Spectrophotometric Titration of Cabbage Extract Using a Lego-Smartphone Spectrophotometer

Introduction

Acid-Base Indicators are compounds that are added to a solution to estimate the solutions pH from the color they exhibit. Acid-base indicators are themselves acids or bases. They are compounds that are selected because they undergo intense and distinctive color changes when they react to form their conjugate acid or base. Acid-base indicators are generally placed in solutions at very low concentrations so that their acid or base character contributes a negligible amount of H^+ or OH^- to the solution and therefore does not measurably affect the pH. The color of the solution created by the indicator reflects the prevailing pH due to the presence of other acids or bases at much higher concentrations.

In this experiment you will be working with *Cabbage Extract* as a pH indicator. Cabbage extract contains compounds known as *Anthocyanins* that are present in many fruits and vegetables giving them their natural color. Anthocyanins are red in solutions that are acidic and change to shades of purplish blue as the solutions become more basic. The different colors of anthocyanin in acidic and basic solutions are shown in Figure 1.

In this experiment you will perform a titration of an NaOH solution into an HCl solution. Both solutions will be prepared with identical (relatively low) concentrations of cabbage extract. Therefore, as the solutions are mixed the concentration of cabbage extract will not change, but the pH will, as the NaOH and HCl react. A graph of *Absorbance of 630 nm versus pH* will be prepared. This graph is a titration curve, which is expected to be roughly "S shaped". The pH at which the inflection point of this curve occurs is approximately the pK_a of the anthocyanins in the cabbage extract because this is the point where B/A ~ 1.0, so

$$pH = pK_a + \log\left(\frac{B}{A}\right)$$

$$pH = pK_a + \log(1)$$

$$pH = pK_a + 0$$

$$pH = pK_a$$

Figure 1. Cabbage extract containing anthocyanins. The cabbage extract solid is sold as a food ingredient to be added to shakes and smoothies. It is an intensely red solid. When dissolved in basic solutions it is a blueish/purple color (left) when dissolved in acidic solutions it is a reddish color (right).

If the data is too "noisy" to clearly locate an inflection point you can estimate the pK_a as the pH where the curve is about halfway between the absorbance of the flat region of low pH and the flat region of high pH.

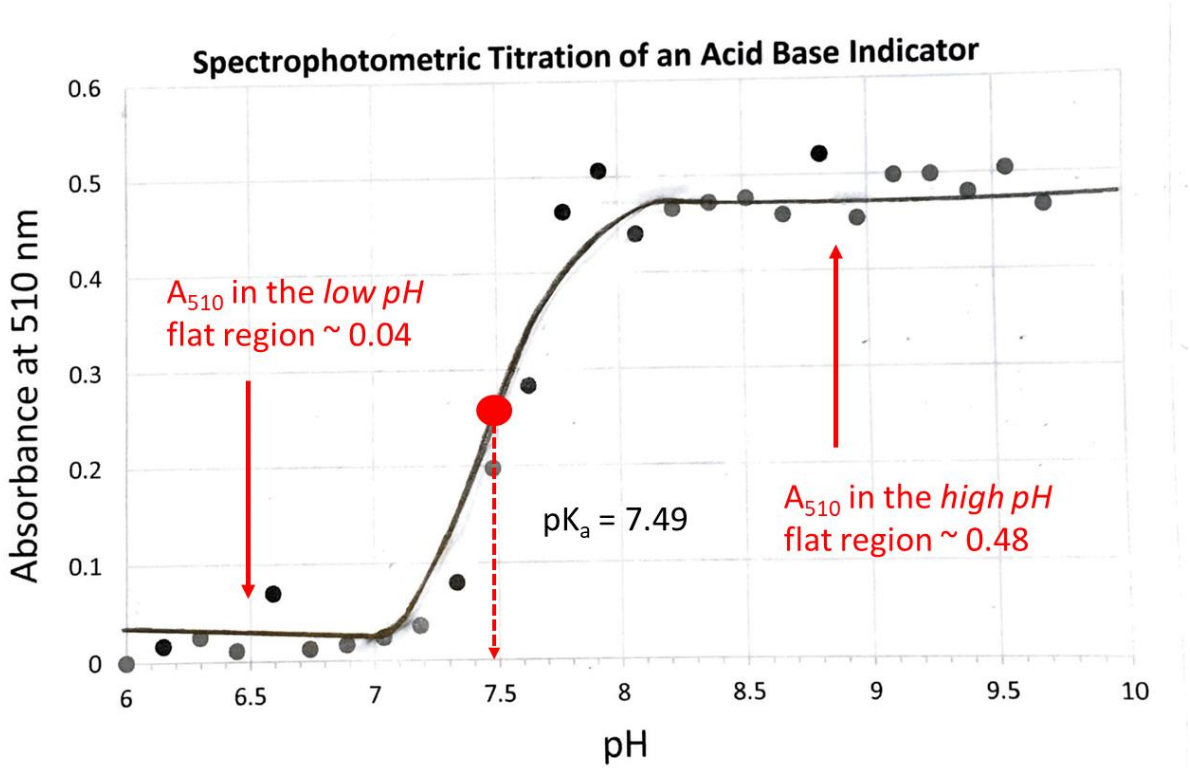

Figure 2. Spectrophotometric titration of an indicator (*not* the one used in this experiment) showing how the pK_a was estimated graphically.

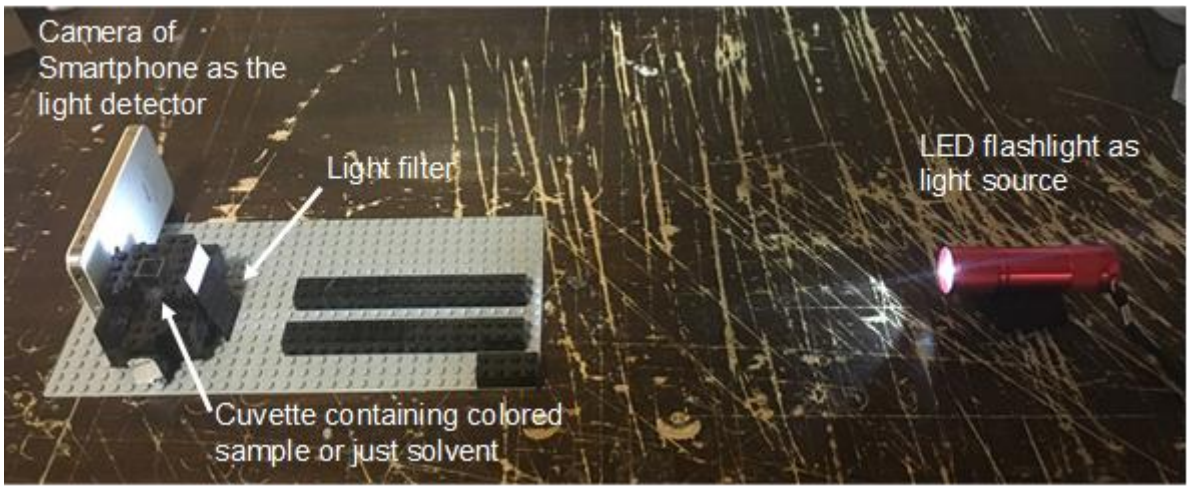

Figure 3. Spectrophotometer built from Legos, LED flashlight, plastic light filter, and a smartphone.

Experimental Procedures

Part I.

Preparation of Stock Solutions of NaOH and HCl with 1.0 mg/mL of Cabbage Extract

1. From your kit obtain the two 50 mL screw lid test tubes with the 1.0×10^{-4} M HCl and the 1.0×10^{-4} M NaOH.

2. Using clean droppers to remove solution from each of these until the total volume remaining is 45 mL as read from the markings on the tubes. Discard the solution removed down the sink.

3. From your kit obtain the Eppendorf tube containing the Cabbage Extract, the digital scale and the 100 mL graduated cylinder.

4. Place the 100 mL graduated cylinder on the scale and turn it on. If it does not read zero press the *Tare* button.

5. Shake all the cabbage extract into the 100 mL graduated cylinder.

6. Record the mass of the Cabbage Extract in the space provided for data.

7. Calculate the volume of solution that this mass of cabbage extract must be dissolved in, in order to prepare a 10 mg/mL solution. The volume should be less than 100 mL. If your calculation is not in this range consult your instructor.

8. Add water to the 100 mL graduated cylinder until the total volume is the volume you calculated in the previous step. Use a dropper to carefully adjust to this volume and not overshoot it.

9. Stir or shake this solution to dissolve the cabbage extract.

10. Using a dropper add 5 mL of this 10 mg/mL solution of cabbage extract to both the 1.0×10^{-4} M HCl and the 1.0×10^{-4} M NaOH by using the markings on the tube. In other words, add until each tube has a total volume of 50. mL

11. Place the caps on these tubes and gently invert the tubes to mix them.

Part II. Alignment and setup of the spectrophotometer

1. Setup/assemble the spectrophotometer as described in the video sent by your instructor for the previous experiment.
2. Use two small pieces of double stick tape to hold down the spectrophotometer to the table.
3. Turn on the app (*Color Assist* for Apple users or *Color Grab* for Android users).
4. Position the flashlight approximately 24 inches from the spectrophotometer pointing at the cuvette holder.
5. Place the 630 nm filter in front of the cuvette holder.
6. Fill a cuvette with the 1.0×10^{-4} M HCl with 1.0 mg/mL cabbage extract you prepared in Part I and Label it *Blank Cuvette* along its opaque edge using your Sharpie.
7. Place the Blank Cuvette in the cuvette holder
8. Turn on the flashlight. Whenever turning the flashlight on or off throughout this procedure gently hold it down with one hand and gently press the on/off button to minimize its movement with the other hand.
9. Adjust the distance of the flashlight from the spectrophotometer so that the image of the light from the flashlight on the screen is smaller than the rectangle in which data is collected.
10. Slide the flashlight slightly left and right and turn it back and forth to obtain the maximum light intensity (the *R* value of the RGB values displayed). Be sure as you move it that the image remains within the data collection rectangle.
11. If the light intensity is over 200 counts place one or if necessary two *Neutral Density Filters* from your kit in the path of the light along with the 630 nm filter. If it is still greater than 200 slide the flashlight further away from the spectrophotometer or if that is not possible due to space limitations tilt it slightly so that it does not point directly at the center of the light filters.
12. Once you have achieved a light intensity between 150 and 200 counts use Scotch tape from your kit to tape down the flashlight to the table so that it moves minimally when you turn it on and off. As soon as it is taped down verify that the light intensity is still in the range 150-200 counts. If it has changed undo the tape, reposition the flashlight and tape it down again. Repeat this if necessary, until the flashlight is taped down and the light intensity is between 150-200.
13. Record the light intensity after it is taped down with the Blank cuvette in the cuvette holder as both I and I_o for *Addition #0 (1.0 × 10-4 M HCl with 1.0 mg/mL Cabbage Extract)*

Part III. Spectrophotometric Titration of Cabbage Extract

1. Measure 22 mL of the 1.0×10^{-4} M HCl with 1.0 mg/mL cabbage extract solution using your 100 mL graduated cylinder. Pour it into a small Dixie cup labeled *Reaction Container #1*. Note: if you have a coffee mug available place the Reaction Container #1 Dixie cup in the mug so that when you place the pH meter into it will not be knocked over easily.

2. Measure 22 mL of the 1.0×10^{-4} M NaOH with 1.0 mg/mL cabbage extract solution using your 100 mL graduated cylinder. Pour it into a small Dixie cup labeled *Reaction Container #2*. Note: if you have a coffee mug available place the Reaction Container #2 Dixie cup in the mug so that when you place the pH meter into it will not be knocked over easily.

3. Calibrate your pH meter by turning it on, placing it in 30 mL of pH 6.86 buffer in a small Dixie cup and holding the *Cal* button for 5 seconds. It should blink *6.86* several times, after it does it is calibrated and ready to use.

4. Rinse the pH meters electrode with distilled water from your squirt bottle.

5. Place the pH meter in the 1.0×10^{-4} M HCl with 1.0 mg/mL cabbage extract in the Dixie cup labeled "Reaction Container #1". Wait 1-2 minutes for the values displayed to stop changing. During this time gently stir the solution by moving the pH meter every 15-20 seconds.

6. Record the pH observed after waiting and stirring as the value for *Addition #0* in Table 1.

7. Rinse the pH meters electrode with distilled water.

8. Approximately half-fill a small Dixie cup with the 1.0×10^{-4} M NaOH with 1.0 mg/mL cabbage extract.

9. Place the pH meter into this small Dixie cup containing the 1.0×10^{-4} M NaOH with 1.0 mg/mL cabbage extract. Wait 1-2 minutes for the values displayed to stop changing. During this time gently stir the solution by moving the pH meter. Every 15-30 seconds.

10. Record the pH as the value for the last trial *1.0×10^{-4} M NaOH with 1.0 mg/mL Cabbage Extract* in Table 1.

11. Rinse the pH meters electrode with distilled water.

12. Fill your other cuvette with the 1.0×10^{-4} M NaOH with 1.0 mg/mL cabbage extract you prepared in Part I. Label this cuvette *Reaction Cuvette* along its opaque edge using your Sharpie.

13. The Blank Cuvette should be in the cuvette holder at this point. If it is not place it there.

Try to perform the next four steps relatively quickly

14. Turn on the flashlight.

15. Record the light intensity with the Blank cuvette in the cuvette holder as both I and I_o for *Addition #0 (1.0 × 10-4 M HCl with 1.0 mg/mL Cabbage Extract)*

16. Taking care to not bump or move the spectrophotometer or the flashlight switch cuvettes and place the *Reaction Cuvette* containing the *1.0 × 10^{-4} M NaOH with 1.0 mg/mL Cabbage Extract* into the cuvette holder. Record this value in Table 1 as *I* for the last trial *1.0 × 10^{-4} M NaOH with 1.0 mg/mL Cabbage Extract* in Table 1.

17. As soon as you have recorded I and I_o turn off the flashlight.

18. Pour the contents of the *Reaction Cuvette* back into the container with the rest of the 1.0 × 10^{-4} M NaOH with 1.0 mg/mL Cabbage Extract.

19. Rinse out the Reaction Cuvette with distilled water and shake out excess water.

20. Take a clean pipette and locate its 3.0 mL marking. Use your *Sharpie* to make the marking very visible. If you pipette has no marking use your ruler and mark with a *Sharpie* about 4 inches from the tip.

21. Fill the pipette up to the 3.0 mL Sharpie mark with 1.0 × 10^{-4} M NaOH with 1.0 mg/mL Cabbage Extract.

22. Add this volume to the small Dixie cup labeled *Reaction Container #1* and gently swirl the Dixie cup to mix the contents.

23. Place the pH meter into this solution. After stirring and allowing the pH meter to equilibrate record the pH as the value for *Addition #1*.

24. Remove the pH meter from the solution and lay it on the tabletop.

25. Fill the cuvette labeled Reaction Cuvette with the solution from the Dixie cup labeled Reaction Container #1 to which you have just added the 2.0 mL of *1.0 × 10^{-4} M NaOH with 1.0 mg/mL Cabbage Extract.*

26. Place the *Blank Cuvette* into the cuvette holder and

Try to perform the next four steps relatively quickly

27. Turn on the flashlight.

28. Read the light intensity and record this value as I_o for *Addition #1* in Table 1.

29. Taking care to not bump or move the spectrophotometer switch cuvettes and place the *Reaction Cuvette* into the cuvette holder. Read the light intensity and record this value in Table 1 as *I* for *Addition #1* in Table 1.

30. Turn off the flashlight.

31. Pour the contents of the *Reaction Cuvette* back into the small Dixie cup labeled *Reaction Container #1*.

32. Repeat steps 20-31 until all of the *1.0×10^{-4} M NaOH with 1.0 mg/mL Cabbage Extract* has been used. *This should be about 9 or 10 additions.*

33. Clean out the dropper you have marked with the 3.0 mL on with the sharpie by drawing up clean water into it, shaking it and squirting the water down the sink several times.

34. Repeat Steps 20-31 except switch to adding the *1.0×10^{-4} M HCl with 1.0 mg/mL Cabbage Extract to Reaction Container #2*. Continue recoding this data in the table right after the last addition recorded in step 32. Perform 10 additions.

35. For all of the additions calculate the Absorbance (A) at 630 nm as:

$$A = -log\left(\frac{I}{I_o}\right)$$

36. Prepare a graph of A versus pH.

37. Estimate the pK_a of the anthocyanins in cabbage extract from the graph as described in the introduction.

Data

Mass of Cabbage Extract_____g

Calculation

Show below how you calculated the volume of solution required to prepare a 10 mg/mL solution of cabbage extract using the mass of cabbage extract above.

pK_a of anthocyanins in cabbage extract estimated from the graph_____

Table 1.

Addition #	pH	I_o	I	A
0 (1.0×10^{-4} M HCl with 1.0 mg/mL Cabbage Extract)				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
1.0×10^{-4} M NaOH with 1.0 mg/mL Cabbage Extract				

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