Experiment

Spectrophotometric Determination of pK_a of an Acid-Base Indicator

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Date: …………………..   **Room Temperature: 25 ºC**

Objective: To determine the pK_a of an indicator (Methyl Red) using a spectrophotometer.

Requirements:

(a) **Apparatus and Glassware:** Spectrophotometer, pH Meter, Analytical electronic balance, Volumetric flasks, Beakers, Pipettes, Tissue paper.

(b) **Chemicals:**

- Stock Solution of 0.1 g L^{-1} g methyl red in 95% C_2H_5OH.
- 0.1 M HCl and 0.01 M HCl.
- 0.1 M CH_3COONa and 0.01 M CH_3COONa
- 0.1 M and 0.01 M CH_3COOH

1. Principle

An acid-base indicator is either a weak acid or a weak base that has distinctly different colors in the ionized and unionized forms. One form of an indicator may be colorless, but the other must be distinctly colored. The dissociation of the indicator can be expressed as given below.

$$HIn \rightleftharpoons H^+ + In^-$$

(Unionized)   (Ionized)
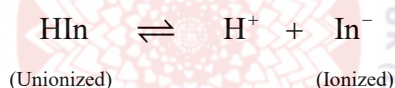
Let us take the example of the indicator methyl red [2-(4-dimethylaminophenylazo)benzoic acid], which is commonly used as an indicator for acid-base titrations in the pH range of 4.4 to 6.2. Methyl red is a weak acid, and the dissociation of methyl red in water can be expressed as given below.

$$HMR \rightleftharpoons H^+ + MR^-$$

(Unionized)   (Ionized)

(Acidic form - Red)   (Basic form - Yellow)
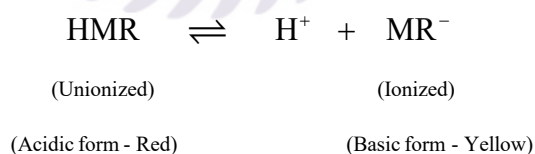
The protonated (in acidic solution) form (HMR) of methyl red is a zwitterion and has a resonance structure between two forms, as shown in Fig. 1. When the base is added, a proton is lost, and anion (MR^-) is formed. This implies that a solution of methyl red will be RED if the pH is lower than 4.8 (unionized) and YELLOW if it is above 6.2 (ionized). On the other hand, if the pH of the solution is in this range (4.4 < pH > 6.2), the color will be an appropriate mixture of both colors.
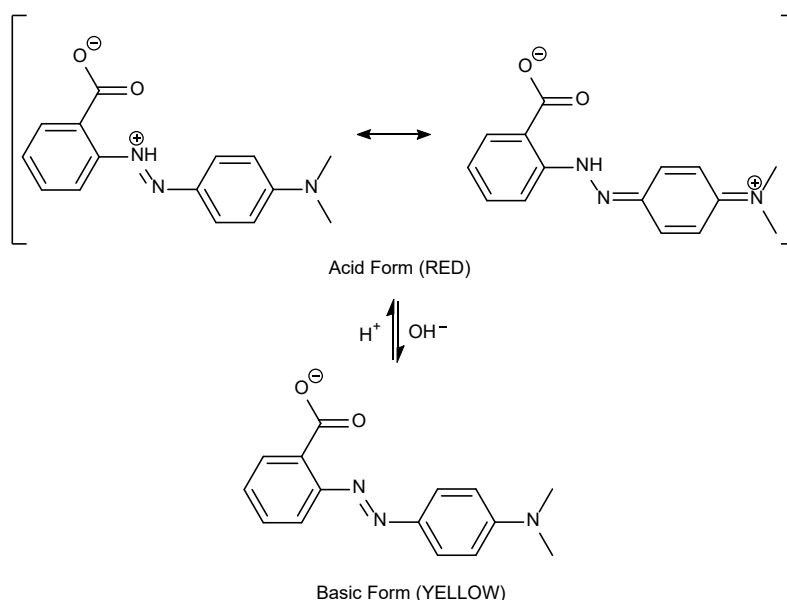
Fig. 1. Acid and base forms of methyl red.

The **Henderson-Hasselbach** equation provides the relationship between pH and pK_a value of an indicator. For methyl red, the Henderson-Hasselbach equation is given below.

$$pK_a = pH + \log\frac{[HMR]}{[MR^-]}$$

$$\text{Or}\quad \log\frac{[HMR]}{[MR^-]} = pK_a - pH$$

Thus, pK_a may be calculated from the measurement of the ratio of $[HMR]/[MR^-]$ at known pH values. Since both the ionized (MR^-) and the unionized (HMR) forms of methyl red are colored, their concentrations can be determined by measuring the absorbances at the wavelengths of maximum absorption of the two forms with the help of a spectrophotometer.

The absorption of light is governed by Beer-Lambert's Law:

$$A = \varepsilon c l$$

where A is the absorbance, ε is the absorption coefficient (depends on λ), l is the path length of the cell in centimeters, and c is the concentration of the absorbing species. The absorbance of mixtures is the sum of the separate absorbencies. In mixtures of the acid and base forms of methyl red, the total absorbance is:

$$A = A_{HMR} + A_{MR^-}$$

Suppose λ_1 and λ_2 are the wavelength of maximum absorbances of the species and HMR and MR^-, respectively. If Beer-Lambert holds, the absorbance of a mixture of HMR and MR^- is represented by:

$$A_{\lambda_1} = \left(\varepsilon_{\lambda_1,\text{HMR}} \times l \times [\text{HMR}]\right) + \left(\varepsilon_{\lambda_1,\text{MR}^-} \times l \times [\text{MR}^-]\right)$$

$$A_{\lambda_2} = \left(\varepsilon_{\lambda_2,\text{HMR}} \times l \times [\text{HMR}]\right) + \left(\varepsilon_{\lambda_2,\text{MR}^-} \times l \times [\text{MR}^-]\right)$$

The four absorbance coefficients $\varepsilon_{\lambda_1,\text{HMR}}$, $\varepsilon_{\lambda_1,\text{MR}^-}$, $\varepsilon_{\lambda_2,\text{HMR}}$, and $\varepsilon_{\lambda_2,\text{MR}^-}$ are obtained from the absorbances at two wavelengths of λ_1 and λ_2 from a series of concentrations of HMR or MR⁻ (contain one component alone) solutions by linear curves of Beer-Lambert's law. Thus, from absorbancy measurements at two different wavelengths, the [HMR] and [MR⁻] can be calculated.

2. Solution Preparation:

2.1   Stock Solution of 0.1 g L⁻¹ methyl red in 95%-C₂H₅OH

Dissolve 0.005 g methyl red indicator in 50 mL of 95%-C₂H₅OH.

(Use microbalance for weighting methyl red)

2.2   0.1 M HCl and 0.01 M HCl

To prepare 0.1 M HCl, take 0.9 mL concentrated HCl to a 100-mL volumetric flask containing 50.0 mL of distilled water. After mixing, the volume is made up by distilled water.

To prepare 0.01 M HCl, dilute 10 mL of the 0.1 M hydrochloric acid solution prepared above to 100 mL with distilled water.

2.3   0.1 M CH₃COONa and 0.01 M CH₃COONa

To prepare 0.1 M CH₃COONa, dissolve 0.82 g anhydrous CH₃COONa in a 100-mL volumetric flask with distilled water. After mixing, the volume is made up by distilled water.

To prepare 0.01 M CH₃COONa, dilute 10 mL of the 0.1 M CH₃COONa solution prepared above to 100 mL with distilled water.

2.4   0.1 M CH₃COOH and 0.01 M CH₃COOH

To prepare 0.1 M CH₃COOH, dissolve 0.6 mL concentrated CH₃COOH in a 100-mL volumetric flask with distilled water. After mixing, the volume is made up by distilled water.

To prepare 0.01 M CH₃COOH, dilute 10 mL of the 0.1 M CH₃COOH solution prepared above to 100 mL with distilled water.

2.5   Acidic solution of methyl red (Solution-A):

5 mL of the methyl red stock solution is taken in a 50-mL volumetric flask. Then, 5 mL of 0.1 M HCl is added, and the obtained solution is diluted to the mark with distilled water and mixed

thoroughly. Make sure the solution is not turbid.

Also, solutions of $\frac{1}{2}, \frac{1}{4}$, and $\frac{1}{8}$ of the initial molarity of Solution-A are to be prepared by successive volumetric dilutions; for each dilution, pipette 5 mL of solution into a 50-mL beaker and add 5 mL **0.01 M HCl**.

2.6 Basic solution of methyl red (Solution-B):

5 mL of the methyl red stock solution is taken in a 50-mL volumetric flask. Then, 5 mL of 0.1 M CH_3COONa is added, and the solution is diluted to the mark with distilled water and mixed thoroughly. Make sure the solution is not turbid.

Also, solutions of $\frac{1}{2}, \frac{1}{4}$, and $\frac{1}{8}$ of the initial molarity of Solution-B are to be prepared by successive volumetric dilutions; for each dilution, pipette 5 mL of solution into a 50-mL beaker and add 5 mL **0.01 M CH_3COONa**.

3. Experimental Procedure

3.1 Determination of the wavelengths of maximum absorption for the HMR and MR⁻

- Record the UV-Vis spectrum of Solution-A and Solution-B between 350–650 nm using an automated computer-interfaced spectrophotometer. (In case the instrument is of manual type, measure the absorption value after every 10 nm over the spectral range, as given in **Table 1**)
  (Distilled water is used in the reference cell for the entire experiment.)
- The plots of absorbance versus wavelength (**Fig. 2**) are prepared for the acid and base solutions and choose two maximum absorbance wavelengths λ_1 and λ_2 for Solution-A and Solution-B, respectively. At one of these, the acidic form HMR has the largest absorbance and at the other, the basic form MR⁻ has the largest absorbance compared with the rest.

3.2 Verification of Beer-Lambert's law for HMR and MR⁻ at λ_1 and λ_2

- Take absorbance at the chosen λ_1 and λ_2 for Solution-A and Solution-B by running dilutions of $\frac{1}{1}, \frac{1}{2}, \frac{1}{4}$, and $\frac{1}{8}$, as given in **Table 2** and **Table 3,** respectively. Serial dilutions are strongly encouraged. Absorbance versus concentration values should then be plotted to check whether Beer's law is obeyed.
- Obtaine four absorbance coefficients the absorbances at two wavelengths of λ_1 and λ_2 from a series of concentrations of HMR or MR⁻ solutions by the linear curves of Beer-Lambert's law (**Fig. 3**).

3.3   Determination of the *pK$_a$*

- Prepare five solutions of the methyl red indicator in buffer solution of different pH values by mixing CH$_3$COONa, CH$_3$COOH, methyl red, and water, as detailed in **Table 4**.

- Measure the absorbances of each of these solutions at λ_1, λ_2 and the pH values also, by using a pH-meter.

- Using equation $pK_a = pH + \log\dfrac{[\text{HMR}]}{[\text{MR}^-]}$, calculate *pK$_a$* from data obtained in **Table C**.

4. Observation Tables and Calculation:

4.1 Determination of the wavelengths of maximum absorption for the HMR and MR$^-$

Table 1: Absorbance values of the solution-A and solution-B at different wavelengths

Wavelength (nm)	Absorbance		Wavelength (nm)	Absorbance		Wavelength (nm)	Absorbance	
	Sol-A	Sol-B		Sol-A	Sol-B		Sol-A	Sol-B
350	0.088	0.205	460	0.239	0.659	570	0.481	0.077
360	0.073	0.269	470	0.332	0.595	580	0.264	0.048
370	0.056	0.348	480	0.447	0.503	590	0.115	0.025
380	0.044	0.438	490	0.574	0.395	600	0.048	0.014
390	0.038	0.53	500	0.698	0.292	610	0.024	0.01
400	0.037	0.608	510	0.807	0.214	620	0.015	0.007
410	0.043	0.663	520	0.876	0.165	630	0.012	0.005
420	0.055	0.693	530	0.884	0.134	640	0.01	0.003
430	0.077	0.706	540	0.843	0.113	650	0.009	0.003
440	0.112	0.707	550	0.784	0.103			
450	0.165	0.695	560	0.671	0.095			
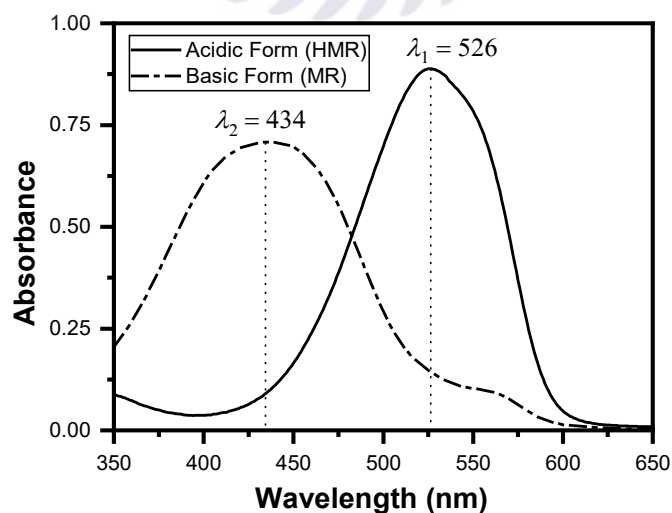
Fig. 2. Spectra of HMR and MR form of methyl red indicator.

The wavelengths of maximum absorption for

$$\text{HMR} = \lambda_1 = \textbf{526 nm}$$

$$\text{MR}^- = \lambda_2 = \textbf{434 nm}$$
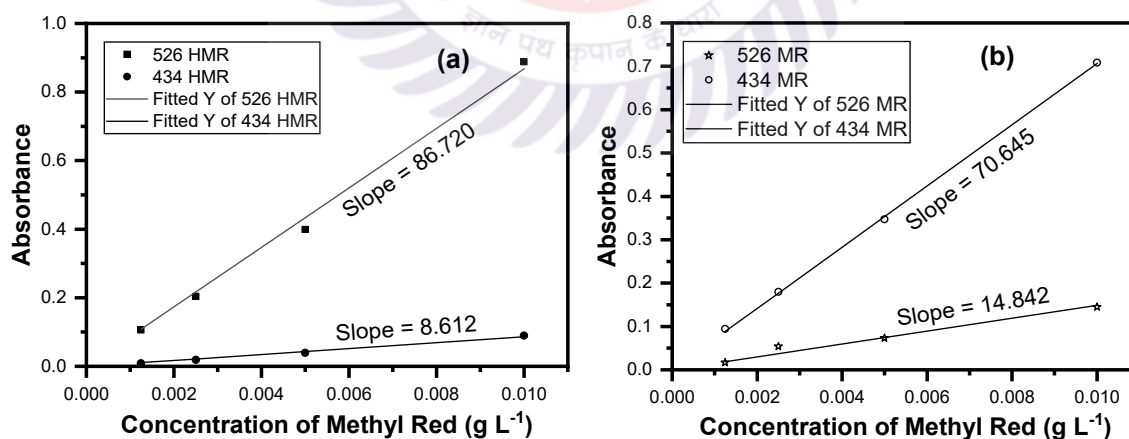
4.2 Verification of Beer-Lambert's law for HMR and MR⁻ at λ_1 and λ_2 and obtaining of four molar absorbance coefficients

Table 2: For unionized (HMR) form (Solution-A)

S. No.	Dilution	Concentration of Methyl Red (g/L)	Absorbance at	
			λ_1	λ_2
A1	1/1	0.01	1.915	0.170
A2	1/2	0.005	0.913	0.082
A3	1/4	0.0025	0.459	0.042
A4	1/8	0.00125	0.233	0.022

Table 3: For ionized (MR⁻) form (Solution-B)

S. No.	Dilution	Concentration of Methyl Red (g/L)	Absorbance at	
			λ_1	λ_2
B1	1/1	0.01	0.121	0.757
B2	1/2	0.005	0.075	0.365
B3	1/4	0.0025	0.049	0.194
B4	1/8	0.00125	0.020	0.100

Fig. 3. Verification of Beer-Lambert's law at λ₁ and λ₂ (a) acidic form (HMR) and (b) basic form MR⁻.

The four absorbance coefficients at two wavelengths of λ_1 and λ_2 for the species HMR and MR⁻ are:

$$\varepsilon_{\lambda_1,\text{HMR}} = 86.720 \text{ L g}^{-1} \text{ cm}^{-1}$$

$$\varepsilon_{\lambda_2,\text{HMR}} = 8.612 \text{ L g}^{-1} \text{ cm}^{-1}$$

$$\varepsilon_{\lambda_1,\text{MR}^-} = 14.842 \text{ L g}^{-1} \text{ cm}^{-1}$$

$$\varepsilon_{\lambda_2,\text{MR}^-} = 70.645 \text{ L g}^{-1} \text{ cm}^{-1}$$

4.3 Calculation of pK_a

Table 4: Absorbance values of the indicator solution in buffer solutions

S. No.	Total Volume*	0.1 M CH₃COONa	CH₃COOH		Methyl Red Stock Solution	Water	pH	Absorbance	
	mL	mL	Molarity	mL	mL	mL		A_{λ_1}	A_{λ_2}
1	50	2.3	0.1	6.5	5	To make up to the mark	4.15	1.265	0.183
2	50	2.3	0.1	3.4	5		4.30	1.247	0.219
3	50	2.3	0.01	15.8	5		4.48	1.410	0.309
4	50	2.3	0.01	6.7	5		4.95	0.870	0.430
5	50	2.3	0.01	2.3	5		5.40	0.494	0.563
***Use a 50-mL volumetric flask for this purpose.**									

4.3.1. Simultaneous Equation (Algebraic) Method

The values $[\text{HMR}]$, $[\text{MR}^-]$, and $\dfrac{[\text{HMR}]}{[\text{MR}^-]}$ can be calculated from the observed absorbance values

at different *pH* values using the following equations:

$$A_{\lambda_1} = \left(\varepsilon_{\lambda_1,\text{HMR}} \times l \times [\text{HMR}]\right) + \left(\varepsilon_{\lambda_1,\text{MR}^-} \times l \times [\text{MR}^-]\right)$$

$$A_{\lambda_2} = \left(\varepsilon_{\lambda_2,\text{HMR}} \times l \times [\text{HMR}]\right) + \left(\varepsilon_{\lambda_2,\text{MR}^-} \times l \times [\text{MR}^-]\right)$$

Solving these simultaneous equations, we get the expressions for the concentrations of the two species as follows. The path length of the cell (*l*) = 1 cm.

$$[\text{HMR}] = \frac{A_{\lambda_1} \times \varepsilon_{\lambda_2,\text{MR}^-} - A_{\lambda_2} \times \varepsilon_{\lambda_1,\text{MR}^-}}{\varepsilon_{\lambda_1,\text{HMR}} \times \varepsilon_{\lambda_2,\text{MR}^-} - \varepsilon_{\lambda_2,\text{HMR}} \times \varepsilon_{\lambda_1,\text{MR}^-}}$$

$$[\text{MR}^-] = \frac{A_{\lambda_1} \times \varepsilon_{\lambda_2,\text{HMR}} - A_{\lambda_2} \times \varepsilon_{\lambda_1,\text{HMR}}}{\varepsilon_{\lambda_2,\text{HMR}} \times \varepsilon_{\lambda_1,\text{MR}^-} - \varepsilon_{\lambda_1,\text{HMR}} \times \varepsilon_{\lambda_2,\text{MR}^-}}$$

$$\frac{[\text{HMR}]}{[\text{MR}^-]} = \frac{A_{\lambda_1} \times \varepsilon_{\lambda_2,\text{MR}^-} - A_{\lambda_2} \times \varepsilon_{\lambda_1,\text{MR}^-}}{A_{\lambda_2} \times \varepsilon_{\lambda_1,\text{HMR}} - A_{\lambda_1} \times \varepsilon_{\lambda_2,\text{HMR}}}$$

This can then be used to obtain the *pK*a value of the indicator by using the Henderson-Hasselbach equation, viz., (**Table 5**)

$$pK_a = pH + \log \frac{[HMR]}{[MR^-]}$$

$$\text{or} \quad pK_a = pH + \log \left[\frac{A_{\lambda_1} \times \varepsilon_{\lambda_2,MR^-} - A_{\lambda_2} \times \varepsilon_{\lambda_1,MR^-}}{A_{\lambda_2} \times \varepsilon_{\lambda_1,HMR} - A_{\lambda_1} \times \varepsilon_{\lambda_2,HMR}} \right]$$

Table 5: Computation of the pKₐ values of methyl red indicator using the Handerson-Hesselbalch equation.

S. No.	pH	A_{λ_1}	A_{λ_2}	$\frac{[HMR]}{[MR^-]}$	$\log \frac{[HMR]}{[MR^-]}$	$pK_a = pH + \log \frac{[HMR]}{[MR^-]}$
1	4.15	1.265	0.183	17.406	1.240	5.391
2	4.30	1.247	0.219	10.279	1.012	5.312
3	4.48	1.410	0.309	6.484	0.812	5.292
4	4.95	0.870	0.430	1.848	0.267	5.216
5	5.40	0.494	0.563	0.596	−0.225	5.175
Average Value of pKₐ						**5.277**

4.3.2. Graphical Method

Plot a graph between pH (*x*-axis) and $\log \frac{[HMR]}{[MR^-]}$ (*y*-axis) (**Fig. 4**). The slope of the plot for the indicator is generally close to zero. Determine the pKₐ value from the point of intersection of the line and the pH axis and also in terms of the intercept on the *y*-axis and report the result.
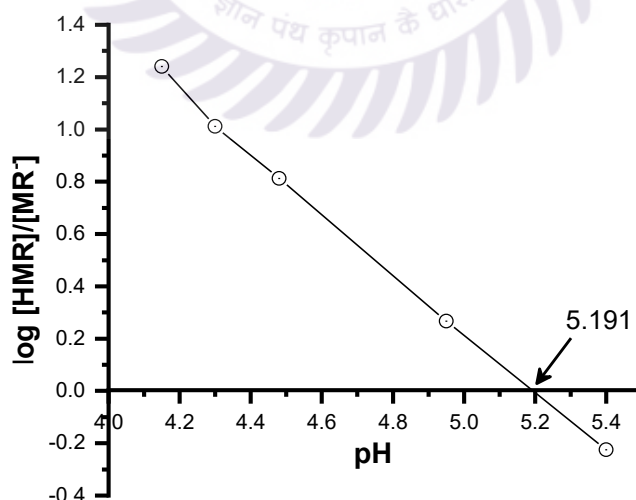
Fig .4 Graph between $\log \left(\frac{[HMR]}{[MR^-]} \right)$ and pH.

5. Results and Discussion

The pK_a value of methyl red using is found under the experimental conditions as follows:

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By the Algebraic Method   **pK_a = 5.277**

By the Graphical Method   **pK_a = 5.191**

</div>

The pK_a values determined by the algebraic and graphical methods are essentially the same. The slopes of the plots (**Fig. 4**) for the indicators are generally close to –1.

Practical Application: This method is useful for studying dyes for use as indicators in acid-base titrations, or by an analogous procedure for indicators for oxidation-reduction titrations.

6. Precaution

- Carefully prepare methyl red stock solution.
- Carefully handle strong acids and bases.

7. Further Reading

(i)   Read up details of the acid-base dissociation constant (pK_a and pK_b).

(ii)   *Atkins' Physical Chemistry*, P. Atkin and J. d. Paula, Ed: 8th, W. H. Freeman and Company, 2006.

(iii)   *Principle of Physical Chemistry*, B. R. Puri, L. R. Sharma, and Pathania, Vishal Publishing Co. Jalandhar.

(iv)   *Experimental Physical Chemistry*, F. Daniels, R. A. Alberty, J. W. Williams, C. D. Cornwell, P. Bender, and J. E. Harriman, 7th Ed., McGraw-Hill, **1970**.

(v)   G. S. Patterson, A Simplified Method for Finding the pK_a of an Acid-Base Indicator by Spectrophotometry, *J. Chem. Edu.*, **1999**, *76*, 395.

Appendix

<div align="center">

pK_a values of commonly used indicators at 25 °C.

J. Chem. Edu. **1999**, *76*, 395.

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Indicators	pK_a ± 0.05
Bromocresol green	4.66
Bromocresol purple	6.12
Bromophenol blue	3.85
Bromothymol blue	7.10
Methyl orange	3.46
Methyl red	**5.0**
Phenolphthalein	9.7
Phenol red	7.81