Determination of dissociation constant of indicators spectrophotometrically

Introduction

Indicator molecules are often applied for marking the endpoint of acid-base titrations (beside instrumental indications). In this case during titrations, the color changes of the indicators indicate the equivalence points were obtained. The color indicators used in acid-base titrations operation is based on the protonation and deprotonation of the indicator molecule. The accuracy of the titration is highly influenced by the correct choice of the indicator. In this case we must know the valid pK_a value of the indicator in the given medium (permittivity, ionic strength).

The indicators are two types of: the first, which includes dyes, having only one color form, while the other form is colorless (e.g. the protonated form of phenolphthalein). The other group includes those, which change the color by pH change.
The pK_a value can be calculated with applying the law of mass action, if the concentrations of the dye's protonated and deprotonated forms are determined beside known H^+ activity at the same time:

$$K_a = \frac{[F^-][H^+]}{[FH]} \tag{1}$$

where FH is the protonated, F^- is the deprotonated form of an indicator. The indicator to be tested belongs in the first group and the deprotonated form is colored. According to the Beer-Lambert law, the absorbance of the indicator at a given wavelength is:

$$A = \varepsilon_\lambda c_F l \tag{2}$$

can be written, where ε_λ is the decadic molar extinction coefficient at the given wavelength, c_F is the concentration of basic form of the dye and, l is the thickness of the solution layer. In practice (for a better signal-to-noise ratio) we proceed, as recording absorption spectra in dilute solution of the basic form, we determine the wavelength at which the dye solution's absorbance is the highest and continue working in this wavelength.
Expressing c_F from equation (2) the amount of protonated dye is

$$c_{FH} = c_T - c_F$$

where c_T is the total concentration of the dye. Substituting the resulting concentrations into equation (1) K_a can be calculated. Usually we do not have the molar decadic absorption coefficient for all wavelengths, so a separate measurement is needed to determine it. Considering equation (2) for this purpose solutions should be made in which the total concentration of dye is different, but in each solution completely deprotonated form is present, ie, $c_F = c_T$. The absorbance of latter solutions is compared with the absorbance of solvent measured in a 1 cm cuvette, and represented a function of the concentration a straight line passing through origin is obtained, whose slope is ε_λ.

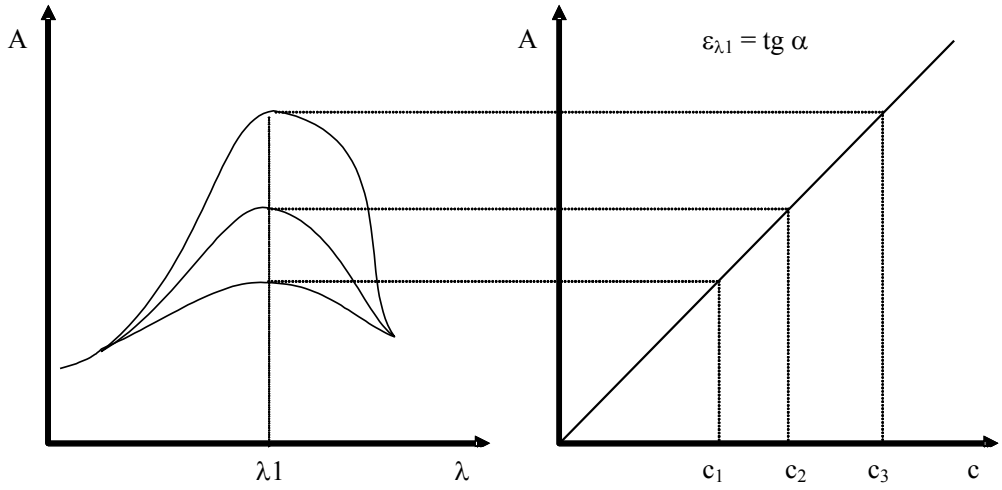

Fig.1 Determination of indicator extinction coefficient
left: schematics of the absorption spectra; right: calibrating plot for the determination of ε_λ

The observed spectrum will show two maximum values (at least) when the protonated and deprotonated forms of the indicator have different colors, and the spectrum is taken in a buffer solution with a pH close to the neutralization pH of the indicator. The absorption band corresponding to FH can have significant absorption also in the region of the absorptions band of the other form (F^-) and vice versa. Accordingly, at any wavelengths, the absorption of the solution can be expressed by the sum of the absorbing species:

$$A_\lambda = (\varepsilon_{FH,\lambda}\, c_{FH} + \varepsilon_{F,\lambda}\, c_F)l \qquad (3)$$

Using the $c_T = c_{FH} + c_F$ equality

$$and\ ^{thus} \qquad c_F = c_T - c_{FH}$$

can be written into equation (3). , after simplification, we gain the following relations:

$$\frac{K_a}{[H^+]} = \frac{c_T e_{FH,\lambda} - A_\lambda / l}{A_\lambda / l - c_T e_{F,\lambda}} \qquad (4)$$

By repeating the measurement with several buffers of different pHs, and by plotting the right side of the expression (4) as a function of $1/[H^+]$, a straight line is observed whose slope is K_a. For the calculation the molar absorption coefficients are needed (at the measuring wavelengths) of both the protonated and the deprotonated forms. Two series of solution of different dye concentrations are prepared. In one series, the dye is completely protonated (acidic solution), in the other series is completely deprotonated (alkaline solution). We measure the absorption of acidic solutions at the previously selected measuring wavelength, plot it a function of the concentration and define ε_{FH}, λ_1 value from its slope. Then the alkaline series are measured on the same wavelength and is calculated ε_F, λ_1 like above.

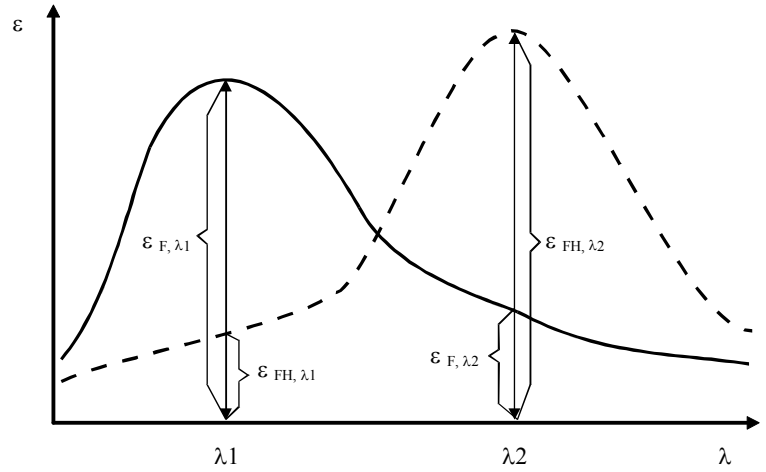

Fig. 2 Wavelenght-dependence of the absorption of an indicator having two absorption bands (solid and dashed lines correspond to the acidic and basic absorption bands of the indicator, respectively)

In case that the total concentration of dye is not known (if you do not know the indicator's the structural formula, molecular weight, etc.), by considering equation (4) it is impossible to calculate the requested dissociation constant. However, it is possible to calculate the ratio of the two forms (protonated and deprotonated), as follows: once we are measuring the absorption at two different wavelengths, we can apply equation (3) two times:

$$A_{\lambda 1} = (\varepsilon_{FH,\lambda 1}\, c_{FH} + \varepsilon_{F,\lambda 1}\, c_F)l \qquad (5.a)$$

$$A_{\lambda 2} = (\varepsilon_{FH,\lambda 2}\, c_{FH} + \varepsilon_{F,\lambda 2}\, c_F)l \qquad (5.b)$$

From these two equations we can calculate the value of C_{FH} and C_F, by knowing the valid molar extinction coefficients of the two forms at the two wavelengths.
Prepare once again two different series of solutions with different indicator concentrations (e.g., by dilution of the stock solution). One of the series acidic, so the dye is protonated, the other an alkaline state including the dye is deprotonated. Measure the absorption spectra of an acidic and an alkaline solution against the solvent. From the two curves select the wavelength of the maximum absorbance for both the acidic and the basic form, and then measure the absorption of all solutions at these two wavelengths. The concentration plotting against the absorbance values give a straight through zero granting the requested decadic molar extinction coefficients, if the measurements were performed in 1 cm cuvettes.

Practice descriptions

Prepare 11 pieces, numbered 2-12 test tubes, add in 3-3 cm^3 of numbered the same pH Britton-Robinson buffer. From the indicator stock solution of known concentration provided by the exercise commander add 1-1 drops of the indicator with an eyedropper into the test tubes. looking From the top, determine the pH range of the equivalence point, select from the attached table the best three match and prepare 50-50 cm^3 of these buffer solutions. Pipette 1-1 cm^3 of the dye stock solution each of the three, 25 cm^3 volumetric flask (marked 1, 2, 3), then fill them up to the mark with the individual buffers. Verify the pH of the residue of buffers with a pH meter certified at two check points.
For the determination of absorption coefficient, pipette 0.4, 0.8, 1.2, 1.6 cm^3 dye solutions into another four 25 cm^3 volumetric flask and fill up to the mark with 0.01 M HCl. Load the middle

solution into a cuvette and record the spectra of 360-620 nm region beside water as comparing liquid. Prepare the deprotonated dye containing sequence, then pipette 0.4, 0.8, 1.2, 1.6 cm^3 of dye solution, but instead of hydrochloric acid use 0.01 M NaOH. If we do not have the analytical concentration of dye stock solution, record the absorption spectrum of the basic form at the given wavelength range. Select the maximum absorption spectrum of the acid form. Measure the other solutions absorption at this wavelength. If the stock solution concentration is not known, carry out the measurements with the basic form at the absorption maximum, and the former model determine ε_{FH}, λ_2 and ε_F, λ_2 values. Than measure at the selected wavelength (or wavelengths) the absorption of solutions marked 1, 2, 3.

The measurement of results

Plot the absorbance of acidic and basic form in function with the concentration and determine ε_{FH}, λ_1 and ε_F, λ_1 values.
As the pH of solutions marked 1, 2, 3 have been determined, in case of a known concentration of dye solution using the equation (4) the right expression can be calculated, and plot in function with 1/H. Determination of the value of K_a from the obtained straight.
In case of an unknown concentration of dye solution, the (5a) - (5b) two-unknown equations must be solved to calculate the pH of the dye steady-state volume of protonated and deprotonated conditions. Then make quotients c_F/c_{FH} belonging to different pH, and then plot a function of 1/H. The slope of the straight is K_a, dissociation constant.

Give the results as the following:

Name of the Dye		equivalence range	
Acidic form of maximum absorption (λ_1):		Basic Form absorption maximum (λ_2):	
ε_{FH}, λ_1 =	ε_{FH}, λ_2 =	ε_F, λ_1 =	ε_F, λ_2 =

Measurements for the determination of the molecular decadic adsorption coefficients

Acetic form	c(M)	A(λ1)	A(λ2)
1.			
2.			
3.			
4.			
Basic form	c(M)	A(λ1)	A(λ2)
1.			
2.			
3.			
4.			

Measurements and calculations for the determination of the dissociation product constants

#	pH	$[H^+]$	A(λ1)	A(λ2)	c_{FH}	c_F
1.						
2.						
3.						

Preparation of Britton-Robinson buffer
The buffers are prepared by adding to 100 cm^3 acidic component (A), an amount of alkaline component (B) should be given in the table. The component A is provided by the tech. Component B: 8.00 g NaOH was dissolved in 1 dm^3

Britton-Robinson buffer solutions composition

pH	**B (cm^3)**	**pH**	**B (cm^3)**	**pH**	**B (cm^3)**	**pH**	**B (cm^3)**
1.81	0.0	4.10	25.0	6.80	50.0	9.62	75.0
1.89	2.5	4.35	27.5	7.00	52.5	9.91	77.5
1.98	5.0	4.56	30.0	7.24	55.0	10.38	80.0
2.09	7.5	4.78	32.5	7.54	57.5	10.88	82.5
2.21	10.0	5.02	35.0	7.96	60.0	11.20	85.0
2.36	12.5	5.33	37.5	8.36	62.5	11.40	87.5
2.56	15.0	5.72	40.0	8.69	65.0	11.58	90.0
2.87	17.5	6.09	42.5	8.95	67.5	11.70	92.5
3.29	20.0	6.37	45.0	9.15	70.0	11.82	95.0
3.78	22.5	6.59	47.5	9.37	72.5	11.92	97.5