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Rapid Communication

Evaluation of acidity constants for sparingly soluble compounds from fluorescence measurements

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Summary

A method to evaluate acidity constants in the ground state of sparingly soluble compounds based on fluorescence measurement is proposed. Mathematical relationships are obtained and presented in a form suitable for easy calculations. Depending on the availability of experimental points at the limiting zones of the fluorescence intensity vs pH graph, three possible cases are considered.

Knowledge of the dissociation constants of compounds is of vital interest both in the analysis of drugs and in the interpretation of their mechanisms of action (Newton and Kluza, 1978; Van Damme, 1978). However the dissociation constants in water of a wide range of pharmaceutically significant substances are either unknown or only very approximately known because of their insolubility in water. Potentiometric or spectrophotometric techniques for evaluating acidity constants are not generally applicable for compounds whose solubility in water is low or which have similar absorption spectra for the conjugate acid-base species (Asuero, 1988).

Fluorimetry is often considerably more sensitive than most analytical methods for pK_a evaluation in cases where the ionization process leads to a change in the fluorescence spectrum. Owing to its great sensitivity, the problem of solubility may be circumvented. On the other hand, fluorimetric procedures are probably more accurate than spectrophotometric techniques because of the generally better separation between fluorescence bands of acid and conjugate base in the spectrum.

Nevertheless, fluorimetry has been rarely employed as a tool in the evaluation of ground-state acidity constants. This state of affairs may result from the fact that the time scale for fluorescence events is comparable in many cases to that of proton transfer in the lowest excited singlet state, leading to a complex fluorimetric pH titration behaviour because the kinetics of excited-state proton transfer as well as the distribution of acid and conjugate base in the ground electronic state are reflected in the dependence of the fluorescence vs pH graph. However, for single protolytic equilibria involving monofunctional aromatic com-

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pounds, all these drawbacks may be conveniently circumvented and therefore, accurate pK_a values could be routinely evaluated fluorimetrically.

Basic relationships: The dissociation equilibrium of a monobasic acid, HR = H + R, is characterized by the apparent acidity constant (at a fixed ionic strength):

$$K_a = [\mathrm{H}][\mathrm{R}]/[\mathrm{HR}]$$

Charges are omitted for simplicity. The fluorescence intensity F at a given pH of a solution containing a low total concentration $C_R = [\mathrm{HR}] + [\mathrm{R}]$, is given by (Rosemberg et al., 1979a)

$$F = 2.303 I_0 l \big(\theta_{HR} \epsilon_{HR} [\mathrm{HR}] + \theta_R \epsilon_{HR} [\mathrm{HR}] + \theta_R^0 \epsilon_R [\mathrm{R}] \big) \qquad (1)$$

where I_0 and l are the intensity of light at the analytical excitation wavelength and the optical depth of the sample, respectively; ϵ_{HR} and ϵ_R are the molar absorptivities of acid HR and conjugate base R, respectively, at the analytical excitation wavelength; [HR] and [R] are the respective equilibrium ground-state concentrations of acid and conjugate base at the pH of the experiment; and θ_{HR}, θ_R and θ_R^0 are, respectively, the quantum yields of fluorescence of HR and R in the occurrence of proton exchange in the lowest excited singlet state and of R in the absence of proton exchange in the lowest excited singlet state.

Defining F_{HR}^0 and F_R^0 as the fluorescence intensities obtained when the test solution is either the pure acid ($C_R = [\mathrm{HR}]$) or the pure conjugate base ($C_R = [\mathrm{R}]$)

$$F_{HR}^0 = 2.303 I_0 l \theta_{HR}^0 \epsilon_{HR} C_R \qquad (2)$$

$$F_R^0 = 2.303 I_0 l \theta_R^0 \epsilon_R C_R \qquad (3)$$

By combining Eqns. 1–3 and rearranging we obtain

$$F = \big(F_{HR}^0 (\theta_{HR}/\theta_{HR}^0) + F_R^0 (\theta_R/\theta_R^0)(\epsilon_{HR}/\epsilon_R) \big) \times f_{HR} + F_R^0 f_R \qquad (4)$$

where f_{HR} and f_R are the molarity fractions defined by

$$f_{HR} = [\mathrm{H}]/([\mathrm{H}] + K_a)$$

and

$$f_R = K_a/([\mathrm{H}] + K_a)$$

Weller (1961) showed, by means of the steady-state kinetics, that if excited-state proton transfer occurs (HR* = R* + H, the asterisk referring to the excited species), then

$$\theta_{HR}/\theta_{HR}^0 = (1 + k_R \tau_R [\mathrm{H}]) / (1 + k_{HR} \tau_{HR} + k_R \tau_R [\mathrm{H}]) \qquad (5)$$

$$\theta_R/\theta_R^0 = k_{HR} \tau_{HR} / (1 + k_{HR} \tau_{HR} + k_R \tau_R [\mathrm{H}]) \qquad (6)$$

where k_{HR} and k_R are the rate constants for dissociation of excited acid and protonation of excited conjugate base, respectively, and τ_{HR} and τ_R are the respective lifetimes of the lowest excited singlet states of the acid and conjugate base in the absence of proton exchange.

By differentiating eqn. 4 twice with respect to pH we obtain

$$\begin{aligned} \mathrm{d}^2F/\mathrm{dpH}^2 &= (2.303)^2 \Bigg[\big(F_R^0 (\epsilon_{HR}/\epsilon_R) - F_{HR}^0 \big) \\ &\quad \times \frac{k_R \tau_R k_{HR} \tau_{HR} [\mathrm{H}] (1 + k_{HR} \tau_{HR} - k_R \tau_R [\mathrm{H}])}{(1 + k_{HR} \tau_{HR} + K_R \tau_R [\mathrm{H}])^3} \\ &\quad \times f_{HR} + f_{HR} f_R (f_{HR} - f_R) \\ &\quad \times \big(F_R^0 - F_{HR}^0 (\theta_{HR}/\theta_{HR}^0) \big) \\ &\quad + F_R^0 (\theta_R/\theta_R^0)(\epsilon_{HR}/\epsilon_R) \Bigg] \end{aligned} \qquad (7)$$

and the condition $\mathrm{d}^2F/\mathrm{d\ pH}^2 = 0$ will locate the points of inflexion in the graph of F vs pH. Taking into account (Rosemberg et al., 1979a) that the maximum possible values of $k_{HR}\tau_{HR}$ and $k_R\tau_R$ are around 1000 and 1000 M^{-1}, respectively, usually, at pH greater than 4, $k_R\tau_R[H] \ll 1$ and therefore, $\theta_{HR}/\theta^0_{HR}$ and θ_R/θ^0_R become independent of pH. If $pK_a \gg 4$, then the second derivative within the interval $pK_a \pm 2$ is substantially

$$\begin{aligned} d^2F/d\,pH^2 &= (2.303)^2\big[f_{HR}f_R(f_{HR}-f_R) \\ &\quad \times\big(F^0_R - F^0_{HR}(\theta_{HR}/\theta^0_{HR}) \\ &\quad + F^0_R(\theta_R/\theta^0_R)(\epsilon_{HR}/\epsilon_R)\big)\big] \end{aligned} \tag{8}$$

So, at the inflexion point $f_{HR} = f_R = 0.5$ and pH $= pK_a$. Accordingly, the pK_a in the ground state may be evaluated from the F vs pH graph.

Method of calculation of p*K*a: Combination of Eqn. 4 and the definitions of molarity fractions and subsequent rearrangent lead to the expression

$$\begin{aligned} K_a = \big(F &- F^0_{HR}(\theta_{HR}/\theta^0_{HR}) \\ &- F^0_R(\theta_R/\theta^0_R)(\epsilon_{HR}/\epsilon_R)\big)[H]/(F^0_R - F) \end{aligned} \tag{9}$$

Recalling that if pH > 4:

$$F^0_{HR}(\theta_{HR}/\theta^0_{HR}) = \text{constant} = c_1$$

$$F^0_R(\theta_R/\theta^0_R)(\epsilon_{HR}/\epsilon_R) = \text{constant} = c_2$$

and taking $A = c_1 + c_2$ and $B = F^0_R$, we have

$$K_a = [H](F - A)/(B - F) \tag{10}$$

It is a simple matter to determine A (c_1 or c_2) as well as B at the analytical emission wavelength by inspection of the fluorimetric titration curve (Rosemberg et al., 1979b). However, taking into consideration that one can obtain an F vs pH curve without the necessary points at the limiting zones which gives A and B, we can develop three typical cases very similar to the classic ones studied in the spectrophotometric evaluation of ionization constants (Navas et al., 1985; Asuero et al., 1986).

Case I: A and B known: This is the simplest case. By taking decadic logarithms in Eqn. 10 we can write

$$pK_a = pH - \log(F - A)/(B - F) \tag{11}$$

Thus, at each experimental point F_i, pH_i, we obtain a value for pK_{a_i}. The average of all values can be a good estimation of pK_a if the dispersions are not too large. This procedure may be regarded as a variation of the method proposed by Albert and Serjeant (1962) in potentiometry.

Case II: either A or B is unknown: Eqn. 10 may be arranged in several ways giving typical relationships that permit the estimation of K_a (and the unknown parameter) by means of linear regression techniques. Among all the diverse possibilities we can write, for instance when A is unknown:

$$F = A + K_a(B - F)/[H] \tag{12}$$

when B is unknown:

$$F = B + 1/K_a[H](F - A) \tag{13}$$

In these cases, the ionization constant may be directly evaluated from the slope of the straight line. From another rearrangement the ionization constant can be dependent on both slope and intercept.

Case III: both A and B are unknown: In this case, Eqn. 10 can be rearranged to give

$$F[H]/K_a - A[H]/K_a - B = -F \tag{14}$$

if we put $x = 1/K_a$, $y = -A/K_a$ and $z = -B$, we can obtain from each of the three points i = a, b, c a system of linear equations

$$F_a[H]_a x + [H]_a y + z = -F_a$$

$$F_b[H]_b x + [H]_b y + z = -F_b$$

$$F_c[H]_C x + [H]_c y + z = -F_c$$

whose solution yields the values of A, B and K_a.

Another possibility is to assume simply a guess value for B (or A) and according to Eqn. 12 (or

Eqn. 13), to apply the regression technique. Because the model equation depends on the quantity $B - F$ (or $F - A$), any error in B (or in A) becomes more serious as the values of [H] become lower (or higher in the respective case). This will lead to curvature in the plot (Ramette, 1967). Only the value of B (or A) which is correct will yield a plot which is straight. The common procedure consists of the minimization of the variance of regression according to the following: for each trial value of B (or A) the intercept of the plot, A (or B), is calculated using least squares, and then the variance of regression is obtained for each point. The best value of B (or A) is defined as that which gives the lowest variance of regression. Once it is true, the slope and intercept of the plot correspond to the true values of K_a (or $1/K_a$) and of A (or B).

Experimental procedure: In order to evaluate the ionization constant of a sparingly water soluble compound (it is assumed that the compound is fluorescent), the corresponding stock solutions are prepared allowing 50 ml of distilled water (pH about 6.8) to stand overnight in contact with the solid compound. Nitrogen should be bubbled continuously through the solution to avoid oxidation. Each saturated solution is conveniently diluted to make stock solutions (note that, although the concentration C_R is not known, the pK_a can be calculated from the data F vs pH according to the proposed technique).

Each fluorimetric titration consists of delivering 1 ml of stock solution to a 10 ml volumetric flsk and diluting to the mark with dilute $HClO_4$ or NaOH. The fluorescence spectra of the solution are then recorded exciting at an isobsestic point in the pH-dependent absorption of the compound (in this case $\epsilon_{HR} = \epsilon_R$) or at a point on the red edge of the longest wavelength absorption band of the R species (where absorption by the HR species is nil) (Rosemberg et al., 1979a).

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