

9. SPECTROPHOTOMETRIC DETERMINATION OF P_{Ka}

Aim: Spectrophotometric determination of acid dissociation constant (P_{Ka}) of Methyl Red (MR), an acid-base indicator.

Theory: An acid-base indicator generally exhibits different colours in acids and bases. The colour changes are believed to be due to structure in acidic and basic medium. Methyl red is an acid base indicator. Its acidic form (HMR) and basic form is (MR⁻).

The equation is in the adjacent page.

The acid dissociation constant k is given by

$$K_a = [H^+][MR^-] / [HMR] \rightarrow (1)$$

$$P_{Ka} = PH - \log [MR^-] / [HMR] \rightarrow (2)$$

The ionisation constant may be calculated from measurement of the ratio of $[MR^-] / [HMR]$ at known pH values.

* Spectrophotometer: When an incident light (I_0) falls on a homogeneous medium, a portion of it is reflected (I_r), a portion is absorbed (I_a) & the remainder is transmitted (I_t).

$$I_0 = I_a + I_r + I_t, I_r \text{ is very small & is eliminated}$$

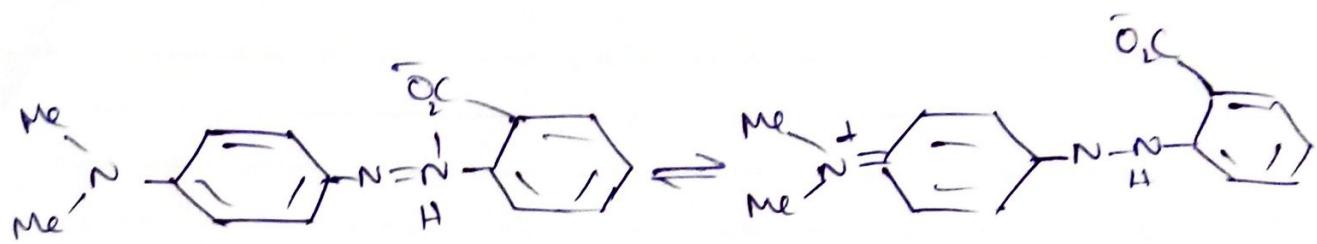
$$\text{Hence } I_0 = I_a + I_t.$$

* Lambert's law: When monochromatic light passes through a transparent medium, the intensity of transmitted light (I_t) decreases exponentially as the thickness of the medium increases arithmetically.

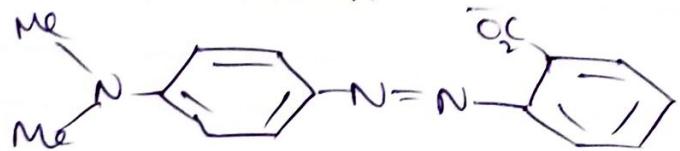
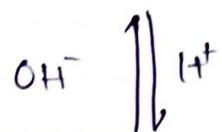
$$I_t = I_0 e^{-kt} = I_0 10^{-0.434kt} = I_0 10^{-kt}$$

$I_t = I_0 10^{-kt}$

k = constant, t = thickness.



HMR (acid form) RED



MR⁻ (Basic form) YELLOW.

Beer's law: When monochromatic light passes through a transparent coloured solution of a substance, the intensity of transmitted light decreases exponentially as the concentration of the substance increases arithmetically.

$$I_t = I_0 e^{-\epsilon c} \quad (c = \text{concentration})$$

$$\log \frac{I_0}{I_t} = \epsilon c \quad (\text{By combining Lambert's \& Beer's law}), \\ \therefore I_t = I_0 e^{-\epsilon c}$$

$$\log (I_0/I_t) = D \quad (\text{optical density / Absorbance}).$$

ϵ = molar absorption coefficient.

ϵ is characteristic constant of the substance at a definite wavelength.

D is generally determined at the wavelength of maximum absorption (λ_{max}).

$pK_a = p_H - \log [MR]/[HMR]$. Both HMR & MR^- have strong absorption peaks in visible region. The colour change interval is pH 4 to pH 6 & can be conventionally obtained with a Sodiumacetate acetic acid buffer system.

Determination of pK_a involves 3 steps:-

1) Evaluation of wavelengths at which HMR (λ_a) & MR^- (λ_b) exhibit maximum absorption.

2) Verification of Beer's law for HMR, MR^- at λ_a, λ_b .

3) Determination of relative amounts of HMR & MR^- in solution as a function of pH.

$$D_a = \epsilon_{aHMR} t[HMR] + \epsilon_{aMR^-} t[MR^-]$$

$$D_b = \epsilon_{bHMR} t[HMR] + \epsilon_{bMR^-} t[MR^-].$$

ϵ_{aHMR} = molar extinction coefficient of HMR at λ_a .

ϵ_{bHMR} = molar extinction coefficient of HMR at λ_b .

$\epsilon_{aMR^-}, \epsilon_{bMR^-}$ are molar extinction coefficients of MR^- at λ_a, λ_b respectively.

Flask number	1	2	3
standard indicator solution MR(ml)	5	5	5
0.04(M) sodium acetate(ml)	12.5	12.5	12.5
0.02(M) acetic acid(ml)	25	12.5	5.0
water(ml)	7.5	20.0	27.5
pH	4.84	5.15	5.5

Δ_1, Δ_2 are directly obtained from colorimeter & $\epsilon_{\text{HMR}}, \epsilon_{\text{bHMR}}, \Sigma_{\text{AMR}}, \Sigma_{\text{bMR}}$ can be calculated from the spectral data. Later, $[\text{HMR}]$, $[\text{MR}^-]$ at different pH are determined & pK_a is calculated.

Reagents:-

- (a) Methyl red solution: Dissolve 0.2 g of pure crystalline methyl red in 60 ml of 95% ethanol & dilute to 100 ml with water.
- (b) Standard solutions:
50 ml of stock solution to 500 ml of 95% ethanol & adding 450 ml of distilled water to make the 1 lit solution.
- (a) Sodium acetate: 0.04M
- (b) Acetic acid: 0.02M, Reagents a, b, c, d are supplied.

Apparatus Required:-

- i) Conical flask: 9 (125 ml)
- ii) Beakers: 1 (100 ml)
- iii) Pipette: 4 (2.5, 10, 25 ml).

Procedure:- (a) Determination Δ_1, Δ_2 .

Solution A is prepared by diluting a mixture of 6 ml of the standard solution of indicator (MR) & 6 ml of 0.1M HCl to 60 ml in a 100 ml conical flask. The pH of the solution is ~2 so that the indicator MR is present entirely as HMR. Using 1cm cell, the absorption spectrum of this solution over a range 400 nm to 600 nm against a blank of distilled water is determined. The absorbance (A) against λ_1, λ_2 at which max. absorbance occurs is plotted. This is ~520 nm. The solution is preserved in the conical flask.

solution	wavelength	Filter no.	Absorbance.
	430nm	4	0.044
A ₁	430nm	4	0.024
A ₂	430nm	4	0.006
A ₃	430nm	4	0.469
A ₁	520 nm	4	0.295
A ₂	520 nm	4	0.416
A ₃	520 nm	4	0.186
B ₁	430 nm	4	0.120
B ₂	430 nm	4	0.044
B ₃	520 nm	4	0.058
B ₁	520 nm	4	0.016
B ₂	520 nm	4	0.003
B ₃	430 nm	4	0.118
C ₁	430 nm	4	0.148
C ₂	430 nm	4	0.198
C ₃	520 nm	4	0.411
C ₁	520 nm	4	0.306
C ₂	520 nm	4	0.173

Solution B is prepared by diluting a mixture of the standard solution of the indicator & 15 ml of 0.04M sodium acetate to 60ml in an 100ml conical flask. The pH of this solution is ~8.80 so that the indicator MR is present almost as MR^- . The absorbance of B is measured over the range 350nm-500nm with manual colorimeter using 25nm steps except for 400-450 nm where 10nm steps ~~are~~ are used. The λ_{abs} of max. absorbance is determined (~430nm). This solution is preserved in conical flask.

- (b) Using solution A, 20ml, 12.5ml, 5ml are measured out into separate 50 conical flasks. The total volume in each case is diluted to 25ml by addition of 0.01M HCl. The resulting solution will contain 0.8, 0.5 & 0.2 times the initial concentration of HMR. Similarly, 20ml, 12.5ml, 5ml are measured out from B to 50 conical flasks. Here these are diluted to 25ml by addition of 0.01M sodium acetate. Resulting solution will contain 0.8, 0.5, 0.2 times respectively the initial concentration of MR^- . The absorption of 3 solution of HMR (same as MR) at λ_a , λ_b and measured. The straight lines equal ϵ_a^{HMR} & ϵ_b^{HMR} respectively. Similarly the absorbance of 3 solutions of MR^- at λ_a , λ_b are measured. The absorbance against the $[\text{MR}^-]$ are plotted. Starting lines will be obtained in each case. From the plots, slopes, $\epsilon_a^{[\text{MR}]}t \cdot \epsilon_b^{[\text{MR}]}t$ are found using $D = \epsilon ct$.

- (c) Absorbance's of each solution made according to the adjacent table are measured at λ_a & λ_b . These solutions contain same concentrations of indicators as in A & B. D_a , D_b at λ_a , λ_b are measured for all.

Calculations :-

From graph,

$$\epsilon_{aHMR} = 0.59$$

$$\epsilon_{aMR^-} = 0.0262$$

$$\epsilon_{bHMR} = 0.067$$

$$\epsilon_{bMR^-} = 0.223.$$

For C₁,

$$0.411 = 0.59 [HMR] + 0.0262 [MR^-]$$

$$0.118 = 0.067 [HMR] + 0.22 [MR^-]$$

$$\Rightarrow [HMR] \approx 0.672, [MR^-] \approx 0.795$$

$$pK_a = p^H - \log \frac{[MR^-]}{[HMR]} = 4.76$$

For C₂,

$$0.306 = 0.59 [HMR] + 0.0262 [MR^-]$$

$$0.148 = 0.067 [HMR] + 0.223 [MR^-]$$

$$\Rightarrow [MR^-] \approx 0.519, [HMR] \approx 0.508.$$

$$pK_a = p^H - \log \frac{[MR^-]}{[HMR]} \approx 5.13$$

For (3)

$$0.173 = 0.59 [\text{HMR}] + 0.0262 [\text{MR}^-]$$

$$0.198 = 0.067 [\text{HMR}] + 0.223 [\text{MR}^-]$$

$$\begin{aligned} \text{pK}_a &= \text{pH} - \log \frac{[\text{MR}^-]}{[\text{HMR}]} = 5.5 - \log \frac{0.820}{0.261} \\ &= 5.00. \end{aligned}$$

$$(\text{pK}_a)_{\text{avg}} = \frac{4.76 + 5.13 + 5.00}{3}$$

$$= 4.96.$$

$$\begin{aligned} D_a &= D_{\text{HMR}(a)} + D_{\text{MR}^-(a)} \\ &= \frac{\epsilon_a[\text{HMR}]}{\text{HMR}} t + \frac{\epsilon_b[\text{MR}^-]}{\text{MR}^-} t \end{aligned}$$

$$\begin{aligned} D_b &= D_{\text{HMR}(b)} + D_{\text{MR}^-(b)} \\ &= \frac{\epsilon_b[\text{HMR}]}{\text{HMR}} t + \frac{\epsilon_a[\text{MR}^-]}{\text{MR}^-} t. \end{aligned}$$

The values of $(\epsilon_a[\text{HMR}]/\text{HMR})t$, $(\epsilon_b[\text{MR}^-]/\text{MR}^-)t$, $(\epsilon_a[\text{MR}^-]/\text{MR}^-)t$ are already determined in (b) & $[\text{HMR}]$ & $[\text{MR}^-]$ can be calculated.

Thus, knowing the values of $[\text{HMR}]$ & $[\text{MR}^-]$ at particular pH , the pK_a is determined using

$$\text{pK}_a = \text{pH} - \log [\text{MR}^-]/[\text{HMR}].$$

Similarly, pK_a of methyl red are calculated at several pH & the mean value is determined.

For operating spectrophotometer:

- 1) The colorimeter is switched on & the cell is filled with distilled water & placed in the cell holder.
- 2) The wavelength knob is turned 430 nm / 520 nm & the zero absorbance is adjusted using Filter A
- 3) The cell is filled with (A, A₂, A₃, B₁, B₂, B₃ & C₁, C₂, C₃) & the absorbance at 430 nm / 520 nm (as per instruction) is measured.

Result: $\epsilon_{\text{HMR}} t = 0.59$

$$\epsilon_{\text{MR}^-} t = 0.0962$$

$$\epsilon_{\text{bHMR}} t = 0.067$$

$$\epsilon_{\text{bMR}^-} t = 0.223.$$

$$(\text{pK}_a)_{\text{avg}} = 4.96.$$

Discussion: The pK_a value is one method used to indicate the strength of an acid. pK_a is the negative log of the acid dissociation constant or K_a value. A lower pK_a value indicates a stronger acid. That is, the lower value indicates the acid more fully dissociates in water.

