

Spectrophotometric determination of the acid dissociation constant of methyl red



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DETERMINATION OF THE ACID DISSOCIATION CONSTANT OF METHYL RED M.

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Philippines Received : September 21, 2011 ABSTRACT This experiment aims to determine the acid dissociation constant value of Methyl Red by means of spectrophotometry.

Ten samples were spectrophotometrically analyzed under a UV-Vis spectrophotometer and from the absorbance values acquired, calibration curves were constructed to determine the concentration of the species of interest particularly HMR(acidic methyl red) and MR-(basic methyl red). The principle of Beer's Law was used since it establishes the direct proportionality between the absorbance and concentration. A pH vs. $\log(\text{MR-}/\text{HMR})$ curve was also constructed to discern the pKa which is the y-intercept of the equation of the line.

A pKa value of 4. 9414 was obtained . By comparing the experimental value to the literature value of pKa which is 5. 00, a 1. 172% deviation was calculated. Based on the aforementioned results, it was confirmed that spectrophotometry is a feasible technique in the determination of the acid dissociation constant of a methyl red . INTRODUCTION Spectrophotometry is a technique used to obtain measurements on how much an analyte absorbs radiant energy at a certain wavelength by passing a beam of light through the analyte.

The analyte absorbs a fraction of the light and transmits the rest. [3]. The absorbed light or Absorbance (A) can be equated to the logarithmic ratio between I_0 and I , the intensity of the monochromatic light entering the sample (I_0) and the intensity of this light emerging from the sample (I). It is represented by the equation: $A = \log (I_0/I)$ (1) By Spectrophotometry one or more species present in the sample can be quantitatively determined which gives it the advantage of having the ability to analyze participating species which cannot be isolated in a solution.

The analysis of two different components in a sample can be done by choosing an optimum wavelength for each of the components wherein one of the species has a higher absorbance compared to the other. [2] The sample analyzed in this experiment is Methyl red which primarily exists in three forms: H_2M^+ , a cation which appears in an intense red color, HMR, a neutral molecule known as a zwitterion (consists of a positive and a negative charge) which also has a red color and MR^- , an anion with a yellow color (which results in the addition of base causing methyl red to lose a proton). [5] These colored forms of methyl red absorb light in the visible region of the spectrum and have minimum interference from other substances. Methyl red is a weak acid widely used as an acid-base indicator. Its ability as an indicator is based on the equilibrium that exists between HMR and MR^- . The chemical equation for the equilibrium is: $HMR \rightleftharpoons MR^- + H^+$ From the chemical equation the acid dissociation constant K_a can be represented as: $K_a = \frac{[MR^-][H^+]}{[HMR]}$ (2)

In order to experimentally determine the acid dissociation constant, the concentration of HMR and MR^- were obtained through the use of a <https://assignbuster.com/spectrophotometric-determination-of-the-acid-dissociation-constant-of-methyl-red/>

spectrophotometer and application of Beer's Law which directly relates the concentration of the colored analyte and the amount of light it absorbs. The equation below shows the relationship between equation 1 and Beer's Law.

$A = \log(I_0/I) = \epsilon b c$ (3) Where: A is the absorbance ϵ is the molar absorptivity coefficient expressed in cm. per M b is the path length in cm (1cm in this experiment) c is the concentration of the absorbing substance Even though there exists a relationship between transmittance and absorbance, Beer's Law is still measured by Absorbance since it is unitless and is proportional to the concentration of the solution. On the other hand, Transmittance is expressed in percentage and is proportional to the intensity of light that entered and was reflected by the solution B Two absorption maxima (λ_1, λ_2) were obtained having the values ϵ_1, ϵ_2 at the first wavelength λ_1 , and ϵ'_1, ϵ'_2 at a second wavelength, λ_2 .

Absorbance is an additive property so that at a particular wavelength, the total absorbance of a solution containing a number of different absorbing components is given by assuming that there is no interaction between the individual components. The total absorbance is represented as: $A = A_1 + A_2 = \epsilon_1 b c_1 + \epsilon_2 b c_2$ (4) $A' = A'_1 + A'_2 = \epsilon'_1 b c_1 + \epsilon'_2 b c_2$ (5) The HMR and MR- concentration were calculated by solving (3) and (4). The pH of samples 7, 8, 9 and 10 were also gathered. The concentrations and pH were supplied in the Henderson Hasselbach equation: $pK_a = pH + \log \frac{[HMR]}{[MR^-]}$ (6)

Through equation 6 pKa was determined which was compared to the theoretical pKa of methyl red which is 5.00. METHODOLOGY A. Preparation of Methyl Red Stock Solution A 50-ml stock solution was prepared by

dissolving 0.0494 grams of methyl red in 35 ml of 95% ethanol and <https://assignbuster.com/spectrophotometric-determination-of-the-acid-dissociation-constant-of-methyl-red/>

transferring the solution into a 50-ml volumetric flask. The solution was

diluted to mark with water. B. Preparation of Methyl Red Standard Solution

The solution was prepared by adding 2.50 ml of the stock solution to 25 ml of 95% ethyl alcohol. The solution was also transferred to a 50-ml volumetric flask and diluted to mark with distilled water.

C. Preparation of 0.04 M and 0.01 M NaOAc solution, 1.0 M and 0.02 M

HOAc and 0.10 and 0.01 M HCl The solutions were prepared accordingly.

Solutions with higher concentrations were prepared first, followed by the reagents with smaller concentrations since certain amounts of higher-

concentration reagents were needed in their preparation. In preparing 0.04

M NaOAc, 0.33 grams of NaOAc crystals was dissolved in distilled water. The

solution was transferred in a 100-ml volumetric flask and diluted to mark

with distilled water. From the 0.04 M NaOAc, 0.1 M NaOAc was prepared by

diluting to mark 12.5 ml of 0.04 M NaOAc with distilled water in a 50-ml

volumetric flask. A 50-ml solution of 1M HOAc was used in the preparation of

0.02 M HOAc wherein 1 ml of the 1M solution was diluted to mark by

distilled water in a 50-ml volumetric flask. Lastly, 50 ml of 0.10 M HCl was

used in the preparation of 0.010 M HCl by diluting to mark 5 ml of 0.10 M

HCl solution with distilled water in a 50-ml volumetric flask. D. Preparation of

Standard solution of Acidic Methyl Red (HMR) This was prepared by the

addition of 5.0 ml of methyl red standard solution (from step B) to 5.00 ml of

0.100 M HCl solution contained in a 50 ml volumetric flask. The solution was

diluted to mark, covered by a parafilm and mixed thoroughly. It was taken to

account that the pH of the solution must be approximately 2. This solution

was used in the preparation of solution 1-3 (in step F). E. Preparation of

Standard solution of Alkaline Methyl Red(MR-) The solution was prepared by the addition of 5.00ml of methyl red standard solution (from step B) to 12.50ml of 0.040 M NaOAc which was contained in a 50-ml volumetric flask.

The solution was diluted to mark, covered by a parafilm and mixed thoroughly. The pH of the solution that was measured was only 6.56 instead of an 8. This solution was used in the preparation of solution 4-6(in step F). F. Preparation of Sample Solutions Appropriate amounts of the prepared solutions in step C-E were used in preparing the sample solutions. Ten 100-ml beakers were used in preparing the 10 samples. The sample solutions were not diluted instead only the volumes corresponding to certain reagents indicated below were added. Sample Solution Number V0.010M HCl (ml) V HMR (ml) 15.0 15.00 210.00 10.00 315.005.00 Sample Solution Number V0.010M NaOAc (ml) V MR (ml) 45.00 15.00 510.00 10.00 615.005.00 Sample Solution Number V Std. Methyl Red (ml) V0.0200M HOAc (ml) V0.040M NaOAc (ml) 76.00 1.20 12.80 86.00 2.40 11.60 96.00 4.80 9.20 106.00 7.20 6.80 G. Measuring the pH of Solutions 7-10 Using a pH meter, the pH of solutions 7-10 were measured before taking their absorbance readings. I. Gathering Absorbance Data The spectra of HMR and MR- were obtained and analyzed using a UV-Vis spectrophotometer under 424-520nm and distilled water was used in the reference cell.

Standard solutions of HMR and MR- were analyzed after the calibration. Followed by the sample solutions. RESULTS AND DISCUSSIONS The prepared solutions were analyzed and absorbance values were obtained. Below is a tabulated representation of the measured absorbance values and calculated concentrations corresponding to the following solutions. Table 1.0
<https://assignbuster.com/spectrophotometric-determination-of-the-acid-dissociation-constant-of-methyl-red/>

Absorbance Values and Sample Concentrations Solution Number Absorbance
Concentration of solutions 1-6(M) MR-(424nm)HMR (520nm) 10. 0370. 2901.
375789083×10⁻⁵ 20. 0280. 1969. 17192722 ×10⁻⁶ 30. 0190. 1024.
58596361 ×10⁻⁶ 40. 1230. 0241. 375789083×10⁻⁵ 0. 0780. 0159. 17192722
×10⁻⁶ 60. 0430. 014. 58596361 ×10⁻⁶ The absorbance of the solutions were
measured using a UV-Vis spectrophotometer. The instrument was calibrated
first by loading distilled water in each cuvette and putting both on the
reference and sample compartment of the instrument. This represented the
reference or blank cells. This procedure was done in order to get a high
accuracy and consistency on the readings by setting the absorbance to “
zero” and if there were no ions and particulate matters present in the
distilled water, this could compensate for the interferences. 4]The
absorbance of the distilled water in the reference cell sets the baseline
value. Thus, the absorbance of the other samples are obtained by the
instrument relative to the absorbance of the distilled water in the reference
cell , which is the initially “ zeroed” substance. After that, the standard HMR
and MR- were loaded into the cuvettes for analysis in order to obtain the two
absorption maxima which is found out to be 424-520 nm. The Concentration
of the samples were obtained by using the dilution formula(in the appendix)
by taking to account all the added solutions.

Only HMR was added in solutions 1-3 and MR- in 5-6. The values tabulated in
Table 1. 0 were used in the construction of the calibration curves. A total of 4
calibration curves were constructed . In solutions 1-3 the concentration of
MR- was assumed to be zero(0) . HMR with pH 2. 00 is added in the solutions
in order to assure that only HMR will be present in the solution since at this

point all Methyl Red is converted to HMR(red soln). This gave more accurate and correct absorbance readings.

In applying the principles of Beer's law, the limits of Beer's law were strictly followed in order to get reliable and significant results. The sample solutions used were dilute as Beer's law only applies to dilute solutions because the molar absorptivity at this concentration level is still independent of the substance whereas in concentrated solutions(> 0.01M) the molar absorptivity is no longer constant due to the close proximity of the molecules which results to electrostatic interactions. At this point, the molar absorptivity becomes dependent on the refractive index of the solution[2].

Beer's Law is also limited to the use of monochromatic light and a UV-Vis spectrophotometer has a monochromator which is responsible for wavelength selection from the polychromatic light coming from the light source or lamps. [1] There are two light source in a UV-Vis : Deuterium and Tungsten Lamps. Deuterium lamp is the UV light source whereas Tungsten Lamp is the Visible light source. In the experiment, the colored solutions absorb light in the Visible portion of the spectrum. Therefore, the lamp that emitted the radiation absorbed by the samples in this experiment is the Tungsten lamp.

The calibration curve for Absorbance under 424 and 520nm were constructed separately. The equation of the line in the calibration curves were compared to the Beer's Law equation: y as the Absorbance(A), m as the Molar Absorptivity coefficient(?) multiplied by the pathlength(b). For Graph 1. 0(in the appendix)the Absorbance and Concentration for Solutions

1-3 under 424 nm were graphically represented. The analysis of the two graphs are shown below respectively. Equation of the line: $y = 1962.5x + 0.01$
Linearity (R^2): 1 Under 424nm, the value of y-intercept is 0.01.

This accounts for the error in the readings. The y-intercept is relatively small because aside from the minimal experimental errors, Matched cuvettes were used all throughout the experiment which lessened the degree of getting error in readings since both cuvettes have similar pathlength and optical properties. The Linearity of the line (R^2) is also 1 (ideal value for a straight line) which also indicates minimal errors and effective calibration. The calculated Molar Absorptivity coefficient using linear regression is the slope of the line (m) which is equal to 1962.5. Equation of the line: $y = 20497x + 0.08$
Linearity (R^2): 1 Under 520nm, the calibration curve still have a low y-intercept and a linearity (R^2) value of 1. The molar absorptivity coefficient is determined to be 20497. In solutions 4-6, the concentration of HMR is assumed to be zero (0). MR- with pH 6.56 was added in the solutions. The ideal pH is 8.00 where all the Methyl Red is converted to MR-. Since the ideal pH was not met during the calibration, there are still unconverted MR- present in the solution. The calibration curve for Absorbance under 424 and 520 were also constructed separately. For Graph 1.3 and 1. (in the appendix) the Absorbance and Concentration for Solutions 4-6 under 424 nm and 520nm were graphically represented. The analysis of the two graphs are shown below respectively. Equation of the line: $y = 8722.3x + 0.0013$
Linearity (R^2): 0.994 Under 424nm, the y-intercept which is 0.0013 is still low but the linearity of the line somehow decreased to 0.994. The calculated Molar Absorptivity coefficient using linear regression is the slope of the

line(m) which is equal to 8722. 3. Equation of the line: $y = 1526.4x + 0.0023$

Linearity (R^2) : 0.9735 Under 520nm, the y-intercept which is 0.0023 is still low but the linearity of the line which is 0.9735, the lowest among the 3

calibration curves. The calculated Molar Absorptivity coefficient using linear regression is equal to 1526.4. Methyl Red was added together with 0.04

NaOAc and 0.02 NaOAc in solutions 7-10. This means that both HMR and

MR⁻ are present in the solutions. The pH of the solutions were determined

first before analyzing the solutions in the UV-Vis spectrophotometer under

the wavelength 424nm-520nm. The table below shows the corresponding pH

and Absorbance values for the solutions. Table 1.1 Absorbance and pH

Readings of Solutions 7-10

Solution Number	Absorbance (424nm)	pH	Absorbance (520nm)
7	0.45	3.24	7.5
8	0.38	3.30	3.80
9	0.45	9.0	4.59
10	0.25	0.4	0.97

2500.8484. 68 The absorbance of the samples were also measured using a

UV-Vis spectrophotometer but the instrument was not calibrated again. The

pH of the solutions were measured using a pH meter. The absorbance of

molecules with acidic or functional groups is dependent on the pH of the

solution. The absorption may vary with the hydrogen-ion concentration.

Applying the additive property of Absorbance the HMR and MR⁻

concentration were calculated using the equations 4 and 5: $A = A_1 + A_2 = \epsilon_1 b c_1 + \epsilon_2 b c_2$ (4) $A' = A'_1 + A'_2 = \epsilon'_1 b c_1 + \epsilon'_2 b c_2$ (5) The calculated

concentration values for solutions 7-9 and the logarithmic ratio between the

concentration of MR⁻ and HMR are shown below. Table 1.2 Calculated Values

of MR⁻ and HMR Concentration and The Logarithmic Ratio of MR⁻ and HMR

Solution Number	[MR ⁻]	[HMR]	Log ([MR ⁻]/[HMR])
7	5.006332917 x10 ⁻⁵⁸		
8	2.22356167 x10 ⁻⁶⁰	7.79273	84.041632619 x10 ⁻⁵¹
9	5.52951748 x10 ⁻⁵⁰		

415399 95. 072945096 x10⁻⁵⁸. 419113336 x10⁻⁶⁰. 779994 101. 968337271 x10⁻⁵³. 990609845 x10⁻⁵⁻⁰. 30694

Using the known Concentration of the MR⁻ and HMR in the following solutions, the pKa can be calculated using the Henderson-Haselbach Equation. The measured pH is also needed. Another method to determine the pKa value which was applied in this experiment is plotting pH against the logarithmic ratio of MR⁻ and HMR Graph 1. 5(in the appendix) . In this method, the pKa was directly determined by finding the equation of the line which is $y = 0.6203x + 4.9414$ with a linearity of 0.4596. The y-intercept in the equation of the line is the pKa with the value of 4.9414. The experimental pKa has a 1.72% deviation from the theoretical pKa which is 5.00. The possible sources of errors or deviation from the pKa values are the following: The calibration of the pH of the Standard MR⁻ did not reach the pH of 8.00. That resulted to incomplete conversion of all Methyl Red to MR⁻ which means that the two forms of Methyl Red which are H₂M⁺ and HMR are still present in the solution. Thus, resulting to discrepancies in the readings due to the H⁺ component of the two forms. The standard Methyl Red solution contained ethanol and the other working solutions also contain other volatile liquids that evaporate when uncovered.

This could yield a higher pH due to the evaporation of H⁺. Improper handling of the cuvettes. Instrumental errors such as instrumental noise and ' Stray' light(which is the reflection of light as it passes through the lenses , mirrors and other components of the instruments.) passing the cuvettes . Variations in temperature also contributed to deviation since acid-dissociation

constants may vary significantly with temperature. [2] Alterations of the <https://assignbuster.com/spectrophotometric-determination-of-the-acid-dissociation-constant-of-methyl-red/>

concentration due to contaminated glass wares and inconsistency in the measurement of reagents. CONCLUSIONS AND RECOMMENDATIONS

After tabulating and interpreting the data gathered in the experiment, a pKa value of 4.9414 was obtained. Only a 1.172% deviation from the theoretical pKa value, 5.00, was calculated. The small deviation indicates that the experiment yielded a positive result. Based on the results, it was attested that spectrophotometry is a suitable and alternative way in finding the equilibrium concentration and constant for complex-forming reactions. These results also showed the effectiveness of Spectrophotometry in the experimental determination of acid dissociation constants and equilibrium concentrations.

It is recommended to apply UV-Vis spectrophotometry using an even more dilute solutions and gaseous solutions loaded in cuvettes with longer pathlengths (more than 1cm) in order to gain a knowledge on how these form of substances were analyzed. It is also recommended to use different methods of determining acid dissociation constant such as NMR chemical shift measurements and Isothermal Titration Calorimetry to determine the degree of accuracy of Spectrophotometry. REFERENCES: [1] Harvey, D.

Modern Analytical Chemistry 1st Ed. , Quebecor Printing Book

Group/Kingsport, 2000, Pgs. 393-394 [2] Harrison G. R. , Lord R.

C. , Loofbourow J. R. Practical Spectroscopy. Prentice-Hall Inc. , New York.

1948. [3] Parkampus, H. H. UV-VIS Spectroscopy and Its Applications.

Springer-Verlag Berlin Heidelberg, Germany. 1992. [4] Skoog, D. A. et al...

Fundamentals of Analytical Chemistry. 8th Edition. Singapore: Thomson

<https://assignbuster.com/spectrophotometric-determination-of-the-acid-dissociation-constant-of-methyl-red/>

Learning Asia, 2004, Pg. 734 [5]Tobey, S. W. , The Acid Dissociation Constant of Methyl Red A Spectrophotometric Determination , American Chemical Society Journal. Volume 35 (Issue 10): p. 514-515, October 1958. APPENDIX I.

SAMPLE CALCULATIONS a. Molarity of the Stock Solution weight of methyl red: 0.0494 volume of solution: 50ml

FW of methyl red: 269.3 g/mol $M = \frac{\text{mass}}{\text{molar mass}} / (L \text{ soln})$ $M = (0.0494 \text{g} / (269.3 \text{ g/mol})) / 0.050 \text{L} = 3.668770887 \times 10^{-3} \text{M}$ b. Molar

Concentration of Methyl Red Standard Solution $M_{\text{std.}} = M_{\text{stock}} V_{\text{stock}} / V_{\text{std.}}$

$= (3.668770887 \times 10^{-3} \text{M} (2.5 \text{ml})) / 50 \text{ml} = 1.834385444 \times 10^{-4} \text{M}$ c. Molar

Concentration of HMR and MR- $M_{\text{working std.}} = M_{\text{std}} V_{\text{std.}} / V_{\text{working std.}}$

$= (1.834385444 \times 10^{-4} \text{M} (5 \text{ml})) / 50 \text{ml} = 1.834385444 \times 10^{-5} \text{M}$ d. Molar

Concentration of Solution Number 1 volume of HCl= 5 ml volume of HMR=

15ml total volume: 20ml $M_{\text{soln}} = M_{\text{HMR}} V_{\text{HMR}} / V_{\text{soln}} = (1.834385444 \times 10^{-5} \text{M}$

(15ml)) / 20ml = $1.375789083 \times 10^{-5}$ e. Concentration of HMR and MR

$A = A_1 + A_2 = \epsilon_1 b c_1 + \epsilon_2 b c_2$ $A' = A'_1 + A'_2 = \epsilon'_1 b c_1 + \epsilon'_2 b c_2$ (0.247 =

20497 [HMR1] + 1526.4 [MR-1]) 1962.5 (0.453 = 1962.5 [HMR2] + 8722.3

[MR-2]) 20497 [MR] = -8,800.4035 -175,785,423.1 [MR] = 5.006332917

$\times 10^{-5}$ 20497 [HMR] = 0.247 - 1526.4 [HMR] = $8.322356167 \times 10^{-6}$ f.

Logarithmic ratio between [MR-] and [HMR] $[\text{MR-}] / [\text{HMR}] = 5.006332917 \times 10^{-5}$

$8.322356167 \times 10^{-6} = 6.015523509$ $\text{Log}([\text{MR-}] / [\text{HMR}]) = \text{Log}(6.015523509)$

= 0.779273428 g. Percent Deviation Experimental pKa= 4.

9414 Theoretical pKa= 5.00 %Deviation= $\frac{\text{Theoretical value} - \text{Actual value}}{\text{Theoretical value}} \times 100$

$\frac{5.00 - 4.9414}{5.00} = 1.172\%$