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Evaluation of partition coefficient and acidity constants of amphoteric substances by graphic differentiation of the distribution ratio against pH curve

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The solution of numerous problems of pharmaceutical interest often requires the knowledge of acidity constants of drugs (Newton and Kluza, 1978). The potentiometric and spectrophotometric methods for evaluating acidity constants are not generally applicable (King, 1965), e.g. to compounds whose solubility in water is scarce or which have very similar absorption spectra for the conjugate acid–base species. On the other hand, the use of the partition coefficient as the extrathermodynamic reference parameter for hydrophobic bonding in biological and pharmacological studies has generated a great interest (Leo et al., 1971; Van de Waterbeemd, 1983) in distribution measurements. Solvent extraction possesses several advantages over other techniques which are used to measure ionization constants of acids and bases and may be applied in the study of amphoteric substances as we will show in the following.

For an amphoteric substance, HR, the distribution ratio between organic and aqueous phase is governed by (charges omitted for simplicity)

$$D = \frac{[\mathrm{HR}]_0}{[\mathrm{R}] + [\mathrm{HR}] + [\mathrm{H_2R}]} = \frac{\dfrac{[\mathrm{HR}]_0}{[\mathrm{HR}]}}{\dfrac{K_{a_2}}{[\mathrm{H}]} + 1 + \dfrac{[\mathrm{H}]}{K_{a_1}}}$$

$$= K_d f_1 \qquad (1)$$

where K_{a_1} and K_{a_2} are the acidity constants defined as $K_{a_1} = [\mathrm{HR}][\mathrm{H}]/[\mathrm{H_2R}]$ and $K_{a_2} = [\mathrm{R}][\mathrm{H}]/[\mathrm{HR}]$, K_d is the partition coefficient or distribution constant (Irving et al., 1978), and f_1 is the molarity fraction ($f_j = [\mathrm{H_jR}]/C_\mathrm{R}$) of species HR ($j = 1$) in the aqueous phase.

By differentiation of Eqn. 1 we have

$$\frac{d^2D}{d(\mathrm{pH})^2} = K_d \frac{d^2 f_1}{d(\mathrm{pH})^2} \qquad (2)$$

Values of $d^2D/d(\mathrm{pH})^2 = 0$ will locate points of inflexion in the graph of D against pH. In a

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previous paper (Asuero et al., 1986) it has been shown that for a polyprotic acid (H_nR)

$$\frac{d^2 f_j}{d(pH)^2} = 2.303^2 f_j \left((j-\bar{n})^2 - \sum_{j=0}^{n} j(j-\bar{n}) f_j \right) \tag{3}$$

($j = 0$ to n), where $\bar{n}$ is the average number of protons bound per R (Bjerrum, 1957)

$$\bar{n} = \sum_{j=0}^{n} j f_j \tag{4}$$

For a diprotic acid ($n = 2$), taking into account that

$$1 - \bar{n} = f_0 - f_2 \tag{5}$$

$$2 - \bar{n} = 2f_0 + f_1 \tag{6}$$

we get

$$\frac{d^2 f_1}{d(pH)^2} = 2.303^2 f_1 (f_0^2 + f_2^2 - f_1 f_2 - f_1 f_0 - 6 f_0 f_2) \tag{7}$$

making

$$\frac{[H'']}{K_{a_1} K_{a_2}} = y \tag{8}$$

and taking into account the relationship between the acidity constants and molarity fractions (Asuero et al., 1986), at the points where the second derivative is zero the following relationship can be shown to be valid

$$y^4 - \sqrt{\frac{K_{a_1}}{K_{a_2}}}\, y^3 - 6y^2 - \sqrt{\frac{K_{a_1}}{K_{a_2}}}\, y + 1 = 0 \tag{9}$$

The values of the inflexion points in the graph of D against pH may be calculated from the two real positive roots of Eqn. 9, which can be factored to give

$$\sqrt{\frac{K_{a_1}}{K_{a_2}}} = \frac{y^4 - 6y^2 + 1}{y^3 + y} = y + \frac{1}{y} - \frac{8}{y + \frac{1}{y}} \tag{10}$$

Taking into account the definition of hyperbolic sine and cosine functions $\cosh x = (e^x + e^{-x})/2$, $\sinh x = (e^x - e^{-x})/2$, it can be shown that the square root of the ratio of K_{a_1} by K_{a_2} depends on the difference between the pH values corresponding to the maximum (pH′) and the points of inflexion (pH″) of the D–pH curve

$$\sqrt{\frac{K_{a_1}}{K_{a_2}}} = 4\sqrt{2}\, \sinh\left(\ln\left(\frac{\cosh(\ln 10\, \Delta pH^*)}{\sqrt{2}} \right) \right) \tag{11}$$

where

$$\Delta pH^* = pH' - pH'' = \log y \tag{12}$$

The pH″ values may be obtained by graphical differentiation (Asuero et al., 1986; Asuero et al., 1987; Asuero et al., 1988) of a smooth curve plotted through experimental values of D obtained at various values of pH. If many data points are obtained, a direct plot of $\Delta D/\Delta pH$ from experimental data against pH will suffice. The maximum and minimum of this representation allows the inflexion points of the D–pH curve to be calculated whereas the point of zero ordinate gives the pH′ value.

Once the value of $\sqrt{K_{a_1}/K_{a_2}}$ is known, the pK_a values may be evaluated from

$$pK_{a_2} = pH' + \log\sqrt{\frac{K_{a_1}}{K_{a_2}}} \tag{13}$$

$$pK_{a_1} = pH' - \log\sqrt{\frac{K_{a_1}}{K_{a_2}}} \tag{14}$$

so that from differentiation of Eqn. 1 (Asuero et al., 1986) we get

$$\frac{\mathrm{d}D}{\mathrm{d(pH)}} = K_\mathrm{d}\frac{\mathrm{d}f_1}{\mathrm{d(pH)}} = -\ln 10 \cdot K_\mathrm{d} \cdot f_1(1-\bar{n}) \tag{15}$$

and thus, in the maximum of the D–pH curve it follows that $1-\bar{n}=0$, and then $f_0 = f_2$, and

$$\mathrm{pH}' = \sqrt{K_{\mathrm{a}_1}K_{\mathrm{a}_2}} \tag{16}$$

Conversely, the knowledge of the ratio of K_{a_1} by K_{a_2} allows to evaluate the difference in pH units between the maximum and the points of inflexion. In effect, from Eqn. 11 we get

$$\Delta\mathrm{pH}^* = \frac{1}{\ln 10}\operatorname{arc\,cosh}(\sqrt{2} \times \exp\left(\operatorname{arc\,sinh}\left(\frac{1}{4}\sqrt{\frac{K_{\mathrm{a}_1}}{2\cdot K_{\mathrm{a}_2}}}\right)\right)) \tag{17}$$

Once the values of $\mathrm{p}K_{\mathrm{a}_1}$ and $\mathrm{pK}_{\mathrm{a}_2}$ are known, the parameter K_d may be calculated from a representation of log D against log f_1 for various pH values

$$\log D = \log K_\mathrm{d} + \log f_1 \tag{18}$$

This method is applicable only to those D–pH curves which exhibit a maximum, and inflexion points that can be accurately located depending on the quality of experimental data. In such cases, the coordinates of the inflexion points are useful parameters that can aid in the evaluation of the acidity constants and partition coefficient (distribution constant) of amphoteric substances.

This paper is inspired by previous work reported by Irving et al. (1955).

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References

Asuero, A.G., Jiménez, A.M., González, G.G. and Marqués, M.L., Spectrophotometric evaluation of acidity constants of two-step overlapping equilibria from the inflexion points of the absorbance versus pH graphs with application to the dipyridylglyoxal mono(2-pyridyl)hydrazone system. *Acta Pharm. Technol.*, (1988) in press.

Asuero, A.G, Jiménez-Trillo, J.L. and Navas, M.J., Mathematical treatment of absorbance versus pH graphs of polybasic acids. *Talanta*, 33 (1986) 929–934.

Asuero, A.G., Navas, M.J., Recamales, A.F. and Herrador, M.A., Numerical evaluation of overlapping acidity constants from the ratio of absorbances at two wavelengths. *Mikrochim. Acta* I (1986) 395–406 (Publ. 1987).

Bjerrum, J., *Metal Ammine Formation in Aqueous Solution (Theory of Reversible Step Reactions)*, R. Haase, Copenhagen, 1957.

Irving, H.M.N.H., Freiser, H. and West, T.S., *I.U.P.A.C., Compendium of Analytical Nomenclature*, Pergamon, Oxford, 1978.

Irving, H., Rossotti, H.S. and Harris, G., The determination of dissociation constants of dibasic acids. *Analyst*, 80 (1955) 83–94.

King, E., *Acid–Base Equilibria*, Pergamon Press, Oxford, 1965.

Leo, A., Hansch, C. and Elkins, D., Partition coefficients and their uses. *Chem. Rev.*, 71 (1971) 525–615.

Newton, D.W. and Kluza, R.B., pK_a values of medicinal compounds in pharmacy practice. *Drug. Intell. Clin. Pharmacol.*, 12 (1978) 546–554.

Van de Waterbeemd, H., The theoretical basis for relationships between drug transport and partition coefficients. *Pharmacochem. Libr.*, 6 (Quant. Approaches Drug Des.) (1983) 183–192.