Chapter 5 -- The Spectrophotometric Determination of the pH of a Buffer	NAME:	
	Lab Section:	
	Date:	Sign-Off:

<mark>Chapter 5 -- The Spectrophotometric Determination of the pH of a Buffer</mark>

Introduction

Weak acids, represented by HA, dissociate readily into cationic and anionic products as shown below.

$$HA \Leftrightarrow H^+ + A^-$$

The dissociation may be expressed arithmetically as shown below.

$$K_d = \frac{[H^+][A^-]}{[HA]}$$

Indicators (used to identify specific end points by color change during titrations with acids and bases) are weak acids and bases which dissociate readily into different colored species. Indicators, represented by HIn, dissociate as show below (the dissociation expression is also shown below):

$$HIn \Leftrightarrow H^+ + In^-$$

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

The most commonly used indicator, phenolphthalein, is colorless until the pH is between 7 and 8, at which point it turns pink.

Interestingly enough, a change in color is what a spectrophotometer measures. A change in color means that some amount of light being shined through a sample will be either decreased by absorption of the light by the colored compound, or not affected by transmission of the light through a colorless solution. How then, does a spectrophotometer work? As you may observe below,
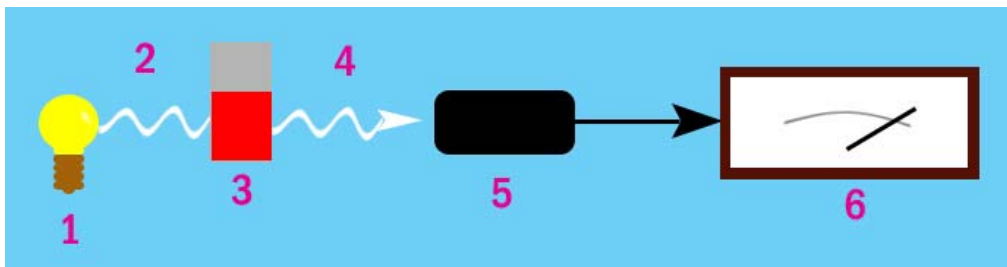
a light source (1) shines light of original intensity I_o (2) through a sample (3). The intensity I (4) which comes through the sample (4) is detected by a detector (5) and then sent to some kind of meter (6) to be quantitated.

How does one make use of this information? It turns out that the ratio of the intensities are logarithmically related to the size of the sample holder (cuvette), the concentration of the sample (moles/liter) and a constant for each molecule (molar absorptivity). The log of the ratio of the two intensities is equal to the absorbance. The absorbance is related to the transmittance (see below for equation), also logarithmically.

$$\frac{\log I_o}{\log I} = A = -\log T$$

Thus far, we have not seen why this information is useful. How is this information useful in determining anything with a spectrophotometer? It turns out that in the late 1800's, Beer, Lambert and Bougier showed that there was a linear relationship between absorbance (A), molar absorptivity (a), the path length of the cuvette (b) and the molar concentration of the analyte (c). The equation below illustrates what has collectively become the Beer-Lambert-Bougier Law:

$$A = abc$$

Briefly, the relationship says that if one plots the absorbance (A) of a known standard versus the concentration (c) of the standard, one gets a "standard curve" which 1) is linear, or straight, over a specific range, and 2) can be used to determine the concentration of unknown solutions.

This still is not really helpful information, for this requires a single wavelength and assumes only 1 form of analyte. Our experiment mandates two chemical forms of the analyte and suggests that if there are two chemical forms of analyte, we may require 2 wavelengths to determine the data necessary to solve our experiment. Indeed, not only will we require two wavelengths, we will have to perform a wavelength scan (see sample scans below) in

order to determine which two wavelengths to use. Additionally, we will scan two forms of our analyte: an acid form and an alkaline form.

Experimental

In this method, the absorptivities of the acid and base forms of an indicator are determined at two wavelengths. The concentration of each form in an unknown buffer is then determined by spectral measurement at two wavelengths. The hydrogen ion concentration can then be calculated from these data.

Reagents: Bromothymol blue indicator (BTB); 0.3 M HCl, 0.3 M NaOH.

Supplies: disposable cuvettes (3), Parafilm, disposable pipets (3), test tube rack, scanning spectrophotometer.

Procedure: Determination of individual absorption spectra. Transfer several drops of your bromothymol blue indicator to two disposable cuvettes. To one cuvette, add approximately 1 mL of 0.3 M HCl; to the other, add about 1 mL of 0.3 M NaOH. Mix each solution thoroughly using Parafilm and holding the cuvette by its top and bottom – not its sides. Obtain the absorption spectra for the acid and base forms of the indicator between 400 and 600 nm, using water or air as a blank. Evaluate the absorptivity for each species at wavelengths that correspond to their respective absorption maxima.

Determination of the pH of an unknown buffer: Transfer several drops of your bromothymol blue indicator to another disposable cuvette (if you plan ahead, you may set up all three cuvettes at the same time). Add about 1 mL of the unknown buffer to your indicator in the cuvette. Measure the absorbance of the unknown solution at the wavelengths for which absorptivity data were calculated. **Report the pH of the buffer** and show your work.

Data Manipulation

Given that:

$$A = abc,$$

it must follow that the total absorbance of a solution is equal to the sum of its parts, i.e., :

$$A_T = A_1 + A_2 + A_n, \text{ and hence,}$$

$$A_T = a_1 bc_1 + a_2 bc_2 + a_n bc_n + ...$$

where "A_T" is total absorbance, A_1 is the absorbance due to the first component, A_2 for the second, *etc.*, and $a_1 bc_1 = A_1$, *etc.*

Therefore, the absorbance of a solution at some specific wavelength, λ, of an indicator must be equal to the sum of the absorbances of the acidic and basic forms at that wavelength:

$$A_T = A_A + A_B$$

Where A_A is the absorbance due to the acidic form of the indicator and A_B is the absorbance due to the alkaline form of the indicator.

Since b is a constant (1 cm), this equation reduces to:

$$A_\lambda = a_A c_A + a_B c_B$$

Where the subscript "A" is the acidic form and "B" is the alkaline (basic) form of the indicator.
If the concentrations are known, and one variable falls out, there is one equation and one unknown: therefore, the molar absorptivity may be solved for.

Let's use the following data derived from the sample scans as an example of solving for the molar absorptivities of the acidic [HCl] and alkaline [NaOH] forms of the indicator (concentration of the acid and base is 0.30M, each; vertical axis is Absorbance; horizontal axis is wavelength in nm):

Acid Form of BTB	Base Form of BTB
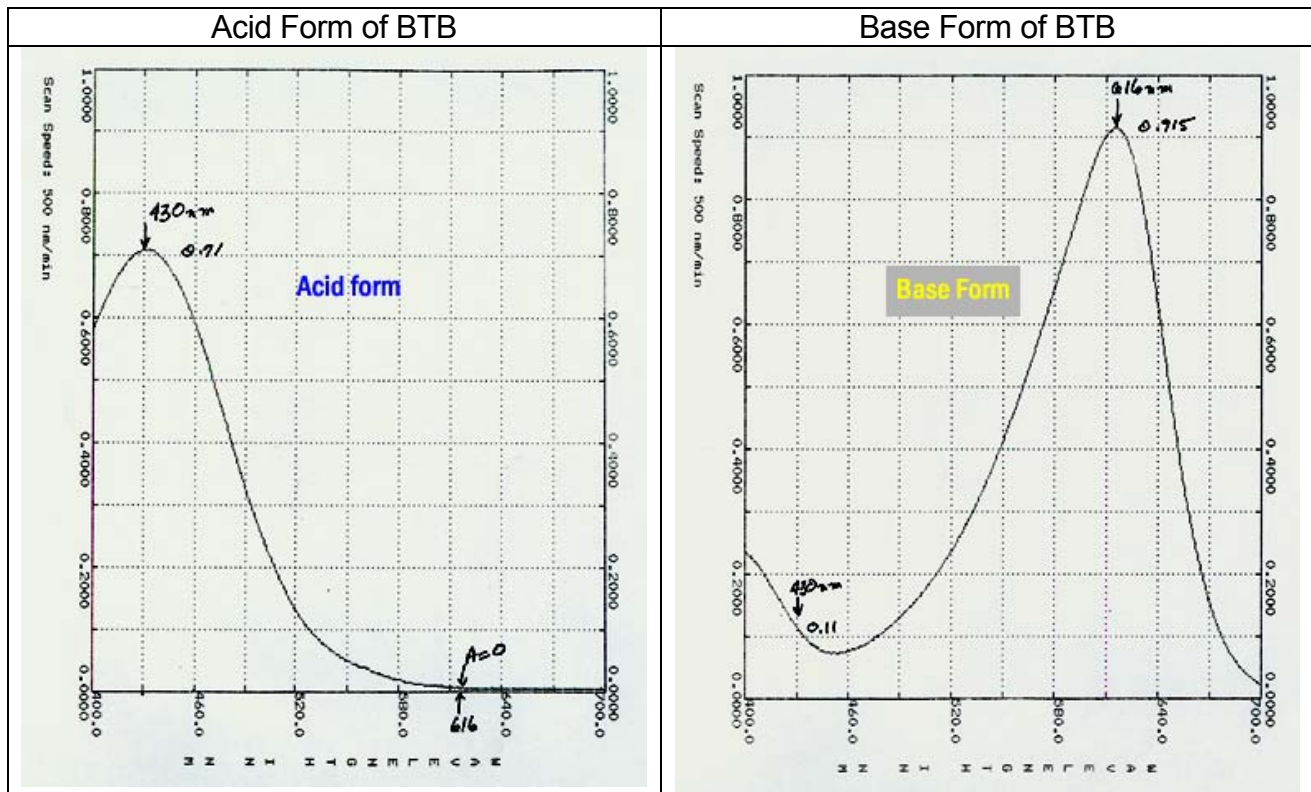
| λ, nm	A_{Acid}	A_{Base}	c_{Acid}	c_{Base}
430	0.71	0.11	0.3M	0.3M
616	0	0.915	0M	0.3M

where c_A is the concentration of HCl and c_B is the concentration of the NaOH.

At 430 nm, $a_{Acid} = 0.71/0.3 = 2.37$ and $a_{Base} = 0.11/0.3 = 0.367$.

Calculations at 616 nm are performed in the same manner, giving the following table:

λ, nm	A_{Acid}	C_{Acid}	a_{Acid}	A_{Base}	C_{Base}	a_{Base}
430	0.71	0.30	2.37	0.11	0.30	0.367
616	0	0	0	0.915	0.30	3.05

The buffer sample had an absorbance of 0.81 at 430 nm and 0.68 at 616 nm (axes as above):

Buffered Form of BTB

$$A_{616} = 0.68 = a_A c_A + a_B c_B$$
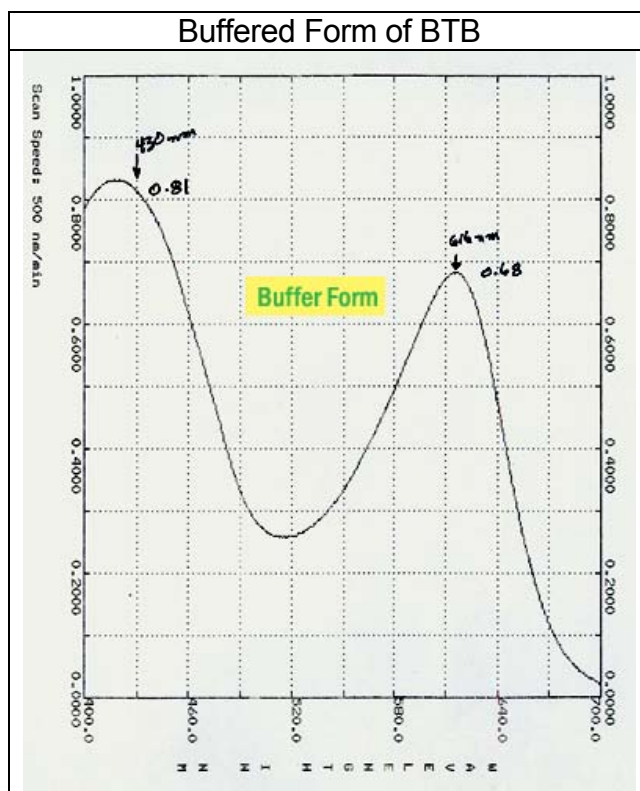
Since a_A = 0, the first term falls out, leaving the second. Hence, the concentration of the base is 0.223 M. Using the concentration of the base from the last equation, solve for the concentration of the acid at 430 nm, inserting the determined C_B into its expression:

$$A_{430} = 0.81 = a_A c_A + a_B c_B$$

$$0.81 = (2.37)c_A + (0.4)(0.223)$$

$$c_A = 0.304 \text{ M}$$

Now substitute in the concentration of HCl for [HIn] and the concentration of NaOH for [In⁻] in the dissociation expression:

$$K_d = \frac{[H^+][In^-]}{[HIn]} = \frac{[H^+]\,(0.223)}{(0.304)}$$

Our example was using bromthymol blue; the K_d = 1.199×10^{-7}.

$$-\log \frac{[(1.199 \times 10^{-7})(0.304)]}{(0.223)} = 6.787$$

Hence, the pH of the buffer solution is 6.787; remember that the expression just previous is actually an expression for [H⁺], and pH = -log[H⁺]. Since the label on the buffer bottle said that the pH was 7.0, how might you explain this discrepancy?

This is how **it's supposed to work**. What happens when you have an indicator in some form which is not in low enough concentration to allow the absorbance, and hence the concentration, of at least one variable to go to zero? That is what is depicted in the following tables of scan data. The first table is from a scan of the acidic, alkaline and buffered forms of phenolphthalein (Note: no scans are illustrated, here):

λ, nm	A_{Acid}	C_{Acid}	a_{Acid}	A_{Base}	C_{Base}	a_{Base}
Phenolphthalein Data						
364	0.70	0.30	2.33	0.27	0.30	0.90
553	0.38	0.30	1.27	0.76	0.30	2.533

Notice that there are no A values which approach zero. This makes the solution of the problem most difficult when the buffered solution is examined at the two wavelengths. The respective absorbances of the buffered solution are 0.48 at 364 nm and 0.22 at 553 nm.

The same sort of data is obtained when doing the same thing with another indicator,

Methyl Orange. Again, the data show there is no single component of the Methyl Orange which approaches zero:

λ, nm	A_{Acid}	C_{Acid}	a_{Acid}	A_{Base}	C_{Base}	a_{Base}
Methyl Orange Data						
464	1.785	0.30	5.95	1.68	0.30	5.60
508	3.43	0.30	11.43	0.91	0.30	3.03

Notice that there are no A values which approach zero. This makes the solution of the problem most difficult when the buffered solution is examined at the two wavelengths. The respective absorbances are 2.345 at 464 nm and 1.173 at 508 nm, see above graphic. The use of augmented matrices does not simplify this process, at all.   Rather, an augmented matrix confounds the results for one of the solutions is *negative*.

After examining the data from the previous two buffers (phenolphthalein and Methyl Orange), it is hopefully painfully obvious that neither are useful in the range of 400-600 nm for this experiment. Hence, in spite of the fact that phenolphthalein is one of the most commonly utilized acid-base indicators in titrations, it is not particularly useful for the spectrophotometric determination of the pH of a solution. This is why bromothymol blue is used in the experiment.

Beckman Operating Procedure for Scans

The following is the operating procedure, a step at a time, for a wavelength scan on the Beckman spectrophotometer following software transfer to a newer computer as of 2 April 2004. Fixed wavelength data acquisition is a slight modification of this procedure -- once you're in the appropriate menu (it follows the old technique by the numbers). NOTE: once I have some time, I'll write an autoexec file to speed the process up.

1. Turn on computer and spectrophotometer -- that includes the appropriate light source, e.g., VIS or UV or both.
2. Once the computer is on, hit "Enter" after the date prompt.
3. Hit "Enter" after the time prompt.
4. At "C:\>", type "cd\datalead", and hit "Enter".
5. At the prompt, type "dc".
6. And hit "Enter".
7. At the menu that comes up, put the cursor on "DU-60" and hit "Enter".
8. At the next menu that comes up, put the cursor on #2 and
9. Hit enter (for the scanning mode).
10. At the menu ON THE COMPUTER, enter your components and
11. Hit "Enter" between each parameter.
12. After the last parameter has been entered,

13. Hit the "F1" key to accept the parameters and
14. Bring up the data screen (an X,Y plotting area on the monitor).
15. Put an empty cuvet in the spectrophotometer and close the lid.
16. On the spectrophotometer's key pad, press "Calb" and
17. Let it run until it resets to your beginning wavelength.
18. Take the cuvet out of the spectrophotometer.
19. Put your blank reagent in the cuvet and
20. Replace in the sample holder in the spectrophotometer.
21. On the spectrophotometer's key pad,
22. Press "Read" and let it run until it resets at the beginning wavelength.
23. Remove the cuvet from the sample well and
24. Replace it with your actual sample.
25. On the spectrophotometer's key pad,
26. Press "Read" and let the scan run –
27. Take the printer "offline" until you're certain that you want this scan -- saves paper.
28. If you're satisfied with the detail on the scan you see on the monitor,
29. Put the printer BACK online so it will print and let it print.
30. When printing is completed, type "N" for "NO" and hit "Enter".
31. Start over with your parameter settings, i.e.,
32. If they are the same, simply confirm that and move on.
33. Put in your next sample, hit "Read" on the spectrophotometer's key pad,
34. Take the printer offline, yadda, yadda, yadda.
35. Repeat as necessary until your samples have been scanned.
36. When you are done scanning, hit "Esc" until you're back at the DOS screen.
37. At the DOS prompt, type "cd\" and hit "Enter".
38. Shut down computer and spectrophotometer.
39. Complete your calculations.