

EXPERIMENT B6: pK OF AN INDICATOR

Learning Outcomes

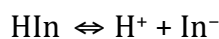
Upon completion of this lab, the student will be able to:

- 1) Collect data using a spectrophotometer.
- 2) Evaluate absorption spectra and choose the wavelengths at which absorbance maxima may be found.
- 3) Measure the pK of an indicator using a spectrophotometer.

Introduction

Indicators are weak acids or bases that undergo color changes in certain pH ranges. These color changes are due to the indicator accepting or releasing a proton (H^+), which in turn causes a significant change in the structure of the indicator. In the titration experiment (CHEM 1A: Experiment A7), phenolphthalein was used as an indicator. Phenolphthalein is colorless in an acidic medium and pink in a basic medium. Likewise, most common indicators used in laboratory experiments exhibit color changes in the visible spectrum, as the purpose of an indicator is to be able to monitor the pH of solution by simple visual observation. Since these color changes occur in the visible spectrum, they can be studied using a UV-Vis spectrophotometer

Suppose that an indicator is a weak acid. The structure of the indicator could thus be represented by the formula HIn . In an acidic medium, the indicator would exist in its protonated form (HIn), while in a basic medium, the indicator would be neutralized and would exist in its conjugate, deprotonated form (In^-). The equilibrium between the protonated and deprotonated forms can be represented as shown below:



At any given time in this equilibrium, both the acid (HIn) and its conjugate base (In^-) will be present. If the acid dissociation constant for the indicator is assumed to be K_a , then:

$$K_a = \frac{[H^+][In^-]}{[HIn]}$$

The above equation can be rearranged to solve for the $[H^+]$ and manipulated as:

$$[H^+] = \frac{K_a [HIn]}{[In^-]}$$

$$-\log[\text{H}^+] = -\log\left(\frac{K_a[\text{HIn}]}{[\text{In}^-]}\right)$$

$$\text{pH} = -\log(K_a) - \log\left(\frac{[\text{HIn}]}{[\text{In}^-]}\right)$$

$$\text{pH} = \text{p}K_a - \log\left(\frac{[\text{HIn}]}{[\text{In}^-]}\right)$$

$$\log\left(\frac{[\text{HIn}]}{[\text{In}^-]}\right) = -\text{pH} + \text{p}K_a$$

Since the acid in question in the above expression is the indicator, the $\text{p}K_a$ may also be written as $\text{p}K_{\text{IN}}$. Therefore the equation is rewritten as shown below:

$$\log\left(\frac{[\text{HIn}]}{[\text{In}^-]}\right) = -\text{pH} + \text{p}K_{\text{IN}} \quad \text{Equation 1}$$

If the protonated and deprotonated forms of the indicator are present in equal concentrations, then the ratio of their concentrations is one, so Equation 1 simplifies as follows:

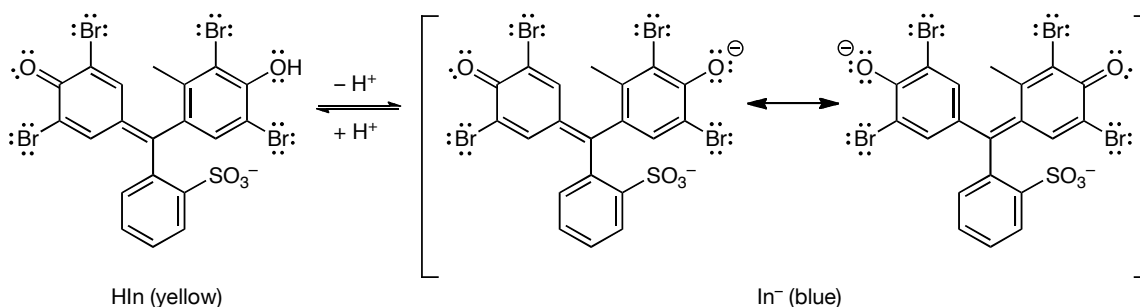
$$\log(1) = -\text{pH} + \text{p}K_{\text{IN}}$$

$$0 = -\text{pH} + \text{p}K_{\text{IN}}$$

$$\text{p}K_{\text{IN}} = \text{pH} \quad \text{Equation 1b}$$

By examining Equations 1 and 1b, it can be shown that if the solution pH is less than $\text{p}K_{\text{IN}}$, then the protonated form of the indicator (HIn) will predominate, while if the solution pH is greater than $\text{p}K_{\text{IN}}$, then the deprotonated form of the indicator (In^-) will predominate.

The indicator that will be studied in this experiment is bromocresol green. The ionization equilibrium of bromocresol green is shown below:



Bromocresol green undergoes a color change when it is deprotonated because of the extensive delocalization found in the resulting anion. As discussed above, the protonated form of bromocresol green (HIn), which is yellow, will be the predominant form at low pH (much below the pK_{IN}) and the deprotonated form of bromocresol green (In^-), which is blue, will be the predominant form at pH values much higher than the pK_{IN} . At all intermediate pH values, both the acid form (HIn) as well as its conjugate base (In^-) will be found. According to Equation 1, the concentrations of HIn and In^- and therefore the ratio $\frac{[HIn]}{[In^-]}$ depend on the pH of the solution.

The concentrations of chemical species that are colored can be determined spectrophotometrically using Beer's law (also referred to as the Beer-Lambert law). According to Beer's law, for substances that absorb light in the visible region of the electromagnetic spectrum (~ 400 to 800 nm), the amount of light absorbed or its absorbance (A) is directly proportional to the molar concentration (C). The mathematical form of this law is shown below:

$$A = \epsilon \times C \times l \quad \text{Equation 2}$$

Aside from absorbance and concentration, the two other quantities in Equation 2 are: ϵ - the molar extinction coefficient and l - the path length through which light travels in the sample. The path length, "l" is usually a fixed quantity for a particular spectrophotometer and is most frequently 1.00 cm. The molar extinction coefficient, ϵ , which represents the sensitivity of the compound to light depends instead on both the sample and the wavelength, λ , at which the absorbance is measured.

So, even though the concentrations of a pure sample of HIn and In^- may be measured relatively easily using a spectrophotometer, assuming ϵ_{HIn} and ϵ_{In^-} are known, the concentration of a mixture of HIn and In^- is more challenging to obtain.

Therefore in order to use Equation 1 to determine the pK_{IN} some amount of mathematical manipulation is required.

Manipulation of Equation 1 to Determine pK_{IN}

$$\log\left(\frac{[HIn]}{[In^-]}\right) = -pH + pK_{IN} \quad \text{Equation 1}$$

At any given time in the equilibrium (other than at extremely low and high pH) both HIn and In^- will be present. Therefore the total concentration of the indicator, In is given as:

$$[In] = [HIn] + [In^-] \quad \text{Equation 2}$$

Assuming that the molar extinction coefficient of the indicator is ϵ , the absorbance of the mixture is then given as:

$$A = \epsilon \times [\text{In}] \times l \quad \text{Equation 3}$$

The total absorbance is a combination of the two forms of the indicator. Therefore the total absorbance A can *also* be written as:

$$A = \{\epsilon_{\text{HIn}} \times [\text{HIn}] \times l\} + \{\epsilon_{\text{In}^-} \times [\text{In}^-] \times l\} \quad \text{Equation 4}$$

Combining Equations 3 and 4:

$$\epsilon \times [\text{In}] \times l = \{\epsilon_{\text{HIn}} \times [\text{HIn}] \times l\} + \{\epsilon_{\text{In}^-} \times [\text{In}^-] \times l\}$$

$$\epsilon \times [\text{In}] = \{\epsilon_{\text{HIn}} \times [\text{HIn}]\} + \{\epsilon_{\text{In}^-} \times [\text{In}^-]\} \quad \text{Equation 5}$$

Keep in mind that only the total concentration of the indicator, $[\text{In}]$, is a measurable quantity and the individual concentrations of the two forms of the indicator, $[\text{HIn}]$ and $[\text{In}^-]$, are not easily measured.

Based on Equation 2, $[\text{In}^-] = [\text{In}] - [\text{HIn}]$. Substituting this in Equation 5:

$$\{\epsilon \times [\text{In}]\} = \{\epsilon_{\text{HIn}} \times [\text{HIn}]\} + \{\epsilon_{\text{In}^-} \times ([\text{In}] - [\text{HIn}])\}$$

$$\{\epsilon \times [\text{In}]\} = \{\epsilon_{\text{HIn}} \times [\text{HIn}]\} + \{\epsilon_{\text{In}^-} \times [\text{In}]\} - \{\epsilon_{\text{In}^-} \times [\text{HIn}]\}$$

$$\{\epsilon \times [\text{In}]\} - \{\epsilon_{\text{In}^-} \times [\text{In}]\} = \{\epsilon_{\text{HIn}} \times [\text{HIn}]\} - \{\epsilon_{\text{In}^-} \times [\text{HIn}]\}$$

$$[\text{In}](\epsilon - \epsilon_{\text{In}^-}) = [\text{HIn}] (\epsilon_{\text{HIn}} - \epsilon_{\text{In}^-})$$

$$[\text{HIn}] = [\text{In}] \left(\frac{\epsilon - \epsilon_{\text{In}^-}}{\epsilon_{\text{HIn}} - \epsilon_{\text{In}^-}} \right) \quad \text{Equation 6}$$

Similarly, based on Equation 2, $[\text{HIn}] = [\text{In}] - [\text{In}^-]$. Substituting this in Equation 5 gives:

$$[\text{In}^-] = [\text{In}] \left(\frac{\epsilon_{\text{HIn}} - \epsilon}{\epsilon_{\text{HIn}} - \epsilon_{\text{In}^-}} \right) \quad \text{Equation 7}$$

Dividing Equation 6 by Equation 7 gives:

$$\frac{[HIn]}{[In^-]} = \left(\frac{\varepsilon - \varepsilon_{In^-}}{\varepsilon_{HIn} - \varepsilon} \right) \quad \text{Equation 8}$$

Substituting the right hand side from Equation 8 into Equation 1:

$$\log \left(\frac{\varepsilon - \varepsilon_{In^-}}{\varepsilon_{HIn} - \varepsilon} \right) = -\text{pH} + \text{pK}_{IN} \quad \text{Equation 9}$$

Three assumptions are required to further manipulate Equation 9.

- 1) The absorbance at pH far below the pK_{IN} , lets say A_L , is mostly from HIn the predominant species at this pH.
- 2) The absorbance at pH much higher than the pK_{IN} , lets say A_H , is mostly from In^- the predominant species at this pH.
- 3) The total concentration of the indicator at low pH is simply the concentration of HIn and the total concentration of the indicator at high pH is the concentration of In^- .

Therefore:

$$A_L = \varepsilon_{HIn} \times [In] \times l \text{ and therefore: } \varepsilon_{HIn} = \frac{A_L}{[In] \times l} \quad \text{Equation 10}$$

$$A_H = \varepsilon_{In^-} \times [In] \times l \text{ and therefore: } \varepsilon_{In^-} = \frac{A_H}{[In] \times l} \quad \text{Equation 11}$$

At all other pH values, the absorbance is:

$$A = \varepsilon \times [In] \times l \text{ and therefore: } \varepsilon = \frac{A}{[In] \times l} \quad \text{Equation 12}$$

Substituting Equations 10, 11, and 12 into Equation 9 gives the following:

$$\log \left(\frac{A - A_H}{A_L - A} \right) = -\text{pH} + \text{pK}_{IN} \quad \text{Equation 13}$$

Experimental Design

In the first part of the experiment the wavelength at which all absorbance values are to be measured will need to be determined. In order to do this, the indicator-bromocresol green should be placed in a solution of the highest pH provided and the visible spectrum of this solution should be obtained by measuring the absorbance at 10nm intervals. The plot of absorbance vs. wavelength will show two wavelengths at which absorbance maxima are seen. The higher of these wavelengths should be used for all subsequent absorbance measurements, as this wavelength corresponds to where the compound is most sensitive to light and therefore most reliably detected.

In the second part of this experiment, the indicator bromocresol green will be placed in a medium of low pH value to determine A_L in Equation 13. The same concentration of the indicator will then be placed in a medium of high pH to determine A_H in Equation 13. Then the total absorbance of the indicator, A will be measured at different pH values; keeping the concentration of the indicator the same throughout the experiment. The data of total absorbance, A vs. pH will be used in Equation 13 to graphically determine the pK_{IN} .

Reagents and Supplies

Bromocresol green, solutions A (boric acid/citric acid) and B (sodium phosphate) (combinations of which will be used to generate solutions of pH values from 1 to 14), spectrophotometer

(See posted Material Safety Data Sheets)

Procedure

PART 1: DETERMINATION OF WAVELENGTH AT WHICH ABSORBANCE VALUES ARE TO BE MEASURED

1. Obtain a spectrophotometer and turn the power on and let the instrument warm up for about 10 minutes.
2. In a large test tube, combine 1.80 mL of solution A, 6.00 mL of solution B, and 0.30 mL of the indicator and thoroughly mix the contents. This will be the "sample".
3. In another large test tube, combine 1.80 mL of solution A, 6.00 mL of solution B, and 0.30 mL of deionized water and thoroughly mix the contents. This will be the "blank".
4. Obtain two cuvettes. Clean and dry the cuvettes and be sure to wipe the sides of the cuvettes with a kimwipes.
5. The instructor will demonstrate the proper use of the spectrophotometer.
6. Set the wavelength of the spectrophotometer to 370 nm.
7. With the sample chamber completely empty, close the lid and adjust the absorbance reading to zero by using the appropriate knob.
8. Place the cuvette containing the "blank" inside the sample compartment, align the guide mark on the cuvette with the guide mark at the front of the sample compartment, close the lid and adjust the absorbance reading to 100 using the appropriate knob..
9. Now place the cuvette containing the "sample" inside the sample compartment, align the guide mark on the cuvette with the guide mark at the front of the sample compartment, close the lid and record the absorbance.
10. Change the wavelength to 380 nm and then repeat steps 7 through 9. Continue this process in 10 nm increments of the wavelength, until absorbance values have been recorded at 700 nm.
11. Empty the contents of the cuvettes into a large beaker labeled as "Waste". Rinse and clean the cuvettes.
12. Plot a graph of Absorbance (y-axis) vs. wavelength (x-axis).

13. From the plot determine the wavelength at which the second absorbance maximum is found. This wavelength will be used for all subsequent measurements.

PART 2: DETERMINATION OF ABSORBANCE OF INDICATOR AT DIFFERENT PH VALUES

1. Obtain a spectrophotometer and turn the power on and allow the instrument to warm up for at least 10 minutes.
2. Obtain 14 large test tubes and number them from 1 to 14.
3. Combine solution A, solution B, and the indicator in each of the test tubes according to the following table. Mix the contents of the test tubes thoroughly. These will be the “sample” test tubes.

Solution Number	pH	Solution A (mL)	Solution B (mL)	Indicator (mL)
1	2.0	7.80	0.00	0.30
2	3.0	7.00	0.80	0.30
3	3.5	6.60	1.20	0.30
4	4.0	6.20	1.60	0.30
5	4.5	5.80	2.00	0.30
6	5.0	5.40	2.40	0.30
7	5.5	5.10	2.80	0.30
8	6.0	4.80	3.20	0.30
9	6.5	4.40	3.50	0.30
10	7.0	4.00	3.80	0.30
11	8.0	3.40	4.40	0.30
12	9.0	2.80	5.00	0.30
13	10.0	2.20	5.60	0.30
14	11.0	1.80	6.00	0.30

4. Obtain 14 large test tubes and number them from 1 to 14 and prepare “blank” solutions corresponding to each sample prepared in step 3.
5. Set the wavelength of the spectrophotometer to the value determined from Part 1 of the experiment.
6. Record the absorbance values of each of the 14 samples (use the method described in Part 1 (steps 7 through 9)).
7. Record the color of each sample solution (labeled 1 to 14)
8. The absorbance value of Solution 1 will be used as A_L .
9. The absorbance value of Solution 14 will be used as A_H .

Data TablePART 1: DETERMINATION OF WAVELENGTH AT WHICH ABSORBANCE VALUES ARE TO BE MEASURED

Wavelength (nm)	Absorbance
370	
380	
390	
400	
410	
420	
430	
440	
450	
460	
470	
480	
490	
500	
510	
520	
530	
540	
550	
560	
570	
580	
590	
600	
610	
620	
630	
640	
650	
660	
670	
680	
690	
700	

PART 2: DETERMINATION OF ABSORBANCE OF INDICATOR AT DIFFERENT pH VALUES

Solution	Color	pH	Absorbance
1		2.0	
2		3.0	
3		3.5	
4		4.0	
5		4.5	
6		5.0	
7		5.5	
8		6.0	
9		6.5	
10		7.0	
11		8.0	
12		9.0	
13		10.0	
14		11.0	

Data Analysis

PART 1: DETERMINATION OF WAVELENGTH AT WHICH ABSORBANCE VALUES ARE TO BE MEASURED

1. Draw a graph of Absorbance (y-axis) vs. wavelength (nm).
2. From the plot, identify the wavelength at which the maximum absorbance occurs. There will be two absorbance maxima in this spectrum. Use the larger of the two wavelengths for all subsequent measurements.

PART 2: DETERMINATION OF ABSORBANCE OF INDICATOR AT DIFFERENT PH VALUES

Much of the analysis for this part can be done using a spreadsheet. The goal is to determine the pK_{IN} and this will be done using Equation 13:

$$\log\left(\frac{A - A_H}{A_L - A}\right) = -pH + pK_{IN}$$

A comparison of the above equation to the equation of a straight line: $y = mx + b$ indicates that the variables y and x in the equation of the line correspond to $\log\left(\frac{A - A_H}{A_L - A}\right)$ and pH , respectively. Therefore, a plot of $\log\left(\frac{A - A_H}{A_L - A}\right)$ (on the y-axis) and pH (x-axis) when fit to the equation of a straight line will yield a slope of -1 (negative one) and a y-intercept corresponding to pK_{IN} .

The value of A_H is the absorbance value of solution 14 and the value of A_L is the absorbance value of solution 1.

The following table shows the entry of information in the spreadsheet.

A	B	C	D	E	F	G	H
A _H	Enter value						
A _L	Enter value						
Solution	Absorbance (A)	A - A _H	A _L - A	(A-A _H)/(A _L -A)	log((A-A _H)/(A _L -A))	pH	log((A-A _H)/(A _L -A))
2	Enter value	Enter =(B6-B1)	Enter =(B2-B6)	Enter =(C6/D6)	Enter =log(E6)	Enter pH value here	Enter =F6
3	Enter value						
4	Enter value						
5	Enter value						
6	Enter value						
7	Enter value						
8	Enter value						
9	Enter value						
10	Enter value						
11	Enter value						
12	Enter value						
13	Enter value						

To plot a graph and obtain the equation of the curve fit line:

1. Select the highlighted cells from the table (the values containing the x and y data that will be plotted).
2. Select "Insert Chart"
3. Choose "XY Scatter"
4. Click on any data point on the scatter plot that appears.
5. From the "Chart" menu, select "Add Trendline"
6. In the "Options" menu, check: 1) display equation and 2) display R-squared value
7. The y-intercept of the equation is the pK_{IN}

Results

1. Include the graph drawn using data from Part 1 of the experiment.
2. The wavelength chosen for measurement of absorbance values = _____ nm
3. Include the spreadsheet and the plot of the data showing the curve fit line.
4. The experimental value of the pK_{IN} for bromocresol green = _____
5. The theoretical value of the pK_{IN} for bromocresol green = _____
(Indicate source material used to obtain this value)

Source:

6. The percent error in the measurement = _____
(Show calculation for percent error)