

Reading:

1. Skoog, Holler and Crouch: Chapter 13 and 14

This experiment was performed last semester in Analytical Chemistry using Spectronic 20. This semester modern UV visible spectrophotometers will be used.

I. INTRODUCTION

Molecular absorption spectroscopy in the UV and visible regions is still extensively used for the identification and quantitative analysis of organic and inorganic compounds. It is inexpensive, can be highly sensitive and does not require expertise. The pH dependence of the electronic spectrum of a visual acid-base indicator and Beer's law are used to determine the concentration of the conjugate acid and the conjugate base at various pH and to evaluate the pKa of bromothymol blue (BTB).

An acid-base visual indicator is a substance that displays different colors at different pH because of the differences between the electronic spectra of its conjugated acid and base. The color of a solution of a mixture of the conjugated acid and base depends on the ratio of the two forms. The ratio depends on the pKa of the indicator and on the pH, as is the case for all weak acids and bases. The relation between the pH, pKa and the concentration of conjugated base and acid is as follows:



$$\log \frac{[\text{In}^-]}{[\text{InH}]} = \text{pH} - \text{pK}_a \quad (1)$$

Equation 1 clearly shows that selection of an appropriate indicator requires knowledge of the value of the pKa of the indicator and the pH at the equivalence point. Ideally the indicator should have a pKa such that the ratio of the two forms of the indicator changes by about 10 around the equivalence point. This means that the equivalence point is located in the middle of the indicator range, the indicator range being the minimum change in pH required to cause a color change.

A simple spectrophotometric method is used to determine the ratio $[\text{In}^-]/[\text{InH}]$. The pKa of the indicator is obtained from graphical analysis of $\log([\text{In}^-]/[\text{Hin}])$ versus pH data. As

indicated by equation 1, the plot $\log \frac{[\text{In}^-]}{[\text{InH}]}$ versus pH, has a slope = 1 and an intercept = -pKa. Note also that when the $[\text{In}^-]/[\text{InH}] = 1$, the log of the ratio is zero and pH = pKa, meaning that X-axis intercept = pKa.

II. PREPARATION

1. Bromothymol blue (BTB) solution. Dissolve 0.5 g of bromothymol blue in 500 mL of 20 % ethanol.
2. 0.2 M solution of NaH_2PO_4 . Dissolve 24 g in 1000 mL.
3. 0.2 M solution of K_2HPO_4 . Dissolve 34 g K_2HPO_4 in 1000 mL.
4. 100 mL of 3 M NaOH.

III. EXPERIMENTAL

Obtain seven 50-mL volumetric flasks. Pipet into each flask 1 mL of the bromothymol blue solution. Then add the volumes of phosphate solutions specified in the table to adjust the pH of the solutions. Dilute to the mark with deionized water and mix thoroughly. Measure and record the pH of each solution using a pH meter.

Flask #	Bromothymol blue, (mL)	NaH_2PO_4 (mL)	K_2HPO_4 (mL)	NaOH	$A(\lambda_1)$	$A(\lambda_2)$
1	1	5	0		$A_a =$	$A_b =$
2	1	5	1			
3	1	10	5			
4	1	5	10			
5	1	1	5			
6	1	1	10			
7	1	0	5	2 drops	$A_b =$	$A_a =$

Determine the spectrum of bromothymol blue at low pH (Spectrum 1)

Use water as the reference to establish the baseline of the spectrophotometer. The pH of solution #1 is low enough so that most of the indicator is in its acid form (HIn). Record the spectrum from 400 to 600 nm.

Determine the spectrum of bromothymol blue at high pH (Spectrum 2)

Use solution # 7 and repeat the procedure described above to obtain the spectrum of the basic form of bromothymol blue (In⁻)

Selection of the working wavelength

Superimpose the two spectra and select on the basic form spectrum 2 a wavelength at which the acid form absorbs least (λ_2). Select on spectrum 1 a wavelength at which the basic form absorbs least (λ_1). Now measure the absorbance of the seven solutions at both wavelengths. Plot the absorbance versus pH data as you measure them.

IV DATA PROCESSING AND QUESTIONS

- 1) Use the data recorded at λ_2 to calculate the ratios of the concentrations of In^- over HIn . If, A_a and A_b are the absorbances of solution #1 and #7 respectively at λ_2 , then the ratio $[\text{In}^-]/[\text{InH}]$ in solutions 2 to 6, is given by equation 2, in which A represents the absorbance of solutions 2-6.

$$\frac{[\text{In}^-]}{[\text{InH}]} = \frac{A - A_a}{A_b - A} \quad (2)$$

- 2) Using data collected at λ_1 where the acidic form of bromothymol blue absorbs most, the ratio

$$\frac{[\text{In}^-]}{[\text{InH}]}$$
 is calculated as follows:

$$\frac{[\text{In}^-]}{[\text{InH}]} = \frac{A_s - A}{A - A_s}$$

- 3) Plot Absorbance versus pH using data obtained at λ_1 and λ_2 .
- 4) Plot $\log \frac{[\text{In}^-]}{[\text{HIn}]}$ versus pH using data obtained at λ_1 and λ_2 .
- 5) Determine the pK_a of the indicator from the four graphs.
- 6) How does the pK_a determined compare with the literature values? (Calculate the % error using a literature value with a least as many significant figures as the experimental value).
- 7) Are the two values significantly different at the 95% confidence level?
- 8) Comment on the accuracy of the values obtained from the two instruments.
- 9) Calculate the extinction coefficient of the conjugate acid of BTB at λ_1 .
- 10) Calculate the extinction coefficient of the conjugate base of BTB at λ_2 .
- 11) How do the values of the extinction coefficients determined compare with literature values. What could be the source(s) of the differences between the literature values and your experimental values?