C 141(Expt. No.____)

NAME : _____ **BATCH :** _____

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VERIFICATION OF BEER - LAMBERT'S LAW & DETERMINATION OF DISSOCIATION CONSTANT (Ka) OF METHYLRED, SPECTROPHOTOMETRICALLY

AIM

To verify Beer - Lambert's law and to determine the dissociation constant (K_a) of methyl red, Spectrophotometrically.

THEORY

Absorption spectroscopy can be used to quantify the absorbing species present in the sample. The greater the quantity of absorbing species present, the greater will be the extent to which the incident light will be absorbed. When a beam of monochromatic light falls on a substance, apart of it is absorbed and the rest is transmitted. The intensity of the transmitted light is decreased.

According to Lambert's law, decrease in intensity (-dI) is proportional to the thickness of the medium (dl) and intensity of incident light (I). Beer extended this law towards solutions (and gases). In such cases decrease in intensity is also proportional to molar concentration (C).

So, $$-dI = K.\ I.\ dl.\ C$$ **(K is a constant)**

Beer - Lambert's law states that for a solution, the absorbance of the sample (A) is given by:

$$A = \log (I_o / I) = \varepsilon\ C\ l = O.\ D.$$

where **I_o** is the intensity of the incident light,
I is the intensity of the transmitted light,
ε is the molar absorptivity (characteristic of the absorbing species),
C is the molar concentration of the absorbing species in the sample,
l is the distance that the incident radiation travels through the sample.
log (I_o / I) = Optical density or Absorbance

Since **ε** depends on wave length of the light and nature of the substance so for a given substance at a given frequency and in a given photochemical cell (l = constant), O.D. = constant * C.

Hence, a plot of **O.D. Vs C** will be a straight line through origin. This is **Beer's law**.
Absorbance has no units. If **'l'** is expressed in **cm**, and **'C'** in **mol dm^{-3}**, then **'ε'** has the units of **mol^{-1} dm^3 cm^{-1}**. A linear plot of absorbance versus concentration would verify the **Beer-Lambert's Law**.

MATERIALS REQUIRED

Spectrophotometer with cells; pH meter with glass electrode, Burette (50 ml); Pipettes (10 ml, 25 ml); Standard Volumetric flasks (100 ml, 50 ml); Conical flasks (100ml); HCl (0.1 M, 0.01 M); Acetic acid (0.02 M); Sodium acetate (0.04 M, 0.01 M); Potassium permanganate stock solution (0.005M); Methyl red indicator stock solution (0.1 %); 95% ethanol, NaOH (0.1 N); oxalic acid (0.1 N); distilled water.

PART – 1

(Verification of Beer – Lambert's Law)

PROCEDURE

1. A stock solution of 0.001 M is to be prepared for 100 ml. From this solution, 10 ml is pipetted out into a 100 ml standard flask and make upto the mark with distilled water. This is the standard solution of 0.0001 M $KMnO_4$ solution.
2. Record the visible spectrum of this solution (0.0001 M KMnO4) in the wavelength range of 350 nm to 800 nm (at the intervals of 10 nm to get maximum wavelength i.e., λ_{max}). Note your results in table 1.
3. A plot of **Absorbance (A)** vs **Wave length (nm)** gives **λmax**.
4. Prepare 10 ml solutions of the following proportions of $KMnO_4$ solution given in the table 2, from the standard solution.
5. Measure the absorbance (A) at λ_{max} for each of the solutions prepared in Step 4, and for the unknown solution of $KMnO_4$ also. Note your results in table 2.
6. Plot a graph between Absorbance (y-axis) vs Concentration (x-axis).

Table 1: **Determination of λ_{max} for 0.0001 M $KMnO_4$.**

S.No.	Wave length, λ (nm)	Absorbance (A)

Table 2: Absorbance of $KMnO_4$ solutions at λ_{max} (.....nm)

S.No	Volume of 0.0001 M $KMnO_4$ (ml)	Volume of Water (ml)	Concentration of $KMnO_4$ (ml)	Absorbance (A)
1	10.0	0.0		
2	7.5	2.5		
3	5.0	5.0		
4	2.5	7.5		
5	1.0	9.0		
6	x	y		

RESULTS

1. λ_{max} for 0.0001 M $KMnO_4$ solution = ...nm
2. Concentration of Unknown solution = ...M
3. Comment on the nature of the graph(s).

PART - II

(Determination of Dissociation Constant)

AIM

To determine the dissociation constant (**Ka**) of methyl red indicator.

THEORY

An acid-base indicator is a compound that has a certain colour in acidic medium, and another colour in basic medium.

An acid- base indicator is generally a water soluble, weakly acidic organic molecule. In aqueous solution, it exists in the following equilibrium :

$$H_2O\ (aq) + HIn\ (aq) \Leftrightarrow H^+\ (aq) + In^-\ (aq) \quad (1)$$

(acid) (conjugate base)

For a very dilute solution, the activity of water is nearly unity.

The dissociation constant of the acid indicator (**pKa**) is given as :

$$K_a = [H^+] \,.\, [In^-] / [HIn] \quad (2)$$

or $$\log K_a = \log [H^+] + \log [In^-] / [HIn] \quad (3)$$

Rearranging equation (3), we get :

$$pH = pK_a + \log [In^-] / [HIn] \quad (4)$$

Absorption spectroscopy can be applied to obtain the value of **pKa** .

When **pH is less than pKa** , the indicator is mainly in the **acidic form**, and the species **HIn** is responsible for the absorbance reading, **AHIn** .

When **pH is greater than pKa** , the indicator is mainly in the **basic form**, and the species $\mathbf{In^-}$ is responsible for the absorbance reading, **AIn-** .

At some intermediate pH value, the absorbance **(A)**, is the sum of the individual absorbances due to

HIn and $\mathbf{In^-}$.

If α is the degree of dissociation of the indicator, then

$$\alpha / (1 - \alpha) = [In^-] / [HIn] \quad (5)$$

and $$A = (1 - \alpha)\ HIn + \alpha\ In^- \quad (6)$$

Rearranging equation (6), we get :

$$\mathbf{\alpha / (1 - \alpha) = (A - HIn) / (In^- - A)} \quad (7)$$

Substituting equation (7) in equation (5) and equation (4), we get :

$$\mathbf{pH = pKa + \log [(A - HIn) / (In^- - A)]} \quad (8)$$

That implies, $$\mathbf{pKa = pH - \log [\alpha / (1 - \alpha)]} \quad (9)$$

A plot of **pH (y-axis) versus log [(A - HIn) / ($\mathbf{In^-}$ - A)] (x-axis)** will be linear, with **pKa** as the intercept on the y-axis.

PROCEDURE

Step – A:

1. Standardize the NaOH solution using standard oxalic acid (0.1 N).
2. Using this NaOH solution, standardize the acetic acid solution.
3. Determine the concentrations of each solution by performing an acid-base titration using phenolphthalein indicator.

Step – B:

1. A **stock solution** of the indicator is prepared by dissolving 0.1 g crystalline methyl red in 30 ml of 95% ethanol and then diluting to 100 ml with distilled water. Then prepare the following solutions carefully.
2. A **standard solution** of the indicator is prepared by transferring 5 ml of the stock solution of indicator made up to 100 ml with distilled water.

3. **Solution A:** 10 ml of the standard solution of the indicator is taken with 10 ml of 0.1 M HCl and the mixture is diluted to 100 ml with distilled water. Its pH will be ~2.0. At this pH, methyl red will be practically in undissociated (molecular) form **(A_{HIn})**.

4. **Solution B:** 10 ml of the standard indicator solution is taken with 25 ml of 0.04 M CH3COONa and the mixture is diluted to 100 ml with distilled water. Its pH will be ~8.0. At this pH the indicator exists almost entirely in the ionic form **(A_{In-})**.

5. **Solution C:** Following 4 buffered solutions (100 ml / 50 ml each) of different pH are made containing the same quantity of the indicator given in table 4, in a clean and dry standard flasks. Determine the pH of each solution using pH meter. Determine the absorbance of Solutions A, B and C against a blank distilled water over the range 400-600 nm at intervals of 10 nm. Plot absorbance aganist wave length and determine the wave length of maximum absorption for each solution. All these curves intersect at a common point known as the **isosbestic point**. At this wave length, the observed absorbance is independent of relative amounts of molecular **(A_{HIn})** and ionic **(A_{In}-)** forms present but depends only on the total amount of indicator present in the solution.

Table 3: Determination of **λmax**

S.No.	Wavelength λ (400 - 600) (nm)	Absorbance					
		Solution A	Solution B	C_1	C_2	C_3	C_4

Table 4:

Sl. No.	Vol. Of Std Ind soln. (ml)	Vol. Of 0.04 M ACONa (ml)	Vol. Of 0.02 M ACOH (ml)	Vol. Of H_2O (ml)	pH	Absorbance A_A (λmax)	Absorbance A_B (λmax)
C_1	10	25	50	15			
C_2	10	25	25	40			
C_3	10	25	10	55			
C_4	10	25	5	60			
C_5	10	25	x	y			

6. Now, measure out 40 ml, 25.0 ml and 10.0 ml of solution A into separate 50 ml volumetric flasks and dilute with 0.01 M HCl solution upto the mark.
7. Similarly, measure out same volumes of solution B into separate 50 ml volumetric flasks and dilute with 0.01 M CH_3COONa solution upto the mark.
8. Determine the absorbance of each of the buffer solutions and of solutions A and B against a blank distilled water over the range 400 nm - 600 nm at intervals of 10 nm.
9. Plot the absorbance versus relative concentration of the indicator for each case in A, B and C and obtain the values of the absorbances at relative concentration of 1.0.
10. Calculate the relative amounts of the acid and base forms of the indicator in the solutions in C as a function of pH and hence calculate the pK_a value of the indicator.

Table 5:

S.No.	Solutions	Relative concentration	Absorbance at λmax Solution A	Absorbance at λmax Solution B
1	A_1	0.8		
2	A_2	0.5		
3	A_3	0.2		
4	B_1	0.8		
5	B_2	0.5		
6	B_3	0.2		

OBSERVATIONS AND CALCULATIONS

Room Temperature = ...^oC.

Selected Filter (or wavelength), **λmax** = ...

From the graph of absorbance versus relative concentration for each of the two sets of solutions A(A_1, A_2, A_3) and B(B_1, B_2, B_3) at the particular wave length of λmax. (from table 3)

dAHIn = slope of absorbance of **A versus relative concentration at λmax (A)**
dAIn- = slope of absorbance of **A versus relative concentration** at **λmax (B)**
dBHIn = slope of absorbance of **B versus relative concentration** at **λmax (A)**
dBIn- = slope of absorbance of **B versus relative concentration** at **λmax (B)**
where **dAHIn , dAIn- , dBHIn , dBIn-** are the absorbance values at relative concentration 1.0.
The relative amounts of **HIn and In⁻** can be calculated from the given equations:

$$A_A = d_{AHIn}[HIn] + d_{AIn^-}[In^-]$$
$$B_B = d_{BHIn}[HIn] + d_{BIn^-}[In^-]$$

By solving the above equations simultaneously, we get

$$[HIn] = \frac{d_{BIn^-} \cdot A_A - d_{AIn^-} \cdot A_B}{d_{BIn^-} \cdot d_{AHIn} - d_{AIn} \cdot d_{BHIn}}$$

$$[In^-] = \frac{d_{BHIn} \cdot A_A - d_{AHIn} \cdot A_B}{d_{BHIn} \cdot d_{AIn^-} - d_{AHIn} \cdot d_{BIn^-}}$$

Table 6:

Solns	[HIn]	[In⁻]	[In⁻]/[HIn]	log [In⁻]/[HIn]	pH	pK_a = pH - log [In⁻]/[HIn]

A plot of **pH versus log[In ⁻]/[HIn]** was obtained. The **intercept on y-axis** gives the value of the **dissociation constant (pKa), i.e., Indicator constant.**

Mean Value of pKa = ...

Ka = antilog (- pKa) = ...

RESULTS

Dissociation constant (Ka) of methyl red indicator = …….. (Theoretical).
= …… …… (Graphical).