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Determination of dissociation constants of sparingly soluble compounds from solubility data

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The solubility of a sparingly soluble drug is normally measured over the whole of the physiological pH range as part of preformulation studies. Knowing the solubility of the neutral species and the apparent solubility at a pH where the molecule is ionized, the dissociation constant may be determined (Albert and Serjeant, 1971). Zimmermann (1983) showed how using the equation:

$$S = S_0\left(1 + \frac{10^{-pH}}{K'_a}\right) \quad (1)$$

(which applies to a weak base) K'_a and S_0, the limiting solubility of the molecule may be determined by unweighted linear regression.

The main problem in this method is the accurate determination of the intercept S_0. The slope S_0/K'_a is easily determined with reasonable accuracy, but if the solubility data have been determined over a wide pH range, the intercept will be close to zero (in relation to most of the data) and the precision of the determination will be low. The reason for this is that unweighted linear regression assumes a constant absolute standard deviation over the whole range of solubility measurements, whereas the absolute standard deviation is likely to be much lower for the lower concentrations being measured. Only for very low concentrations where the background noise level is significant will the standard deviation of the measured solubility be constant. According to Aarons (1982) the inappropriate use of unweighted linear regression will have the following effects: (a) a good estimate of the slope; (b) a poor estimate of the intercept S_0. The error may not be large in absolute terms but since the intercept is close to the origin the relative error can be considerable and will be transmitted to the estimate of the dissociation constant; and (c) estimates of the errors will be poor.

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This method of determination of the ionization constant is rarely as precise as spectrophotometric or potentiometric methods but for even moderate success the most accurate possible estimate of the solubility of the uncharged molecule should be obtained. The most general solution to this problem is to modify the above treatment by using weighted linear regression. Each value of the solubility is weighted by a factor $W = 1/\sigma^2$ where σ is the standard deviation determined either directly at each concentration, or as a function of concentration determined by extended least-squares (Steiner and Beal, 1980; Aarons, 1982).

Should precision data not be available, we consider it justifiable to assume a constant relative standard deviation. This assumption tends to force the regression line closer to the lower solubilities to give a better estimate of the limiting solubility. The effect on the estimate of the slope will be slight. The procedure may underestimate error of the estimated limiting solubility if the latter is so low that instrumental noise becomes significant.

The procedure is carried out as follows. If the relative standard deviation of the solubility (y) is constant, the variance of y is proportional to y^2. Substituting a weighting factor y^{-2} in the equations for weighted linear regression, conveniently listed by Cvetanović et al. (1979), we obtain for the intercept and slope, and their estimated standard deviations:

$$\text{slope:} \quad m = \left(\sum_i \frac{1}{y_i^2} \sum_i \frac{x_i}{y_i} - \sum_i \frac{x_i}{y_i^2} \sum_i \frac{1}{y_i} \right) D^{-1} = \frac{S_0}{K'_a} \tag{2}$$

$$\text{intercept:} \quad I = \left(\sum_i \frac{1}{y_i} \sum \frac{x_i^2}{y_i^2} - \sum_i \frac{x_i}{y_i} \sum \frac{x_i}{y_i^2} \right) D^{-1} = S_0 \tag{3}$$

where y_i = measured solubility at $pH = -\log_{10} x_i$

$$\text{and} \quad D = \sum_i \frac{1}{y_i^2} \sum_i \frac{x_i^2}{y_i^2} - \left(\sum_i \frac{x_i}{y_i^2} \right)^2 \tag{4}$$

$$\text{Standard deviation of slope} = \sqrt{\frac{1}{n-2} \left\{ \frac{n \sum_i \frac{1}{y_i^2} - \left(\sum_i \frac{1}{y_i} \right)^2}{D} - m^2 \right\}} \tag{5}$$

$$\text{Standard deviation of intercept} = \sqrt{\frac{1}{n-2} \left\{ \frac{n \sum_i \frac{1}{y_i^2} - \left(\sum_i \frac{1}{y_i} \right)^2}{D} - m^2 \right\} \frac{\sum \frac{x_i^2}{y_i^2}}{\sum \frac{1}{y_i^2}}} \tag{6}$$

The use of this method is illustrated for the data in Table 1, relating to an

imidazopyridine derivative of very low solubility. These are poor data, which serve to amplify the differences between the two methods of linear regression. Using unweighted linear regression:
intercept = -9.8 mg/litre (standard deviation = ± 10.7 mg/litre); and
slope = 4.06×10^4 mg/mol (standard deviation = ± 414.0 mg/mol).
The intercept is an unbiased estimate of the solubility, but the precision is very low, and clearly the negative value is physically meaningless. Thus no value can be calculated for the pK'_a.

Using weighted linear regression (Eqns. 2–6) we obtain:
intercept = 1.7 mg/litre (standard deviation = ± 0.4 mg/litre);
slope = 2.7×10^4 mg/mol (standard deviation = $\pm 6.4 \times 10^3$ mg/mol; and
$pK'_a = 4.17$ (standard deviation = 0.12).
These are physically realistic results. The considerable increase in the standard deviation of the slope and the corresponding decrease in the standard deviation of the intercept, the result of the redistribution of weighting in favour of points near the intercept, should be noted. Examination of the degree of scatter of the data at pH 4 and above suggests that the precision of the limiting solubility may be overestimated, —i.e. the standard deviation should be more than 0.4 mg/litre—by this simplified weighted linear least-squares method.

These results are summarized in Table 2 where they may be compared with the results of treating rather more precise data by different methods.

Krebs and Speakman (1945) calculated the first and second pK'_a values of the amphoteric compound, 2-sulphanilinamidopyrimidine (sulphadiazine), from solubility data. They calculated the limiting solubility S_0, then calculated the pK'_a for each data point and took the average value. Appropriate modification of Eqn. 1 (Albert and Serjeant, 1971) was necessary for calculating the first pK'_a which represents the loss of the proton. The superiority of weighting the solubilities to carrying out a simple linear regression is clearly shown by the better precision of the results, and the agreement of the values of the limiting solubility determined from the two sets of data ($pH \geqslant 4.59$ and $pH \leqslant 3.06$). The large differences between the two dissociation constants permits these simple methods, which can easily be programmed on a pocket calculator, to be used.

TABLE 1

SOLUBILITY–pH PROFILE OF THE IMIDAZOPYRIDINE DERIVATIVE

pH	Solubility (mg/litre)
1.14	2940
2.07	274
3.02	24.9
4.03	3.13
5.31	3.20
6.11	1.27
6.98	2.10
7.70	3.87

TABLE 2

COMPARISON OF 3 METHODS FOR CALCULATING THE DISSOCIATION CONSTANT FROM SOLUBILITY DATA

		Method of Krebs and Speakman (1945)	Unweighted linear regression	Weighted linear regression
Imidazopyridine derivative (data from table 1)	S_0	2.1 (0.65) [a] mg/litre	not calculable	1.7 (0.4) mg/litre
	pK'_a	4.24 (0.13) [c]		4.2 (0.14)
Sulphadiazine [c] (Krebs and Speakman, 1945)	S_0	61.6 mg/litre	78 [b] (41) mg/litre	61.5 [b] (5.4) mg/litre
			54.2 [c] (7.3) mg/litre	61.0 [c] (1.6) mg/litre
	$pK'_a(1)$	2.08 (0.09)	1.95 (0.26)	2.06 (0.05)
	$pK'_a(2)$	6.45 (0.02)	6.42 (0.05)	6.44 (0.01)
Pyrazolic acid (Zimmermann, 1983)	S_0	23 (8.3) [a] mg/litre	49 (22) mg/litre	18.6 (3.6) mg/litre
	pK'_a	4.85 (0.13)	5.35 (0.16)	4.90 (0.09)
Lisuride hydrogen maleate (Zimmermann, 1983)	S_0	19.5 (1.2) [a] mg/litre	20.6 (3.0) mg/litre	18.4 (0.6) mg/litre
	pK'_a	7.26 (0.03)	7.24 (0.06)	7.29 (0.02)

[a] The method of calculation differs slightly from that of Albert and Serjeant (1971) in that an iterative procedure was used to calculate S_0.

[b] Calculated from solubilities in acid pH $\leqslant$ 3.06 The value of the limiting solubility determined at higher pH was also included in this calculation.

[c] Calculated from solubilities at pH $\geqslant$ 4.89.

[d] Dissociation constants for sulphadiazine measured potentiometrically are reported by Krebs and Speakman (1945). They considered it to be too weak a base for pK'_a to be satisfactorily determined by this method [a]. A value $pK'_a = 6.40 \pm 0.06$ was determined in 0.1 M NaCl.

[e] Values in parentheses represent standard deviations.

The weighted and unweighted least-squares methods are also compared using Zimmermann's (1983) data for pyrazolic acid and lisuride hydrogen maleate. For pyrazolic acid the results of the two methods, compared in Fig. 1, are very different. It is interesting to note that the very large percentage difference between experimental and calculated solubility for the datum at pH 5, when using unweighted linear regression, is dramatically reduced when weighted linear regression is used. There is a corresponding improvement in the precision of the limiting solubility estimate, though the limits are still very wide owing to the lack of data points below pH 5. This effect is seen for the other compounds and is the most significant difference between the results obtained by the two linear regression methods in the case of lisuride hydrogen maleate.

Determination of the dissociation constant from the solubility–pH profile can be a useful method in certain circumstances. As has been shown above, weighted linear regression is probably the best method of calculation, but the necessary analytical data are seldom available. The method described in this paper assuming constant relative standard deviation as a model is more satisfactory than standard unweighted linear regression, which can give misleading results for all but the most precise data. The method of Krebs and Speakman (1945) also appears to be satisfactory.

It should be stressed that determination of the pK'_a by the above method depends greatly on the accurate determination of the limiting solubility. This is often an experimentally difficult procedure, and this difficulty cannot be overcome by

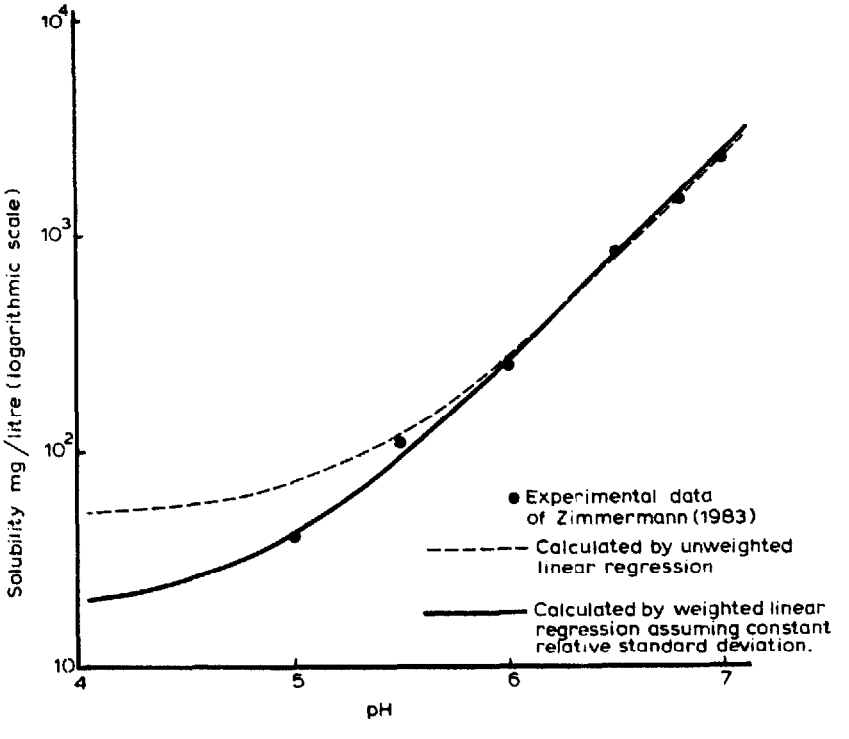

Fig. 1. Comparison of unweighted and weighted linear least-squares regression on solubility–pH data for pyrazolic acid.

relatively more precise measurements at pH values where the solubility is higher. Improved methods for solubility determination become necessary, preferably with a considerable number of measurements. Because of the influence of more soluble impurities, even at very low levels in the sample, phase solubility analysis, or chromatographic techniques should be used. Amongst the latter are the injection of large volumes or on-column sample concentration (May, et al., 1978). It is under such circumstances of very low solubility that the method becomes important for the determination of the pK'_a of the molecule rather than a reassurance that the pH–solubility profile fits the correct theoretical relationship.

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