From the plot, the vol. ratio of Fe (III) and S.A. at maximum absorbance = ···:···

∴ empirical formula of the complex is ···

Original concn. of Fe (III) in this solution ··· (M)

Table 6: Preparation of solutions for determination of stability constant and O.D. readings at λ_{max} = ... nm

Test tube no.	Vol. of 0.002 M Fe^{3+} soln. (ml)	Vol. of 0.002M S.A. soln. (ml)	Vol. of 0.002 M HCl soln. (ml)	Total vol. (ml)	Conc. of Fe^{3+} (M)	O.D.
I	1	5	4	10	···	...
II	2	5	3	10	···	...
III	3	5	2	10	···	...
IV	4	5	1	10	···	...
V	5	5	0	10	···	...

The plot of O.D. vs. conc. of Fe (III) is shown in Fig. 5.6.

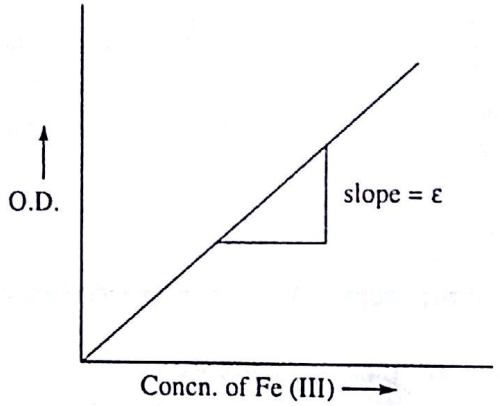

Fig. 5.6

Experiment 3

Determination of—(a) Isobestic point of bromocresol green indicator, and (b) Indicator constant of the indicator.

Theory: An acid-base indicator is a weak acid or base which exhibits two distinct colours in dissociated and undissociated forms of the molecule.

If the indicator be represented as HIn, then its ionisation in aqueous solution may be shown as

$$HIn(aq) \rightleftharpoons H^+(aq) + In^-(aq) \quad (1)$$

In strong alkaline solution, the colour is due to $In^-(aq)$ form and in strong acid medium, the colour is due to the undissociated form of $HIn(aq)$.

When two different spectra of the indicator in strong alkali and in strong acid medium is studied using long range of filters (in visible region) and the O.D.s are plotted against wavelengths, the two curves will cut at a point. This point is called as the **isobestic point** of the indicator.

Hence isobestic point of an indicator is defined as a wavelength at which the absorbance of the two forms (dissociated and undissociated) is equal. The graph will appear as shown in Fig. 5.7.

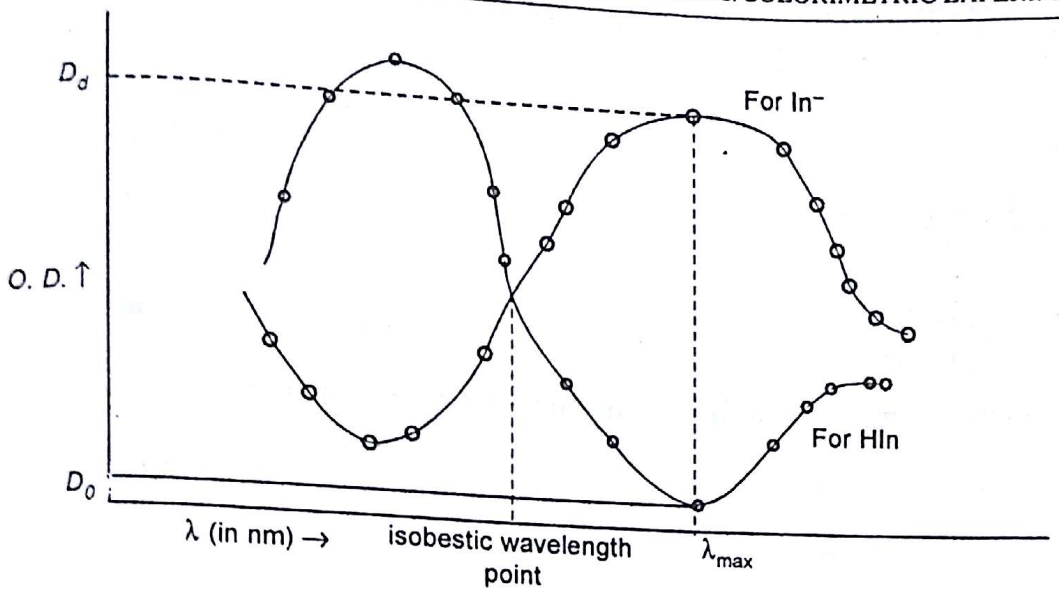

Fig. 5.7

From equation (1), the indicator constant, K_{In} may be written as

$$K_{In} = \frac{[H^+][In^-]}{[HIn]} \tag{2}$$

Taking logarithm on both sides

$$\log K_{In} = \log[H^+] + \log\frac{[In^-]}{[HIn]}$$

or $$-\log[H^+] = -\log K_{In} + \log\frac{[In^-]}{[HIn]}$$

or, $$pH = pK_{In} + \log\frac{[In^-]}{[HIn]} \tag{3}$$

or, $$pH = pK_{In} + \log\frac{[\text{basic form}]}{[\text{acidic form}]} \tag{4}$$

Let us consider ε_d = molar extinction coefficient of the species In^-,
and ε_u = molar extinction coefficient of the species HIn,
α = degree of dissociation of the indicator in the basic In^- form, and
$(1-\alpha)$ = amount of undissociated HIn.

Then according to Lambert-Beer's law, the optical density (D) of the indicator, when both In^- and HIn forms are present, is given by

$$D = \varepsilon_d cl\alpha + \varepsilon_u(1-\alpha)cl \tag{5}$$

when $\alpha = 0, (1-\alpha) = 1$, then

$$D_u = \varepsilon_u cl$$

And when $\alpha = 1, D_d = \varepsilon_d cl$

Thus, $$\varepsilon_u = \frac{D_u}{cl} \text{ and } \varepsilon_d = \frac{D_d}{cl} \tag{6}$$

Substituting (6) in (5),

$$D = \alpha D_d + (1-\alpha) D_u$$

or, $$\alpha = \frac{D - D_u}{D_d - D_u}$$

or, $$\frac{\alpha}{1-\alpha} = \frac{D - D_u}{D_d - D}. \quad (7)$$

Considering the degree of dissociation, equation (4) may be written as

$$\text{pH} = pK_{In} + \log \frac{\alpha}{1-\alpha}$$

or, $$\boxed{\text{pH} = pK_{In} + \log \frac{D - D_u}{D_d - D}}$$ from equation (7)] (8)

So, if we plot $\log \frac{D - D_u}{D_d - D}$ vs. pH, a straight line with positive slope, having intercept, pK_{In}, will be obtained. Thus K_{In} can be calculated from the intercept.

Equipments and materials: Spectrophotometer, ten clean test tubes (20 ml), 100 ml beakers, conical flasks, burette, graduated pipette (10 ml), bromocresol green, oxalic acid, NaOH, HCl, CH_3COOH.

Procedure

1. Prepare 50 ml 0.4N standard oxalic acid solution. Weight required = 1.26 g.
2. Prepare about 150 ml ~ 0.5N acetic acid solution. Dissolve 4.5 ml acetic acid from original bottle (~17N) in water (~ 145 ml).
3. Prepare about 200 ml ~ 0.5 N NaOH solution. Dissolve ~4.0 g NaOH in 200 ml water.
4. Prepare 100 ml ~0.1 M HCl solution. Dissolve ~ 1 ml conc. HCl in 100 ml water.
5. Standardise NaOH solution by oxalic acid solution and then acetic acid solution by standardised NaOH solution taking NaOH solution in burette. Use phenolphthalein indicator.
6. Prepare 100 ml exact 0.4 N solution each of NaOH and AcOH by proper dilution from the above-standardised solutions.
7. Mark the nine hard glass test tubes with 1-9 and prepare the buffer solutions as in table 4.
8. Prepare 50 ml 0.1N NaOH solution from 0.4 N solution by proper dilution.
9. Prepare ~ 0.1% bromocresol green indicator solution.
   Dissolve ~ 0.1 g solid indicator in 100 ml alcohol.
10. Take two beakers—one containing 10 ml of 0.1 N NaOH solution and the other 0.1 N HCl (need not be standardised as HCl is a strong acid).
11. Add 2 drops of indicator solution to each beaker, mix well and take O.D. reading starting from 450 nm-650 nm of each solution, at an interval of 10 nm.
12. Plot in a same graph paper, O.D. vs. λ and find the isobestic point (Fig. 5.7). Choose λ_{max} from the graph where the O.D. values-difference is maximum after the isobestic point.

    λ_{max} = ··· nm

    Isobestic point = ··· nm.

13. Measure O.D. values of the indicator in nine buffer solutions at λ_{max}. The corresponding values are 'D' in equation (8).
14. Select D_d and D_u from Fig. 5.7.
15. Plot $\log\frac{D-D_u}{D_d-D}$ vs. pH and calculate pK_{In} and hence K_{In} from the intercept (Fig. 5.8).

The nature of graph will be as shown in Fig. 5.8

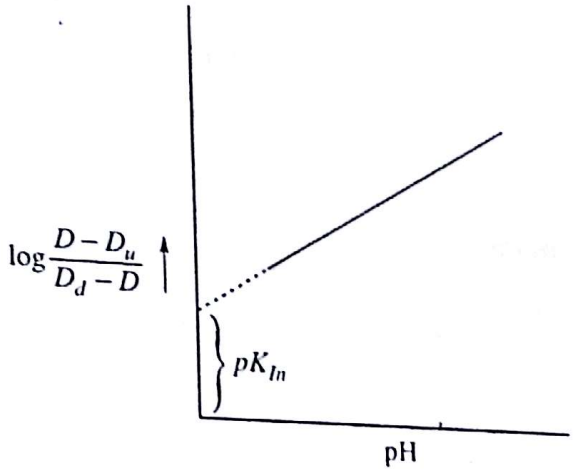

Fig. 5.8

Results and Calculations

Table 1: Recording of laboratory temperature—usual table.

Table 2: Preparation of 50 ml 0.4 N oxalic acid solution—usual table.

Table 3: Standardisation of NaOH solution by standard oxalic acid solution—usual table.

Table 4: Standardisation of AcOH solution by NaOH solution—usual table.

Preparation of 100 ml 0.2 N AcOH solution: Take 50 ml 0.4 N AcOH solution and dilute to 100 ml with distilled water.

Preparation of 100 ml 0.2 N AcONa solution: Mix 50 ml each of 0.4N AcOH and 0.4N NaOH solution together.

Table 5: Preparation of different buffer solutions

pK_{AcOH} = 4.74 at 25 °C temperature

Test tube no.	Vol. 0.2 N AcONa soln. (ml)	Vol. of 0.2 N AcOH soln. (ml)	pH of the buffer	No. of drops of 0.1% BCG
1	1	9	3.78	2
2	2	8	4.14	2
3	3	7	4.37	2

Test tube no.	Vol. of 0.2 N AcONa soln. (ml)	Vol of 0.2 N AcOH soln. (ml)	pH of the buffer	No. of drops of 0.1% BCG
4	4	6	4.56	2
5	5	5	4.74	2
6	6	4	4.91	2
7	7	3	5.11	2
8	8	2	5.34	2
9	9	1	5.69	2

Table 6: O.D. readings for Isobestic point

λ (nm)	O.D. for alkaline solution (In^-)	O.D. for acidic solution (HIn)
450	...	...
460	...	...
470		
⋮		
⋮		
650		

Table 7: O.D. readings for K_{In} value

$D_d = \cdots$; $D_u = \cdots$ $\lambda_{max} = \cdots$ nm

Test tube no.	pH	D	$D - D_u$	$D_d - D$	$\log \frac{D - D_u}{D_d - D}$
1	3.78	...	...	...	...
2	4.14	...	...	...	...
3	4.37	...	...	...	...
4	4.56	...	...	...	...
5	4.74	...	...	...	...
6	4.91	...	...	...	...
7	5.11	...	...	...	...
8	5.34	...	...	...	...
9	5.69	...	...	...	...

Results

(a) From graph 1 (Fig. 5.7), the isobestic point of bromocresol green = ... nm

(b) From graph 2 (Fig. 5.8), the intercept = pK_{In} = ... ,

$\therefore$ $K_{In} = \cdots$.