I. Introduction

The UV - VIS spectrophotometer will be used to determine the pKₐ of two indicator solutions, methyl red and bromocresol green. The method is a simultaneous photometric determination, using Beer's Law and the Henderson - Hasselbalch equation to determine the pKₐ of the indicator.

II. Theory

If a beam of parallel radiation passes through a layer of solution having a thickness, b, and a concentration, c, of a species, the transmittance, T, is defined as the ratio of the power of the beam after passing through the substance to the power in the incident beam. This difference in power is due to interactions between the photons and the molecules in the solution, with this absorption attenuating the beam. The absorbance is defined as:

$$A = - \log (T) = \log(P_0/P)$$

Beer's law relates the absorbance, the concentration of the absorbing species and the path length, such that:

$$A = \varepsilon \cdot b \cdot c$$

where A is the absorbance, ε, is the extinction coefficient, b is the path length (normally 1 cm) and c is the concentration of the absorbing species. Beer's law applies to solutions containing one or more absorbing species, if there is no interaction between the various species in the solution. In the case of a solution containing n species which absorb, the above equation becomes:

$$A_{\text{tot}} = A_1 + A_2 + \ldots + A_n = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \ldots + \varepsilon_n bc_n$$

Beer's law in the case of a fixed path length, b, and extinction coefficient, ε, is a linear relationship between absorbance and the concentration. This is not generally the case. Beer's law is successful in describing the absorption behavior of dilute solutions. One of the fundamental assumptions in the derivation of the law is that the average distance between atoms is large enough such that the charge distributions of neighboring atoms or molecules are not affected by those of its neighbors. This can alter a species' ability to absorb a given wavelength of radiation. This causes a deviation from the linear relationship because the extent of interaction depends on concentration. A similar situation can occur when the concentration of the absorbing species is low compared with the concentrations of other species. The effects of
these molecular interactions become negligible at concentrations below 0.01M. Dilute solutions must therefore be used.

If the weak acid is HAc, then it has a conjugate base Ac\(^-\), which exists in solution and the following equilibrium will be established:

\[
\text{HAc} + \text{H}_2\text{O} \leftrightarrow \text{H}_3\text{O}^+ + \text{Ac}^{-}
\]

The color of the indicator depends on the concentration of H\(_3\)O\(^+\) in solution. The above equilibrium depends on the equilibrium constant, \(K_a\), which is defined as:

\[
K_a = \frac{[\text{H}_3\text{O}^+][\text{Ac}^{-}]}{[\text{HAc}]}
\]

The Henderson - Hasselbach equation is an expression which relates the pH, the pK\(_a\) and the concentrations of the acid and base species in a buffer solution:

\[
\text{pH} = \text{pK}_a - \log \left(\frac{c_A}{c_B}\right)
\]

where \(c_A\) is the concentration of the acid form and \(c_B\) is the concentration of the basic form.

In order to find the pK\(_a\) from the Henderson - Hasselbach equation, the concentrations of the acid and base forms of the indicators must be known. Beer's law and the spectrophotometer can be used to find these concentrations.

A spectra is first run on the indicator solution of known concentration, \(c_A\), which has been completely converted to the HIN (acidic) form. From this spectra, the wavelength that corresponds to the maximum absorbance is found and denoted \(\lambda_1\). A spectra is then run on indicator of known concentration, \(c_B\), that has been completely converted to In\(^-\) (basic) form and the wavelength of maximum absorbance is found and denoted \(\lambda_2\). The absorbance of both the acid form solution and the basic form solution is then measured at both \(\lambda_1\) and \(\lambda_2\) and denoted \(A_{1A}\), \(A_{1B}\), \(A_{2A}\), and \(A_{2B}\). This means that:

\[
\begin{align*}
\text{c}_A\varepsilon_{A_1} &= A_{1A} \\
\text{c}_B\varepsilon_{B_1} &= A_{1B} \\
\text{c}_A\varepsilon_{A_2} &= A_{2A} \\
\text{c}_B\varepsilon_{B_2} &= A_{2B}
\end{align*}
\]

The values of the extinction coefficients are now calculated for the acidic species and the basic species at both wavelengths. Knowing all four of the extinction coefficients and the fact that Beer's law is additive makes the simultaneous determination of the pK\(_a\) possible.

The absorbance of an indicator solution of known (buffered) pH (and unknown concentration) is next measured at both \(\lambda_1\) and \(\lambda_2\), and denoted \(A_1\) and \(A_2\). Because Beer's law is additive, two linear equations may be written:
These two equations may easily be solved for $C_A$ and $C_B$.

$$
C_A = \frac{(A_1 \varepsilon_{A1}) - (A_2 \varepsilon_{B1})}{(\varepsilon_{B2})(\varepsilon_{A1}) - (\varepsilon_{B1})(\varepsilon_{A2})}
$$

$$
C_B = \frac{(A_1 \varepsilon_{A2}) - (A_2 \varepsilon_{A1})}{(\varepsilon_{B1})(\varepsilon_{A2}) - (\varepsilon_{B2})(\varepsilon_{A1})}
$$

The concentrations of the acid form and the basic form are now known and the pK_a can be calculated using the Henderson - Hasselbach equation:

$$
pK_a = pH - \log\left(\frac{C_A}{C_B}\right)
$$

III. Use of the Hewlett Packard Spectrophotometer

Before the samples are run, a blank must be run. A spectrophotometric cell is filled with distilled deionized water and placed in the holder in the spectrophotometer such that the arrow is in the path of the light. The option on the computer to scan a blank should be chosen. Now that a blank has been scanned, the measurement of spectra may begin. The samples will be placed in cells and placed so that the arrow is in the path of the light and the option is chosen on the computer to scan (F1 key). After the spectrum appears on the screen, choose the cursor (F2) and use the arrow keys to go to the desired wavelength or maximum point. Then select F1 again to mark that point and go onto the next point or choose F10 to exit. Now obtain a printout by pressing F9. The printout will list both the ordinate and abscissa values for the points that were marked. Add the next sample and repeat.

IV. Experimental Procedure

A laboratory stock solution of methyl red and bromocresol green should be prepared (check with the instructor regarding the concentrations of each).

Methyl red is a weak organic acid which can be used as an indicator in the pH range of 4.8 to 6.0. A solution of methyl red will be red if the pH is lower than 4.8, yellow if it is above 6.0 and a mixture of both if 4.8 < pH < 6.0. Bromocresol green is a weak organic acid which can be used as an indicator in the pH range of 3.8 to 5.4. The following is the procedure to be used for both indicators.
A. Preparation of Working Solution

A working solution of the indicator is prepared by diluting 10 mL of the stock indicator solution to 25 mL in a volumetric flask.

B. Determination of Working Wavelengths

Prepare acidic and basic species of the indicators using the working solution and the following.

Acidic Form of Indicator: (approximate pH of 2)
10 mL of working indicator solution
10 mL of 0.1 M HCl
Dilute up to volume in a 100 mL volumetric

Basic Form of Indicator (approximate pH of 8)
10 mL of working indicator solution
25 mL of 0.04 NaAc
Dilute up to volume in a 100 mL volumetric

Measure the spectra using the spectrophotometer and find at which wavelength the absorption was maximum for each sample. These are the two wavelengths to be used, $\lambda_1$ and $\lambda_2$. The two peaks must not overlap. Measure the absorbances for each at both wavelengths.

C. Determination of the Molar Absorptivities.

Use solutions A and B from above to prepare a series of diluted solutions. For the three solutions, dilute 50, 25 and 10 ml portions of solutions A and B to 100 ml. Measure the absorption of each solution at the wavelengths selected above.

From the linear plot of absorption vs. concentration determine the the molar absorptivities of the acid and base forms of the indicator at each wavelength.

D. Calibrate the pH meter using standard buffer solutions.

E. Determination of $pK_a$

Prepare a second working solution by diluting 10 mL of the stock solution to 250 mL. Transfer the working solution to a clean, dry 400 mL beaker. Add 1.36 g of sodium acetate and stir on a magnetic stirrer until dissolved. Lower the probe from the pH meter into the solution, keeping the end well clear of
the stir bar. Add 1.0 M HCl to the beaker dropwise until the pH of the solution is approximately 6.5 for methyl red or 5.3 for bromocresol green. Transfer a portion of the solution to a cuvette and measure the absorbance of the samples each of the two previously determined wavelengths, $\lambda_1$ and $\lambda_2$. Continue adjusting the pH of the solution in increments of 0.3 pH units and measure the absorbance spectrum at each new pH until a lower limit of 3.8 is reached.

F. Calculations

The extinction coefficients are first calculated for the pure acid and pure base forms at each of the two wavelengths using the absorbances and the known concentrations from part B. The measured absorbances are now used with the calculated extinction coefficients to solve linearly for the concentrations of the acid and base forms.

G. Data Presentation

After these values are calculated for the five solutions, make a plot of pH versus $\log_{10} ([\text{HAc}]/[\text{Ac}^-])$. The y-intercept of this line is the experimental $pK_a$. This line should be fitted using linear regression to find the y-intercept. Tables should be included which contain the values for the absorbances and the corresponding 4 extinction coefficients from part B. A table should also be made containing the values for the absorbances, the concentrations and the pHs of the buffered solutions. The plot of the pH vs $\log([\text{HAc}]/[\text{Ac}^-])$ with linear regression analysis should be included.

V. Discussion

The following questions should be addressed in the lab report.

1.) How accurate are your results? What do you feel is the major cause of error in this lab? Why? Discuss.

2.) Is the choice of a buffer solution important? Why?

3.) Why is a blank sample of water analyzed before the samples are measured.

4.) What is the importance of the pure acid and pure base peaks being separated when determining the wavelengths to be used?

5.) How would you improve the procedure. Elaborate.

6.) Compare your value to the literature value. Discuss any discrepancies.
7.) Discuss other studies/determinations which can be one using simultaneous spectrophotometry.
Acidic Solution

Annotated Wavelengths:
1: Wavelength = 614 Result = 0.080154
2: Wavelength = 444 Result = 0.773132

Basic Solution

Annotated Wavelengths:
1: Wavelength = 444 Result = 0.131897
2: Wavelength = 614 Result = 2.007599
Blank

pH 4.08

Annotated Wavelengths:
1 : Wavelength = 444  Result = 0.800812
2 : Wavelength = 614  Result = 0.288544
pH 4.38

Annotated Wavelengths:
1: Wavelength = 614   Result = 0.476303
2: Wavelength = 444   Result = 0.711197

pH 4.53

Annotated Wavelengths:
1: Wavelength = 444   Result = 0.664856
2: Wavelength = 614   Result = 0.597916
pH 4.67

Annotated Wavelengths:
1 : Wavelength = 610  Result = 0.703949
2 : Wavelength = 614  Result = 0.717300
3 : Wavelength = 444  Result = 0.604858

pH 4.77

Annotated Wavelengths:
1 : Wavelength = 444  Result = 0.582489
2 : Wavelength = 614  Result = 0.822418