

Short Communication

Evaluation of acidity constants of two-step overlapping equilibria of amphoteric substances from solubility measurements

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Introduction

The therapeutic activity of drugs is related to the concentration of free unbound drug in plasma [1, 2] which is dependent on, among other factors, the ionization and solubility of the drug. In addition, a knowledge of the acidity constants of compounds of pharmaceutical interest is required for the solution of many drug-related problems [3]. However, the choice of method for determining the ionization constants of drugs is severely restricted in a number of cases by poor solubility which does not allow application of normal potentiometric and conductimetric techniques for the determination of the constants in water. In such cases, solubility measurements in combination with any appropriate quantitative analytical technique [4–15] may be used. Although hydroalcoholic solutions may be employed to increase the solubility, studies intended to be of clinical relevance should be conducted in aqueous buffers at physiological temperature and ionic strength. In this report, an equation is proposed that allows the overlapping acidity constants of amphoteric substances to be calculated from solubility measurements.

Theory

On the basis of the definition of apparent acidity constants, $K_{a1} = [\text{HR}][\text{H}^+]/[\text{H}_2\text{R}^+]$, and $K_{a2} = [\text{R}^-][\text{H}^+]/[\text{HR}]$ (where square brackets represent concentrations and (H^+) indicates the hydrogen ion activity), the solubility s of a sparingly soluble, amphoteric substance HR, is given by

$$s = [\text{R}^-] + [\text{HR}] + [\text{H}_2\text{R}^+] = s_0 \left[1 + \frac{(\text{H}^+)}{K_{a1}} + \frac{K_{a2}}{(\text{H}^+)} \right] \quad (1)$$

where $s_0 = [\text{HR}]$ is the limiting or intrinsic solubility of HR, assumed to be constant and independent of pH, whose determination is extremely difficult [13, 16–18].

Equation (1) may be easily converted into:

$$s = s_0 \left[1 + \sqrt{\frac{K_{a2}}{K_{a1}}} \left(\sqrt{\frac{K_{a1} K_{a2}}{(\text{H}^+)}} + \frac{\sqrt{K_{a1} K_{a2}}}{(\text{H}^+)} \right) \right] \quad (2)$$

By differentiating equation (1) with respect to pH and setting $ds/d(\text{H}^+)$ equal to zero the value of (H^+) may be calculated, which makes the solubility minimum

$$(\text{H}^{+'}) = \sqrt{K_{a1} K_{a2}} \quad (3)$$

A combination of equations (2) and (3), as $\cosh x = (e^x + e^{-x})/2$, gives

$$s = s_0 + 2 s_0 \sqrt{\frac{K_{a2}}{K_{a1}}} \cosh (2.303 \Delta\text{pH}) \quad (4)$$

where ΔpH is the difference between the pH which corresponds to a minimum solubility pH' and a given pH

$$\Delta\text{pH} = \text{pH}' - \text{pH} \quad (5)$$

A plot of s against $2 \cosh (2.303 \Delta\text{pH})$ is a straight line $y = a_0 + a_1 x$, the intercept (a_0) being equal to s_0 , and the ratio of slope ($a_1 = s_0 \sqrt{K_{a2}/K_{a1}}$) to intercept being equal to the square root of the ratio of K_{a2} to K_{a1} . The acidity constants may be evaluated from the ratio of slope intercept as follows

$$\text{p}K_{a1} = \text{pH}' + \log (a_1/a_0) \quad (6)$$

$$\text{p}K_{a2} = \text{pH}' - \log (a_1/a_0) \quad (7)$$

The stepwise formation of protonated species, often ignored in studies of this type, can be readily appreciated.

In order to increase the precision in the evaluation of the pH' , the ratio

$$\frac{\Delta \log s}{\Delta \text{pH}} = \frac{\log s_{n+1} - \log s_n}{\text{pH}_{n+1} - \text{pH}_n} \quad (8)$$

may be plotted against $(\text{pH}_{n+1} + \text{pH}_n)/2$, where pH_n and s_n denote the pH and s values, respectively, for the n th point. The pH value which satisfies the condition $\Delta \log s / \Delta \text{pH} = 0$ is then taken as pH' . Such curves are obtained by graphical differentiation of the $\log s$ against pH curve by using very small increments in pH.

The standard deviation of $\text{p}K_{ai}$ values ($i = 1, 2$) is given by

$$s_{\text{p}K_{ai}} = \sqrt{s_{\text{pH}'}^2 + 0.4343^2 \frac{s_0^2}{Q^2}} \quad (9)$$

where $Q = \sqrt{K_{a2}/K_{a1}}$, and s_0 is equal to

$$s_0 = \sqrt{\frac{s_{a_1}^2}{a_0^2} + \frac{a_1^2}{a_0^4} s_{a_0}^2 - 2 \frac{a_1}{a_0^3} \text{cov} (a_0, a_1)} \quad (10)$$

so that Q is calculated as the ratio of slope to intercept of the straight line indicated above; s_{a_0} and s_{a_1} are the standard deviations of intercept and slope, respectively, and $\text{cov}(a_0, a_1)$ is the covariance between the intercept and slope of the straight line obtained by single linear regression.

To check the validity of the proposed procedure, it has been applied to the evaluation of acidity constants of niflumic acid [2] and yellow fluorescein [18].

Application

The solubility of niflumic acid (HR) as a function of pH [2] is given in Table 1. The value of pH' for this system, obtained by graphical differentiation of the $\log s$ -pH curve, was found to be 3.60 ± 0.02 . On the basis of the results obtained (Table 2) by using different pairs of (pH, s) data, the most reliable values found for the unknown parameters were: $s_0 = 22.57 \pm 1.65 \text{ mg l}^{-1}$, $\text{pK}_{a1} = 2.13 \pm 0.04$, and $\text{pK}_{a2} = 5.07 \pm 0.04$. Points number 10 and 11 (Table 1) were considered to be anomalous. The values for pK_a that were obtained were in good agreement with those found by Bres *et al.* [2]: $\text{pK}_{a1} = 2.15$ and $\text{pK}_{a2} = 5.05$. The intrinsic solubility of niflumic acid was not given in the paper by Bres *et al.* [2].

The solubility-pH data for yellow fluorescein (H_2R) [18] are shown in Table 3. For this compound $\text{K}_{a1} = [\text{H}_2\text{R}](\text{H}^+)/[\text{H}_3\text{R}^+]$, $\text{K}_{a2} = [\text{HR}^-](\text{H}^+)/[\text{H}_2\text{R}]$, and $s = [\text{H}_3\text{R}^+] + [\text{H}_2\text{R}] + [\text{HR}^-]$; equations (2)–(10) are also applicable. A pH' value of 3.30 ± 0.02 was obtained in this case. Least-squares treatment of the data for yellow fluorescein yielded intrinsic solubility and pK_a values that varied with the number of data points used (Table 4). The variability of the values obtained for the data points corresponding to high pH values was attributed to the occurrence of an additional deprotonation having a pK_a value (pK_{a3}) of 6.36 [18]. In fact, the solubility curve for yellow fluorescein as a function of pH was not symmetrical about the pH of minimum solubility. Consequently points number 10 to 14 (Table 3) had to be eliminated from the calculations and thus a significant contribution of the doubly-charged anion $\text{R}^{=}$ to the

Table 1
Solubility of niflumic acid as a function of pH at 20°C [2]

Number of sample	pH	$s(\text{mg l}^{-1})$	Number of sample	pH	$s(\text{mg l}^{-1})$
1	1.20	211	7	4.30	26.9
2	1.55	116	8	4.75	32.1
3	2.15	42.9	9	5.35	59.3
4	2.55	31.4	10	5.70	125
5	3.20	26.9	11	6.20	250
6	3.65	23.6			

Table 2
Values for the intrinsic solubility and acidity constants of niflumic acid

Data points	pK_{a2}	pK_{a1}	s_{pK_a}	$s_0 \pm s_{s_0}$
1–8	5.06	2.14	0.05	22.17 ± 1.90
1–9	5.07	2.13	0.04	22.57 ± 1.65
1–10	5.07	2.13	0.05	22.80 ± 1.78
1–11	5.27	1.93	0.11	29.21 ± 5.85

Table 3
Solubility of yellow fluorescein as a function of pH at $23 \pm 0.5^\circ\text{C}$; ionic strength 0.10 [18]

Number of sample	pH	$s \cdot 10^4 \text{ M}$	Number of sample	pH	$s \cdot 10^4 \text{ M}$
1	1.10	72.2	8	4.45	3.92
2	1.53	21.1	9	4.37	5.84
3	2.07	7.82	10	4.90	14.0
4	2.33	6.62	11	5.18	21.5
5	2.69	5.29	12	5.34	33.1
6	3.01	4.81	13	5.53	47.2
7	3.39	3.85	14	6.03	180

Table 4
Values for the intrinsic solubility and acidity constants of yellow fluorescein

Data points	pK_{a2}	pK_{a1}	s_{pK_a}	$(s_0 \pm s_{s_0}) \cdot 10^4$
2-9	4.36	2.24	0.05 ₇	3.38 ± 0.30
2-10	4.37	2.23	0.06 ₀	3.40 ± 0.33
2-11	4.46	2.14	0.07 ₆	3.74 ± 0.47
2-12	4.44	2.16	0.06 ₇	3.62 ± 0.44

solubility occurred at those pH values. Points number 1 was also eliminated because it was found to be anomalous. Thus, the most reliable values found for the unknown parameters were: $s_0 = (3.38 \pm 0.30) \times 10^{-4} \text{ M}$, $\text{pK}_{a1} = 2.24 \pm 0.06$ and $\text{pK}_{a2} = 4.36 \pm 0.06$. Values of $s_0 = 3.80 \times 10^{-4} \text{ M}$, $\text{pK}_{a1} = 2.13$ and $\text{pK}_{a2} = 4.44$ were reported by Diehl and Markuszewski [18] who made various approximations in their calculation.

The method described here appears to be a valuable technique that has not been applied before to the study of amphoteric equilibria.

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