Chapter 3 Theoretical

The pK_a determination method described here aims to find pK_a values that enable accurate fluorescein absorbance measurement interpretation. The three-pK_a fluorescein model was used in this investigation (Model A of Klonis and Sawyer, 1996) and this approach is expected to yield improvements only in cases where a single pK_a value, or incorrect pK_a values have been used.

3.1   Assumptions

The choice of formulas used to curve-fit the data and calculate the final values, was based on a number of assumptions. While these assumptions make it easier to solve for precise pK_a values, they also limit the pK_a value applicability to situations requiring similar or lower levels of accuracy. These assumptions are:

1. Fluorescein behaves according to the laws of equilibrium chemistry.

2. Although dissolved fluorescein has at least six different ionic forms, the lactone, zwitterion and quinonoid forms can be grouped and treated as a single neutral species. This leaves a total of four different species: cation, neutral, monoanion and dianion.

3. The different species have characteristic absorptivities and obey Beer's Law within the test concentration range.

4. The presence of a small amount of fluorescein has a negligible effect on the pH of a buffered solution.

5. The sodium atoms of disodium fluorescein dissociate upon dissolution, leaving a fluorescein molecule indistinguishable from the dissolved form of the free acid at the same pH.

6. Activity effects alter the interactions between the different dissolved species.

7. Activity effects do not affect the interactions between the dissolved species and light.

8. At the ionic strength of the test buffer, the activity coefficient of an uncharged species is equal to one.

9. The absorptivity factor temperature response is analogous to the fluorescence intensity temperature response.

3.2 Equilibrium equations and species formulas

Standard equilibrium expressions are used to develop a concentration formula for each ionic form. These different ionic species are a cation, neutral species, monovalent anion, and divalent anion (Klonis and Sawyer, 1996). Fluorescein is described in the acid form because the titration starts at a low and ends at a high pH.

The ionic reactions of fluorescein are listed below, with "Flu" denoting the deprotonated fluorescein molecule:

$$H_3Flu^+ \leftrightarrow H^+ + H_2Flu \qquad\qquad (1)$$

$$H_2Flu \leftrightarrow H^+ + HFlu^- \qquad\qquad (2)$$

$$HFlu^- \leftrightarrow H^+ + Flu^{2-} \qquad\qquad (3)$$

Relative equilibrium concentrations are denoted by the ionisation constant K_a, with the numerical subscript indicating the ionic reaction and the square brackets denote molar concentrations (Equations 4, 5 and 6). As the use of molar concentrations is only appropriate for dilute solutions, activity corrections will be developed later in Section 3.4.

$$K_{a1} = [H^+][H_2Flu]/[H_3Flu^+] \qquad\qquad (4)$$

$$K_{a2} = [H^+][HFlu^-]/[H_2Flu] \qquad\qquad (5)$$

$$K_{a3} = [H^+][Flu^{2-}]/[HFlu^-] \qquad\qquad (6)$$

A fluorescein mass balance gives:

$$[Flu_{Total}] = [H_3Flu^+] + [H_2Flu] + [HFlu^-] + [Flu^{2-}] \qquad\qquad (7)$$

Rearrangement of Equations 4, 5 and 6 followed by their substitution into Equation 7 produces four Equations. These are:

$$[H_3Flu^+] = [Flu_{Total}]/(1 + K_{a1}/[H^+] + (K_{a1}K_{a2})/[H^+]^2 + K_{a1}K_{a2}K_{a3}/[H^+]^3) \qquad (8)$$

$$[H_2Flu] = [Flu_{Total}]/([H^+]/K_{a1} + 1 + K_{a2}/[H^+] + K_{a2}K_{a3}/[H^+]^2) \qquad (9)$$

$$[HFlu^-] = [Flu_{Total}]/([H^+]^2/(K_{a1}K_{a2}) + [H^+]/K_{a2} + 1 + K_{a3}/[H^+]) \qquad (10)$$

$$[HFlu^{2-}] = [Flu_{Total}]/([H^+]^3/(K_{a1}K_{a2}K_{a3}) + [H^+]^2/(K_{a2}K_{a3}) + [H^+]/K_{a3} + 1) \qquad (11)$$

These last four Equations, i.e. 8 to 11, allow the precise concentration of each species to be calculated as long as the total fluorescein concentration, pH and individual K_as are known.

3.3 Absorbance formulas

The third assumption listed in Section 3.1 is fundamental to quantitative absorbance spectrophotometry (Braude *et al.*, 1950) and is that each species has its own characteristic

influence on the absorbance at a particular wavelength, and that the absorbance and fluorescein concentration relationship follows the Beer-Lambert law:

$$\text{Absorbance} = \varepsilon b c \tag{12}$$

Where ε is the molar absorptivity constant at that wavelength, b is the path length of the light beam through the sample and c is the fluorescein species concentration. As the same analytical instrument and wavelength is used throughout this study the molar absorptivity constant is combined with the instrument path length to produce a factor for each of the four ionic species (F_1, F_2, F_3 and F_4). The total absorbance is the sum of the absorbencies of the different fluorescein species, such that:

$$\text{Total absorbance} = F_1[H_3Flu^+] + F_2[H_2Flu] + F_3[HFlu^-] + F_4[Flu^{2-}] \tag{13}$$

Or where Eq.# symbolises the right-hand side of Equation #…

$$\text{Total absorbance} = F_1(Eq.8) + F_2(Eq.9) + F_3(Eq.10) + F_4(Eq.11) \tag{14}$$

If the total fluorescein, the pH and total absorbance are known there will be seven unknowns: K_{a1}, K_{a2}, K_{a3}, F_1, F_2, F_3 and F_4. During the solving process these seven values are adjusted until the calculated total absorbance matches the measured total absorbance.

3.4   Activity corrections

In this study the activity corrections are based on an approximation in which the "mean ionic diameter" parameter of the extended Debye-Hückel formula is set to 3.0Å (Guggenheim and Schindler, 1934). This has the effect of eliminating two of the terms in the extended Debye-Hückel formula and simplifies its application. This approximation is justified here because the ions used in this investigation have ion sizes ranging from 3 to 4.5 Å (Kielland, 1937). Also the Guggenheim and Schindler (1934) approximation is useful for mixtures of electrolytes up to ionic strengths of about 0.1 (Guggenheim, 1935) and the buffers in this investigation are designed to yield ionic strengths lower than 0.1 throughout the titration.

The Guggenheim and Schindler (1934) approximation describes the relationship between the activity coefficient (γ) of a particular ion, and the solution ionic strength (μ) as:

$$\log \gamma = -0.5Z^2 \frac{\sqrt{\mu}}{1+\sqrt{\mu}} \tag{15}$$

The ionic strength being:

$$\mu = 0.5 \sum_i C_i Z_i^2 \tag{16}$$

Where C is the concentration and Z the charge of a particular species in a solution containing i different species. The 0.5 value used in Equation 15 is the value suggested by Guggenheim and Schindler (1934) but it is also coincidentally close to the correct average value i.e. 0.502 (Manov et al., 1943), for this investigation's test temperatures.

The correct name of Equation 15 is not clear because although Guggenheim and Schindler (1934) did suggest this approximation and Kielland (1937) and Scatchard (1936) attribute it to Guggenheim and Schindler (1934), Sawyer, McCarty and Parkin (1994), Snoeyink and Jenkins (1980), and Stumm and Morgan (1970) refer to the same formula as the Güntelberg approximation. As the last three publications provide no reference it was not possible to confirm this, but Güntelberg (page 58, 1938) also appears to attribute the approximation to Guggenheim (1935). The situation is further complicated in that Bjerrum (page 55, 1926) credits Güntelberg with an important but different simplification in electrolyte thermodynamics.

Once the ionic strength of the solution is known the activity correction is made by multiplying the species concentration by its activity coefficient. Thus for monoionic species such as protons, the activity coefficient formula simplifies to:

$$\gamma_1 = 10^{\left(-0.5\frac{\sqrt{\mu}}{(1+\sqrt{\mu})}\right)} \tag{17}$$

And the activity coefficient formula for a diionic species is:

$$\gamma_2 = 10^{\left(-2\frac{\sqrt{\mu}}{(1+\sqrt{\mu})}\right)} \tag{18}$$

As pH meters measure the proton *activity*, pH readings are already activity corrected. This means that the *mixed* K_a' found after using the proton activity in combination with uncorrected molarity data must be activity corrected (and standardised to 25°C) to find the thermodynamic K_a. Equations 4, 5 and 6 can be expanded to include this activity correction.

$$K_{a1} = \{H^+\}[H_2Flu]/(\gamma_1[H_3Flu^+]) = \{H^+\}[H_2Flu]/[H_3Flu^+] \times 1/\gamma_1 = K_{a1}' \times 1/\gamma_1 \tag{19}$$

$$K_{a2} = \{H^+\}(\gamma_1[HFlu^-])/[H_2Flu] = \{H^+\}[HFlu^-]/[H_2Flu] \times \gamma_1 = K_{a2}' \times \gamma_1 \tag{20}$$

$$K_{a3} = \{H^+\}(\gamma_2[Flu^{2-}])/(\gamma_1[HFlu^-]) = \{H^+\}[Flu^{2-}]/[HFlu^-] \times \gamma_2/\gamma_1 = K_{a3}' \times \gamma_2/\gamma_1 \tag{21}$$

Where the curved brackets denote activity, the square brackets denote molarity, and the activity of uncharged species (e.g. H_2Flu) is unity. These last three Equations, i.e. 19 to 21, are used to activity correct the *mixed* ionisation constant and this value must then be standardised to 25°C to find the thermodynamic pK_a.

3.5   pK$_a$ Temperature corrections

The K$_a$ of solutes are normally standardised to 25°C and can be temperature sensitive. Equation 22 was used to compensate for changes in ionisation constant due to temperature changes. This is the integrated form of the Gibbs-Helmholtz equation where the Gibbs free energy equation is $\Delta G° = -RT \ln(K)$, and the enthalpy is assumed constant (Sawyer *et al.*, 1994).

$$\ln\left(\frac{K_{aT2}}{K_{aT1}}\right) = \frac{-\Delta H^0}{R}\left(\frac{T_1-T_2}{T_1T_2}\right) \tag{22}$$

Equation 22 was rearranged to give:

$$K_{aT2} = K_{aT1}e^{\left(\frac{-\Delta H^0}{R}\left(\frac{T_1-T_2}{T_1T_2}\right)\right)} \tag{23}$$

Where H^0 is the standard enthalpy of reaction, R is the gas constant, K$_{aT1}$ is the thermodynamic ionisation constant at 25°C (T$_1$ = 298.15K), and K$_{aT2}$ is the ionisation constant at the experimental temperature T$_2$. Equation 23 predictions were checked for this investigation's temperature range and were accurate for water, acetate, and the second ionisation constant of phosphate giving ionisation constants within 0.8% of the Lange's Handbook (1992) data, but Equation 23 underestimated the first ionisation constant of phosphate by 5.0% at 5°C.

Equation 23 was used throughout the pK$_a$ determination process to predict buffer composition and pH changes with temperature. While this formula is recognised to be an approximation (Sawyer *et al.*, 1994) it does offer a degree of temperature compensation where greater accuracy may not be warranted.

The standard enthalpy of reaction for each buffer and salt (apart from sodium acetate and fluorescein), was calculated using the CODATA (Cox, Wagman and Medvedev, 1989) values as quoted in the CRC Handbook (1992). The standard enthalpy of reaction for acetate was calculated by substituting ionisation data of acetate at various temperatures (CRC Handbook of Chemistry and Physics, 1992) into Equation 24, which is a further rearrangement of Equation 22. Equation 24 was also used to determine the standard enthalpy of reaction for fluorescein but used the ionisation data produced by this investigation.

$$\Delta H^0 = -R\left(\frac{T_1T_2}{T_1-T_2}\right)\ln\left(\frac{K_{aT2}}{K_{aT1}}\right) \tag{24}$$

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3.6 Absorptivity temperature corrections

Molar absorptivity values are temperature standardised in this investigation by using an analogous formula to that used to compensate for fluorescence intensity (Feuerstein and Selleck, 1963).

$$F_t = F_s e^{n(t-t_s)} \tag{25}$$

Where F_t is the absorptivity factor at the test temperature, F_s is the absorptivity factor at the standard temperature of 0°C, t_s is the standard temperature, t is the measurement temperature and n is the temperature coefficient. The fluorescein fluorescence intensity temperature coefficient of $-0.0036°C^{-1}$ reported by Feuerstein and Selleck (1963) must be recalculated in this investigation to determine the equivalent absorbance temperature coefficient.

When absorbance factor data are collected at two different temperatures then the temperature coefficient can be calculated because the standard absorbance factor is a constant and the t_s term can be eliminated. Equation 25 can then be rewritten as:

$$F_s = \frac{F_{t1}}{e^{nt_1}} = \frac{F_{t2}}{e^{nt_2}} \tag{26}$$

Where F_{t1} and F_{t2} are the absorbance factors at temperatures t_1 and t_2. Solving for n gives:

$$n = \frac{\ln(\frac{F_{t2}}{F_{t1}})}{(t_2 - t_1)} \tag{27}$$

Once the temperature coefficient is known for each ionic species, standardised absorptivity factors can be calculated by substitution into the absorptivity equivalent of Equation 25.