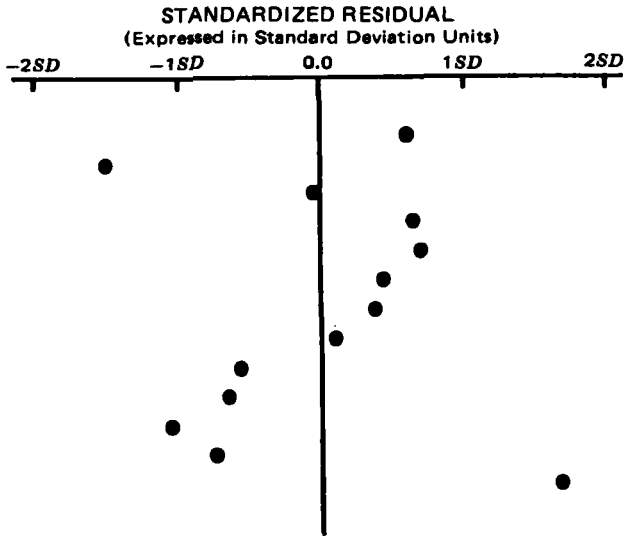
where ϕ_i is the volume fraction of component i in the solution. The solubility parameters of the saturated solutions, δ_{app}, are listed in Table I. The two solubility parameters, δ_1 and δ_{app}, are almost identical when the mole fraction solubility is <0.1 (volume fraction of dioxane is <70%). The solubility parameter of the saturated solution is a little higher (less than one unit) than that of the reference solvent when the solvent mixture contains >80% of dioxane. In the extended Hildebrand approach, (log α_2)/A is regressed on the solubility parameter of the solvent in a polynomial equation. A similar approach can be taken by regressing (log α_2)/A against δ_{app}, the solubility parameter of the solution. These two solubility

Table II—Demonstration of Trial-and-Error Process for the Back-Calculation of *p*-Hydroxybenzoic Acid in Water Using Eqs. 18 and 19

Step	X_2	$f(X_2)$
1	0.001	−100323
2	0.0001	342188
3	0.0005	32358
4	0.0006	−2594
5	0.00055	14082
6	0.00058	3902
7	0.00059	626
8	0.000595	−990
9	0.000592	−21
10	0.0005917	75
11	0.0005919	10
12	0.00059195	−5
13	0.00059193	0

[11] It should be noted that K as defined by Walker (13) and used here does not have the same meaning as K employed in another previous paper (14). In Ref. 14, K is a constant for a solute over the entire composition of a binary solvent. Here, and in other papers of this series, K varies across the solvent composition. The constant in Ref. 14 should probably be designated by another symbol, such as κ (kappa) to prevent confusion.

[12] [83.94 (Phenylene) + 72.61 (CO_2H) + 78.33 (OH)]$^{1/2}$ = (234.88)$^{1/2}$ = 15.3 (cal/cm^3)$^{1/2}$.



No.	$\frac{\log \alpha_2}{A}$ (calc)	Residual	SD units
1	15.0454	0.9115	0.59
2	2.5640	-2.3186	-1.50
3	-10.8254	-0.0393	-0.03
4	-16.7334	0.9857	0.64
5	-19.3055	1.0808	0.70
6	-21.5499	0.6871	0.44
7	-23.4366	0.5954	0.38
8	-24.8546	0.1567	0.10
9	-26.1580	-0.8886	-0.57
10	-25.9339	-0.9994	-0.65
11	-24.9973	-1.6036	-1.04
12	-23.4310	-1.1537	-0.75
13	-21.0603	2.5860	1.67

Figure 3—*Plot of residuals obtained from cubic regression equation, Eq. 17, for* p*-hydroxybenzoic acid in dioxane–water mixtures at 25°.*

parameter systems yield the same results except for rounding-off error.

Extended Hildebrand Approach Using Polynomial Regression and Scattergrams—The $(\log \alpha_2)/A$ for *p*-hydroxybenzoic acid was regressed against δ_1 values of dioxane–water mixtures at 25°. Three polynomial equations and associated statistical parameters[13] are quadratic:

$$\frac{\log \alpha_2}{A} = 21.02\ (\pm 10.36) - 7.6338\ (\pm 1.3152)\delta_1 + 0.31866\ (\pm 0.03984)\delta_1^2 \quad \text{(Eq. 16)}$$

$n = 13, s = 2.30, r^2 = 0.972, F = 173, F(2, 10, 0.01) = 7.56$

cubic:

$$\frac{\log \alpha_2}{A} = 142.51\ (\pm 34.32) - 31.582\ (\pm 6.683)\delta_1 + 1.82625\ (\pm 0.41788)\delta_1^2 - 0.0303307\ (\pm 0.0083899)\delta_1^3 \quad \text{(Eq. 17)}$$

$n = 13, s = 1.55, r^2 = 0.989, F = 259, F(3, 9, 0.01) = 6.99$

and quartic:

$$\frac{\log \alpha_2}{A} = 564.07\ (\pm 54.45) - 142.18\ (\pm 14.12)\delta_1 + 12.396\ (\pm 1.339)\delta_1^2 - 0.46689\ (\pm 0.05504)\delta_1^3 + 0.0065807\ (\pm 0.0008284)\delta_1^4 \quad \text{(Eq. 18)}$$

$n = 13, s = 0.55, r^2 = 0.999, F = 1550, F(4, 8, 0.01) = 7.01$

The residual plots (scattergrams) (7) of these equations are shown in Figs. 2–4 for quadratic, cubic, and quartic, respectively. In the figures, the horizontal axis represents the residuals standardized by dividing each by its standard deviation, while the vertical axis represents $(\log \alpha_2)/A_{calc}$ against which the residuals are plotted. The residual, or deviation of an estimated value, $(\log \alpha_2)/A_{calc}$, from the observed value, $(\log \alpha_2)/A_{obs}$, indicates which regression equation is the most suitable. As can be seen in Fig. 2, the residuals are not randomly scattered along the vertical axis. Therefore, the quadratic equation is not satisfactory, although R^2 is very high. In Fig. 3 the ordered arrangement of the points of Fig. 2 has mostly disappeared, and in Fig. 4 the residuals are scattered randomly along the vertical axis. None of the points in Fig. 4 is outside of a range of 2 σ. By comparing the statistics of the quadratic, cubic, and quartic equations (Eqs. 16, 17, and 18), it is observed that the quartic equation is the best choice due to its high r^2 and F values, and its small s value. The random scattering of points in the scattergram also shows Eq. 18 to be superior.

[13] The statistical quantities associated with each equation are: r^2 is the squared correlation coefficient (index of determination); s is the standard deviation or average of deviations about the arithmetic mean; F is the Fisher F ratio; n is the number of cases; and $F(k, n-k-1, 0.01)$ is the table value of F with k-independent variables; and k and $(n-k-1)$ degrees of freedom at the 99% confidence level. The ± values in parentheses associated with each regression coefficient, B, is the standard error of B.

In the back calculation, $(\log \alpha_2)/A_{calc}$ obtained from Eq. 18 was multiplied by A and the antilog was taken to obtain the activity coefficient, α_{2calc}. The estimated solubility was obtained from $X_{2\ calc} = X_2^i/\alpha_{2\ calc}$.

The values of A as listed in Table I are calculated (see Eqs. 3 and 4) from experimental solubilities and molar volumes. The back-calculated solubilities may be obtained using Eqs. 3 and 4 and an iteration procedure (18) beginning with a value of 1.0 for ϕ_1 and iterating until X_2 or ϕ_1 no longer changes by more than some desired small value. However, a simple iteration procedure does not always behave in a well-conditioned manner. Assuming that the quartic equation (Eq. 18) is a function of δ_1, *i.e.*, $f(\delta_1)$, and combining it with Eqs. 3 and 4, one obtains:

$$f(X_2) = (\log X_2{}^i - \log X_2)(2.303RT)[V_1(1-X_2) + V_2X_2]^2 - V_2[V_1(1-X_2)]^2 f(\delta_1) = 0 \quad \text{(Eq. 19)}$$

X_2 can now be solved by a trial-and-error root finding method. For example, in pure water, $f_D = 0$, $\delta_1 = 23.45$, $\delta_2 = 15.3$ (cal/cm^3)$^{1/2}$, $V_1 = 18.08$, $V_2 = 94.35$ and $\log X_2{}^i = -2.1267$, and substituting 23.45 for δ_1 in Eq. 18 one obtains $f(\delta_1) = (\log \alpha_2)/A_{calc} = 16.020$. Table II lists the steps during the trial-and-error procedure. This computation can be carried out easily by use of a programmable hand calculator. The root finder subroutine, ZBRENT, found in IMSL (19) can also be used for the computation; the earlier iteration procedure of the extended Hildebrand solubility approach has been replaced in this study by the root finder method (19). The back-calculated solubilities from Eq. 18 are listed in Table I and also plotted in Fig. 1. The results compare well with the observed solubilities, having errors <7%.

Log Solubility and Volume Fraction of the Cosolvent—A log linear relationship between moles/liter or mole fraction solubility and volume fraction of the cosolvent has been proposed (20, 21). The log solubility of *p*-hydroxybenzoic acid in units of moles/liter and mole fraction were plotted against the volume fraction of the cosolvent, dioxane, in Fig. 5 to test whether this relationship holds for *p*-hydroxybenzoic acid solutions. Also marked along the horizontal axis (top of the figure) is the solubility parameter of the mixed solvents. It is evident from Fig. 5 that a log linear relation is not observed in the binary solvent mixture, dioxane and water, as the curves are quite nonlinear. They can, however, be reproduced by use of the following quadratic expressions:

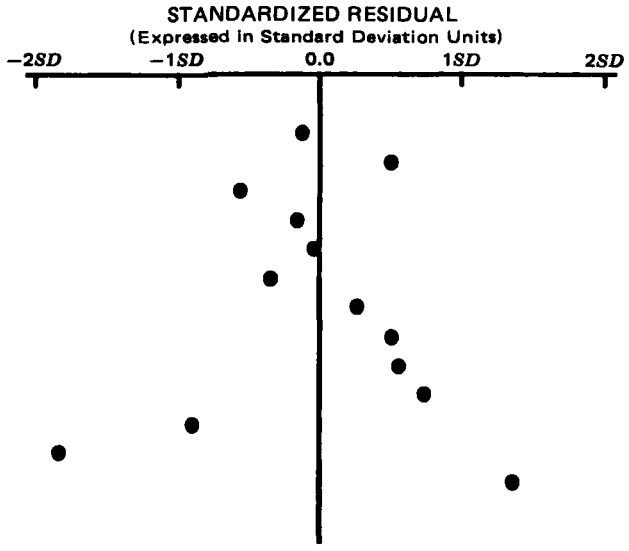
$$\log S_2 = -1.430(\pm 0.050) + 4.874(\pm 0.192)f - 3.374(\pm 0.175)f^2$$
$$r^2 = 0.990, s = 0.056, F = 474, F(2, 10, 0.01) = 7.56 \quad \text{(Eq. 20)}$$

and

$$\log X_2 = -3.178(\pm 0.051) + 5.226(\pm 0.194)f - 3.047(\pm 0.176)f^2$$
$$r^2 = 0.994, s = 0.057, F = 806, F(2, 10, 0.01) = 7.56 \quad \text{(Eq. 21)}$$

where S_2 is the molar and X_2 the mole fraction solubility of *p*-hydroxybenzoic acid in dioxane–water mixtures with various volume fractions f of dioxane at 25°.

In another study (22) it was shown that the log linear rule often holds for drugs in binary solvents of water combined with an organic cosolvent particularly when the cosolvent interacts strongly with the solute and when the solubility parameter of the cosolvent is several units above that



No.	$\frac{\log \alpha_2}{A}$ (calc)	Residual	SD units
1	16.0248	-0.0679	-0.12
2	-0.0327	0.2781	0.50
3	-10.5570	-0.3077	-0.56
4	-15.6597	-0.0880	-0.16
5	-18.2040	-0.0206	-0.04
6	-20.6693	-0.1935	-0.35
7	-22.9922	0.1510	0.27
8	-24.9750	0.2771	0.50
9	-27.3573	0.3107	0.56
10	-27.3418	0.4085	0.74
11	-26.1098	-0.4911	-0.89
12	-23.5708	-0.0139	-1.84
13	-19.2316	0.7572	1.39

Figure 4—*Plot of residuals obtained from quartic regression equation, Eq. 18, for* p*-hydroxybenzoic acid in dioxane–water mixtures at 25°. The almost complete scatter here indicates that Eq. 18 is superior to Eqs. 16 and 17 (Figs. 2 and 3).*

of the solute. Although dioxane appears to interact with *p*-hydroxybenzoic acid and elevates the solubility of the drug above the ideal solubility line, X_2^i, dioxane does not solvate or complex the solute sufficiently to yield a straight line relationship. Furthermore, the solubility parameter of *p*-hydroxybenzoic acid lies between the solubility parameters of water and dioxane and this militates against a log linear relationship. Instead, these conditions create a peak value on a plot of log solubility *versus* volume fraction of cosolvent or *versus* solubility parameter of mixed solvent.

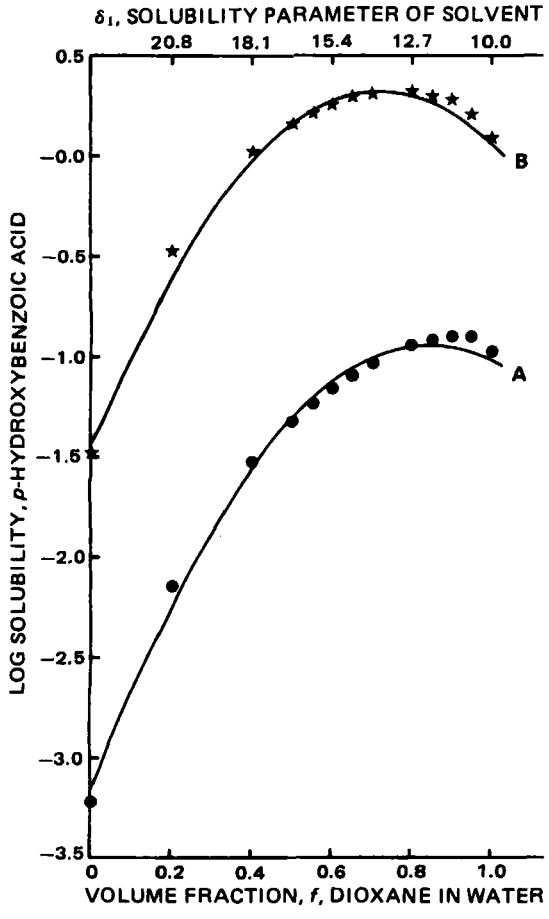


Figure 5—*Log solubility of* p*-hydroxybenzoic acid in dioxane–water mixtures at 25°. Curves A and B are back-calculated from Eqs. 21 and 20, respectively. Key: (★) moles/liter; (●) mole fraction.*

CONCLUSIONS

The solubility parameter of *p*-hydroxybenzoic acid was estimated by determining the peak in the solubility profile, Fig. 1, and by a group-contribution method (15). Interaction of the solute with the cosolvent, dioxane, may have unduly lowered the δ_2 value obtained by the peak solubility technique. The two methods do not yield values that agree closely; the δ_2 value was taken as ~15 $(cal/cm^3)^{1/2}$, a reasonable value for this highly polar compound.

The effect of a saturated solution of the solute on the solubility parameter of the mixed solvent was tested in this system and was found not to influence δ_1 markedly. In predicting solubilities using the extended Hildebrand solubility approach, the slight effect on δ_1 does not change the back-calculated solubility values.

To estimate solubilities of *p*-hydroxybenzoic acid in mixtures of dioxane and water, $(\log \alpha_2)/A$ was regressed in a polynomial on δ_1 of the solvent mixtures according to the extended Hildebrand solubility approach. The residual plots (scattergrams) for the quadratic, cubic, and quartic equations serve together with the standard deviation, r^2 and F values to indicate whether a quadratic equation is satisfactory or whether a cubic, quartic, or higher-order polynomial is required to fit the data. From an analysis of r^2, F, and the scattergram, it was observed that the quartic equation was required to reproduce the solubility data of *p*-hydroxybenzoic acid.

The iteration procedure for the back-calculation of solubilities was studied in detail in this paper, because it was found to be a problem in earlier works, causing an oscillation between two values or a divergence rather than convergence to the proper mole fraction solubility. A root finder method, involving trial-and-error, or better, use of an IMSL subroutine [ZBRENT (19)] proved to be the solution to this problem.

The solubility of *p*-hydroxybenzoic acid in dioxane–water mixtures was observed not to follow a log linear relationship: a regularity earlier observed for some drugs in water combined with nonaqueous cosolvents. The failure of the log linear relationship is expected (22) in this case, however, since the solubility parameter of *p*-hydroxybenzoic acid ($\delta_2 \simeq 15$) lies between that of water ($\delta_1 = 23.4$) and the cosolvent, dioxane ($\delta_1 = 10$).

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Molecular Interaction Between Riboflavin and Salicylic Acid Derivatives in Nonpolar Solvents

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Abstract □ The interaction of riboflavin-2′,3′,4′,5′-tetrabutyrate (I) with salicylic acid (II), aspirin (acetylsalicylic acid, III), and salicylamide (IV) has been spectroscopically investigated to determine the binding mechanism. NMR and absorption spectra were measured in nonpolar solvents. The association constant K of the formation of complex was calculated from the absorption spectra. Compounds I and II form a 1:1 cyclic hydrogen-bonded dimer through the N-3 proton and the C-2 carbonyl oxygen of the isoalloxazine ring, and the carboxylic hydroxyl proton and carbonyl oxygen of II. Compounds I and III form a 1:1 cyclic hydrogen-bonded dimer by the same mode. Compound IV forms a 1:1 cyclic hydrogen-bonded dimer with I through the N-3 proton and the C-2 carbonyl oxygen of the isoalloxazine ring, and the amino proton and the carbonyl oxygen of IV. Salicylates produce marked changes in the absorption spectra of I. These spectral changes are attributed to the formation of the hydrogen-bonded dimer. It appeared that the strongest complex was formed with salicylic acid, a weaker one with aspirin, and an even weaker one with salicylamide.

Keyphrases □ Riboflavin—molecular interactions with salicylic acid derivatives, NMR and absorption spectroscopy in nonpolar solvents, association constant determinations for hydrogen-bonded dimers □ Salicylates—molecular interactions with riboflavin, NMR and absorption spectroscopy in nonpolar solvents, association constant determinations for hydrogen-bonded dimers

Salicylate, one of the oldest synthetic drugs, remains the most widely used analgesic and antipyretic agent. It is known that the hydrogen bonding of salicylates in biological systems is related to their drug action (1). It has been determined experimentally that higher concentrations of salicylates result in marked stimulation of respiration while low concentrations depress this function (2–7). Salicylic acid derivatives act as uncoupling agents on the isolated mitochondrial respiration and lower the respiration rate of dinitrophenol-uncoupled mitochondria; inhibition of state 3 mitochondrial respiration by salicylic acid derivatives is accompanied by an increase in the oxidized state of all electron transport systems (8).

The electron-transfer from nicotinamide adenine dinucleotide (NADH) to flavoprotein, or the charge-transfer complex thus formed, was studied by a number of authors to give an account of the function of the respiratory chain (9–13). It has been determined that reduced NAD-coupling enzyme[1] complex converts spontaneously to the hypothetical intermediate as oxidized NAD-coupling enzyme[2], which is considered indispensable to the formation of adenosine triphosphate (ATP) in the respiratory chain (14). Simultaneously, the electrons in $(NADox)^{-2}$ can be transferred to flavoprotein. It is generally agreed that salicylates, which act as uncoupling agents, cause the breakdown of some high-energy intermediate involved in the phosphorylation process. However, the mechanism of such a breakdown has not previously been determined. The mechanism of action of salicylate could be described as: (*a*) salicylate associates with the adenine moiety of flavin adenine dinucleotide (FAD) or NAD, (*b*) salicylate inhibits the interaction of the flavin moiety with the adenine moiety of FAD or NAD, (*c*) salicylate affects the electronic environment due to an association with flavin, or (*d*) salicylate inhibits the interactions of FAD and the flavin mononucleotide (FMN) with apoprotein. Therefore, it seems worthwhile to examine the molecular interaction between salicylate and riboflavin. In this paper a detailed analysis of the NMR and absorption spectra of the complex will be presented and a structure of the complex will

[1] Enzyme-coupled reduced nicotinamide adenine dinucleotide.
[2] Enzyme-coupled oxidized nicotinamide adenine dinucleotide.

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