Lab Manual

Chem 332L
Experimental Analytical Chemistry

Fall 2010

TABLE OF CONTENTS

PRINCIPLES FOR SAFETY IN THE CHEMICAL LABORATORY

Safe practices in the chemical laboratory are of prime importance. A student should consider it an essential part of his or her educational experience to develop safe and efficient methods of operation in a lab. To do this, one must acquire a basic knowledge of properties of materials present in the lab, and one should realize the types of hazards that exist and the accidents and injuries that can result from ignorance or irresponsibility on the part of the student or a neighbor.

Regulations

1. Wear safety goggles at all times while in the laboratory.

2. Report all accidents to the instructor or lab assistant immediately.

3. **NEVER** eat, drink, chew, or smoke in the laboratory.

4. **NEVER** leave an experiment unattended. Inform the lab assistant if you must leave the lab.

5. After the experiment is completed, turn all equipment off, making sure it is properly stored, and clean your area.

Failure to comply with these regulations is cause for immediate dismissal from lab.

Precautions

1. Approach the laboratory with a serious awareness of personal responsibility and consideration for others in the lab.

2. Become familiar with the location of safety equipment, such as acid-base neutralizing agents, eye wash, fire extinguisher, emergency shower, and fire blanket.

3. Pay strict attention to all instructions presented by the instructor. If something is not clear, do not hesitate to ask the instructor or lab assistant.

4. Clean up all chemical spills immediately.

5. Be aware of all activities occurring within a reasonable proximity of yourself since you are always subject to the actions of others.

6. To avoid contamination of community supplies, do not use personal equipment such as spatulas in shared chemicals and replace all lids after use.

7. Avoid unnecessary physical contact with chemicals; their toxic properties may result in skin irritation.

8. Use all electrical and heating equipment carefully to prevent shocks and burns.

9. **NEVER** handle broken glassware with your hands; use a broom and a dust pan.

10. Wash your hands at the end of the laboratory.

Personal Attire

Choice of clothing for the laboratory is mainly left to the discretion of the student. Because of the corrosive nature of chemicals, it is in your best interest to wear comfortable, practical clothing. Long, floppy sleeves can easily come into contact with chemicals. A lab coat is suggested to help keep clothes protected and close to the body. Accessories also need consideration. Jewelry can be ruined by contact with chemicals. Open toed shoes do not adequately protect one against chemical spills. If hair is long enough to interfere with motion or observation, it should be tied back. Remember that your clothes are worn to protect you.

Assembling Equipment

Equipment should be assembled in the most secure and convenient manner. Utility clamps are provided to fasten flasks, etc., to the metal grid work located at the center of each bench. This keeps top-heavy or bulky equipment away from the edge where it can be knocked easily off the bench. Consider the safe location of the hot plate. Keep it near the grid work to minimize chances of contact with the body. If the aspirator is being used, locate your apparatus near the sink for convenience.

Handling Glassware

Laboratory glassware is usually fragile, and if it is not properly handled, serious injuries may result Do not force glass tubing or thermometers into a rubber stopper. Lubricate the tubing or thermometer with glycerol or water, wrap it in a towel, and gently insert it into the stopper by using pressure in a lengthwise direction while rotating it. Always grasp the tubing near the stopper. When removing the tubing, remember to protect your hands with a towel. If there are difficulties with this procedure, ask for the instructor's assistance. Apparatus that can roll should be placed between two immobile objects away from the edge of the bench. Chipped or broken glassware cannot be used. There are special receptacles near each bench for these waste materials. After the experiment is completed, all glassware should be emptied, rinsed, and cleaned.

Acids and Bases

In this lab sequence, you will come in contact with several acids and bases. As with all chemicals, caution must be taken to prevent contact with the skin. When handling these chemicals, keep hands away from the eyes and face until they have been thoroughly washed. If an acid or base comes in contact with your skin, flush the area with large quantities of clean, cold water. Eyes are extremely sensitive. Use the eye wash provided in the laboratory, or wash with water for at least 10 minutes. Again, the instructor must be notified immediately. To insure your safety, neutralize acid or base spills before cleaning them up. Boric acid solution is available to neutralize base spills, and carbonate powder is provided to neutralize acids.

Attention:

Students are advised against wearing contact lenses while observing or participating in science laboratory activities. While hard contact lenses do not seem to aggravate chemical splash injuries, soft contact lenses absorb vapors and may aggravate some chemical exposures, particularly if worn for extended periods.

Please take your contact lenses out prior to entering the laboratory.

Contact Lens Administrative Policy and Waiver Form

Students are advised against wearing contact lenses while observing or participating in science laboratory activities. While hard contact lenses do not seem to aggravate chemical splash injuries, soft contact lenses absorb vapors and may aggravate some chemical exposures, particularly if worn for extended periods. You are asked to please remove your contact lenses prior to entering the laboratory.

If you do not wish to comply with this recommendation, you must fill out the next page, which is a waiver form.

Waiver of Liability, Indemnification and Medical Release

I am aware of the dangers involved in wearing contact lenses in a science laboratory setting. On behalf of myself, my executors, administrators, heirs, next of kin, successors, and assigns, I hereby:

a. waive, release and discharge from any and all liability for my personal injury, property damage, or actions of any kind, which may hereafter, accrue to me and my estate, the State of South Dakota, and its officers, agents and employees; and

b. indemnify and hold harmless the State of South Dakota, and its officers, agents and employees from and against any and all liabilities and claims made by other individuals or entities as a result of any of my actions during this laboratory.

I hereby consent to receive any medical treatment, which may be deemed advisable in the event of injury during this laboratory.

This release and waiver shall be construed broadly to provide a release and waiver to the maximum extent permissible under applicable law.

I, the undersigned participant, acknowledge that I have read and understand the above Release.

Name _____ Age _____

Signature _____ Date _____

Is there any health information you would like us to know if there is an accident?

LABORATORY NOTEBOOKS

You are required to use a bound notebook in Chem 332L lab to record all primary data and observations. You should prepare your notebook each week before coming to lab by writing the title of the experiment on a new numbered page, summarizing relevant equations from the lab manual, and starting calculations involving molar masses, etc. Take note of theoretical ideas and special instructions given by your instructor at the start of each experiment. Your notebook should be a complete record of your work in lab. You or other chemists should be able to understand the notes in the future, not just during the current experiment Good note taking in lab is a valuable skill that you can learn with a little effort and practice.

Guidelines to be Followed:

1. Always bring your notebook with you to lab. You will be graded on the completeness of your previous note taking and your preparation for the current experiment. You may use your notebook during a lab quiz.

2. Number the pages sequentially and reserve space at the beginning for a table of contents.

3. Take your notebook to the balance room, etc. and record values directly in it - not on loose scraps of paper.

4. Specify each measured quantity by name and include the units.

5. If you make a mistake in your notebook, simply draw a solid line through the error and write the correction nearby.

6. Tables greatly simplify data entry; they should be set up before coming to lab.

7. Write down all observations such as color and phase changes - don't rely on your memory.

8. Save time by doing trial calculations in your notebook before filling out any report sheets.

9. Save time by making preliminary sketches of graphs on the ruled lines in your notebook.

Experiment 1: **STATISTICS**

Reading Assignment

All of Chapters 3&4. This lab will introduce the student to some of the basic statistics that will be used in the lab.

Materials

Each student will be given a package of M&M's (plain). Each student is to count the number of each color and determine the fraction (# color / # total) of each color for his or her pack of M&M's **before they are eaten.**

After you have entered these calculations in your notebook, a sheet will be passed around for you to enter your data into so we can tabulate the results for the entire lab. The sheet will look like this:

Desk #	Brown	Blue	Red	Orange	Yellow	Green
1	.55	.10	.50	.12	0.00	.18
2						
3						
4						
.						
.						
.						

When the sheet is complete we will write down the lab's results on the board for you to copy into your notebook.

The student takes their numbers, and those of the next 3 higher groups as 4 experimental determinations of fraction color. (Students with the highest group will cycle to groups 1 and 2)

For this lab I want you to do all of the following eight statistical calculations. About five minutes after you have started these calculations you will notice that there are many repetitive and tedious calculations here. It will reduce both the tedium and your chance of error if you get the manual of your calculator out and **figure out how to make the calculator do the calculations instead of cranking them out by hand** (You can also use computer spreadsheet calculations). But don't depend entirely on the calculator. **Show an example equation for each calculation, and show how one set of data is used in this equation.**

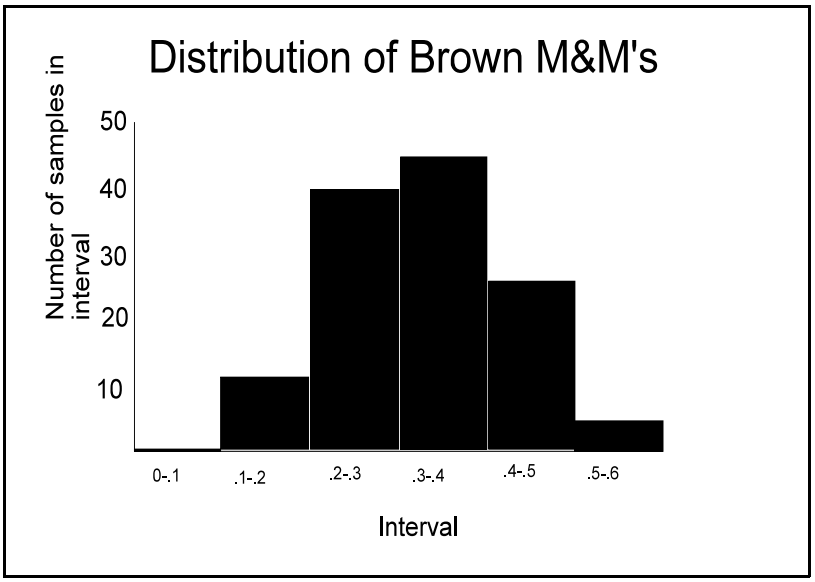
NOTE - Because this lab is not well suited to being written up in your lab notebook, no notebook entries are required for this portion of the lab.

Questions an calculations to be completed for lab write-up (50 points)

1. Determine the mean, standard deviation, and relative standard deviation (Coefficient of Variation) for each color fraction based on your four measurements.

2. Looking at your data (4 measurements) do you have any you think should be rejected? If so, does the Q test allow it to be rejected? (Even if you don't think you need to, try the Q-test on at least two sets of data.)

3. In 1991 the distribution of brown M&M's based on all the data collected looked like this:



Using the data from the entire lab make two similar plots, one for the distribution of brown M&M's and one for the distribution of blue M&M's. On each plot mark the mean and standard deviation you determined for your lab section and for your three samples. How do these values compare to the observed distributions?

4. Using data from the entire lab, what is the mean and the 95% confidence interval around the mean for each color?

5. I like the red M&M's. If I made each person in the lab give me 2 red M&M's before the lab started, what kind of error would that place on our numbers. Likewise, what would have happened if I told everybody to eat 5 M&M's before they started counting, what would have happened to our error. (This is not a calculation, identify the kind of error)

6. Assume that I went through and randomly took a single M&M from your sample or added an M&M. This introduces some uncertainty into your fraction numbers. When you report your fraction color, how many significant digits should you use to represent this uncertainty? Why? (Show me a calculation to prove your answer)

7. I wish to use the fraction Brown and fraction Blue in the following strange calculations. Using what you were taught about propagation of random error, what is the uncertainty in the outcome of these calculations, based on your experimental data? (Use the LAB data average values for fraction brown and fraction blue, and use the standard deviation as the uncertainties in these values.)

Fraction dark = fraction brown + fraction blue

ratio = fraction blue / fraction brown

8. In 1996 my class results were as follows:

Color	Mean	Standard Deviation	
Brown	.323	.093	
Blue	.067	.047	
Red	.201	.075	
Orange	.113	.076	
Yellow	.212	.086	
Green	.086	.054	n=127

Use the appropriate statistics to see if M&M's have changed their color distributions for Brown, Orange, or Green. (Do all three cases. Use a 95% confidence interval. If N is too large for a given table, use the highest N available on your table)

Experiment 2: **AN INTRODUCTION TO COMPUTER SPREADSHEETS**

Reading Assignment

Section 3-5,3-6,4-6 of your text

To analyze a set of X & Y data, you need to fit a set of points to a straight line. Many of you have calculators that will do this, but not all of these 'canned' calculator programs will give you all the statistics I require. If you do the statistics the long way they show in the book, there are lots of places for calculation errors or round off errors.

There are computer programs called Spreadsheets that also do these kinds of calculations. Besides doing just statistics they can be extremely flexible and can do a wide variety of mathematical tasks. In this lab you will get introduced to the Microsoft spreadsheet, Excel. I hope you learn to use it, because once you have, you can use it for every other calculations in this class, as well as lots of exercises in the text, and lots of problems outside of class.

If you have a PC of your own and you are familiar with a different spread sheet, go ahead and use the tool you already know.

Starting the spreadsheet

Go to that upper bar on the windows screen and click on the x symbol. You now get a window that looks something like the Word window; a lot of buttons that do various mysterious functions across the top, and a large working area. This time the working area is divided into rectangles called cells. The way a spread sheet works is that you put data into some of the cells, and formulas into other cells, and the computer does calculations based on the data and the formulas you have given it.

Each cell has a unique position. A number on the left hand side determines its row position and a letter along the top edge determines its column position. Also along the bottom there are pages for additional sheets as well.

For the first part of this exercise we will determine the average and standard deviation of a set of 10 numbers. Click the mouse on **cell A1** then type **95**. You will see 95 entered into a work area along the top edge of the sheet as well as in the cell itself. Now fill in **92, 88, 100, 78, 90, 93, 95, 89, 92** into positions 2 through 10 of the A column. The easiest way to this is to simply hit the down arrow on your key pad after each entry so you don't have to grab the mouse each time. Now go back through the list and check. Any mistakes? If so, simply click the mouse on the mistake and type it in again.

Now for the fun. Let's calculate the mean and place it in cell A11.
Click on cell A11 and type: **=average(a1:a10)**
then hit the return button. The average now appears at that position! Just for grins go back and misspell average 'avrage' What happens? You get a cell that looks like #name? This means that the computer doesn't understand what you typed. So the next time you get this error, take a look at what you typed. Also note that the program understands average but not mean. Now let's go to cell a12 and try for the standard deviation. Type **=stdev (a1:a10)**

Wasn't that faster than doing it on your calculator? If you want to see what other statistical function are available on Excel hit the **f_x** button. This button is the key to lots of different mathematical tools in lots of different fields. After you hit the f_x button you get a menu, click on **statistics** and on the left hand side, and on the right side will be displayed all the statistical function available. Many more than we have covered in class! Pick out a function you like, click on it, then click on **help**. The computer now tells you how that function works, its formula, and how to use it. You can also use the f_x button to call the function wizard that can help you set up calculations with that function. IF you want to use the function wizard press the next button. You can play with that for yourself, but I won't go into that here, so for now, hit **cancel**.

Now that you have a feel for a spreadsheet, let's try something a little more complicated. First, clear the present worksheet by clicking on **file..close..no**. Did you see the 'close' command under the file menu? If not, that is because Word likes to hide menu pieces it doesn't think you need. To get ALL the commands under a window click on the double arrows pointing down at the bottom of the window. Do you see the close command now??

To start a new window click **file .. New**. Go to section 3-6 of your text and do the first exercise dealing with plotting a function. Try to reproduce figure 3-4 from your text.

Now how do you plot the data to get a figure like 3-5 from your text?

1. Click on cell b4 and hold the mouse button down. Move the mouse to cell c11 and release, both columns should be outlined in dark.

2. Click on the little button that looks like a bar graph on your icon bar. This is the 'graph wizard'. In 4 steps it will make a graph for you.

Step 1 is setting up the type of graph you want

-Find Scatter plot in the left hand column and click to chose this plot. Whenever you have paired X-Y data you must always use the scatter plot.

- Now on the right column select the kind o plot you want, points, lines, curves, etc.

- Now hit the Next button on the bottom of the window

Step 2 defines the data set up. You should be OK here. You only need ths window if your X and Y is reversed or your data is in some other format that the computer need to have explained

-Hit the Next button

Step 3 defines char options

each tab lets you set different options like labels, axes, appearance of the

plot. Try different things, flip from one tab to the next until you have just what you want, then
-Hit the Next button

Step 4 defines where you want the plot put. I am happy to have it put right on the data shet I am working with so simply
-Hit the Next button

Your plot should appear on the speadsheet. Use the mouse to move the plot where you want it, and use the mouse to grab the corners and edges of the plot to make it jsut the size you want. You can also try double clicking on the plot or parts of the plot to see what things you can go and change

Now click on the littler printer and make a copy of this page for your lab report.

(The fifth button from the left)

Now try to make a spreadsheet like that shown in section 4-6 to do a Least squares fit of X-Y data. First try to make this spreadsheet using the data given in the text example, so you can see if the computer is actually do the calculations it is supposed to.

Note: the only numbers you should be entering are a2:c5. For the rest of the numbers you should be entering the formula and letting the computer calculate for you. Also be sure you include the documentation cells that help explain the calculations. Also be sure you go back to the text and see if you can figure out which equation from the text each calculation corresponds to.

One thing I want you to notice is the definition of A12 given in cell d11. Do you see the expression “D= A10*E9-B9*B9"? What do you think the $ means? To explain this, let’s go back to the calculation you used for column D. How did you get the xy term on column D. In cell D2 you should have used the expression “=B2*C2". Then, as you used the fill command for cells c3,c4,c5,etc, the computer automatically changed the expression to use A3&B3, A4&B4, A5&B5, etc.

In column F2 you want to use two numbers (the slope and intercept of the line) that are constant. Since you <u>don’t</u> want the computer to automatically change these numbers with each answer cell, you use the $ to tell the computer to keep this value constant.

Final Note

In science never, NEVER, **NEVER** calculate a line of best fit and derive values from it without actually looking at your data. Use Excel to make a plot of absorbance vs concentration for your actual data and see how it looks. Any wild values? Next plot your line of best fit on the same plot. How close is the line of best fit to the actual data. Also, check by eye the value you have derived for the concentration of your unknown. Does it look reasonable?

Final Task

Now for the final test, below is a set of data that was acquired in my lab this summer. The X values are thiamine concentrations in a standard sample, and the Y values are the total fluorescence these samples had in my HPLC system. I have a sample with a total fluorescence of 1027599, what the concentration of thiamine and the uncertainty in that value? (Plot the data, do a least square analysis of the line, use the line of best fit to calculate thiamine for the unknown sample and the uncertainty in that value.

[Thiamine]	Fluorescence
0	31997
0	1023720
5.11×10^{-8}	530670
1.02×10^{-7}	967970
2.05×10^{-7}	1996240
2.05×10^{-7}	1984635

Experiment 3: **A SIMPLE PRECIPITATION TITRATION**

Read section 6-6 from your text (pages 123-125) this lab is based on the demonstration of the Fajans method that is given as Demonstration 6-1 on page 124

I. Preparation of ~0.05M $AgNO_3$ Standard
Dry about 5.0 g of $AgNO_3$ (FM 169.87) at 105°C for one hour *(get this in the oven first, and then work on the other solutions). Cool in a desiccator for 30 minutes with minimum exposure to light. Accurately weigh about 4.2 g of the $AgNO_3$ and place in a 500 ml volumetric flask. Add water to bring to the line and mix throughly.
* Don't leave for more than an hour, it will decompose. Even after only one hour may be slightly discolored but titration will not be affected

II. Preparation of Unknown
I will give you an unknown sample containing about 0.5 g NaCl and a spike of $NaNO_3$ so you cannot simply weigh the unknown to determine the Cl^-. content. Place this sample in a 250 ml volumetric flask. Rinse your weighing boat several times with deionized water to make sure the entire sample has been transferred to the flask, then fill to the mark and mix throughly.

III. Preparation of Indicator (if not provided)
Weigh out about .2g of dichlorofluorescein and place in a 100 ml beaker. Add 75 ml of ethanol and 25 ml of water. Once this has dissolved transfer to a small bottle with an eyedropper cap.

IV. Titration
Rinse your buret once with the $AgNO_3$ solution, then fill and record your initial volume. Use a volumetric pipet to remove a 50 aliquot of your unknown and place this aliquot in a 250 ml beaker with a stirring bar. Add about 50 ml of deionized water, 0.2g of dextrin, and 5 drops of indicator. Place this beaker on a magnetic stirrer under your buret so you can add titrant and stir the solution at the same time. Titrate to the endpoint.* For the first run you can be fairly sloppy and add large amounts of titrant (1-3 ml shots) to find out approximately where the endpoint is. Do three more runs as accurately as possible, trying to hit the endpoint to within a drop or two (.05 ml)
* Do not do titration in direct sunlight, light will make the complex decompose.

V. Calculations (Show an example for each calculation)

Part A: Experimental results
1. What is your unknown number?

2. (5 points) What was the molarity of your $AgNO_3$ standard solution?

3. (5 points) For each of your good titration runs, calculate the molarity of NaCl in your unknown solution. (Show one example calculation, then show final molarity for all runs)

4. (5 points) Calculate the amount of NaCl (in grams) of your unknown using each of the different concentrations listed in question 3 above.

5. (5 points) Based on values listed in question 4, calculate your average grams of NaCl, the standard deviation of your amounts of NaCl, and the relative standard deviation for your set of NaCl data

Part B: Error analysis
6. (10 points) Considering the experimental error in weight of $AgNO_3$ and the experimental error in volume of the volumetric flask, what is the uncertainty in the above molarity of $AgNO_3$ (Question 2)?

7. (10 points) Considering the experimental uncertainty in the molarity of $AgNO_3$ (Question 6), and the experimental uncertainty in the volume of the unknown, and the uncertainty in the volume of the titrant, what is the relative uncertainty in the molarity of the NaCl (Question 3)? For this error analysis chose any single run.

8. (10 points) Based on the uncertainty in the molarity of NaCl (Question 7) and the uncertainty in the volume of the volumetric flask you made the solution in, what is the uncertainty in your final g amount of NaCl in your unknown (Question 4)?

9. How does your experimental uncertainty (relative standard deviation - Question 5) compare to your theoretical uncertainty (answer question 8) Is your experimental technique good, or could it be improved? If so, how?

10. Other - To be determined by instructor.
    40 points - How close you are to actual value of NaCl.
    10 points - How large is your experiment relative error.

Experiment 4: **DIRECT TITRATION OF TRIS WITH HCl**

Preparation of Standard HCl
Prepare and standardize 0.1M HCl using the procedure given your text on pages 215 and 216. Use 6 drops of indicator instead of 3 drops listed in procedure. Also, since the color is hard to see, do the blank titration ***first*** so you can see the endpoint color you are trying to achieve at the end of the titration.

Preparation of Unknown Tris Base
Obtain a sample of Tris (Tris [hydroxymethyl]aminomethane, $NH_2C(CH_2OH)_3$,MW 121.14). This sample will be about 2 grams. Transfer this unknown to a 250ml volumetric, fill to the mark with water and mix throughly.

Determination of Unknown
Use your 50 ml volumetric pipet to transfer an aliquot of your unknown base to a 250 ml Erlenmeyer flask. Add 6 drops of Bromocreosol green indicator and again titrate with the standard HCl until you see a green color. Again you should do a blank titration first and have this flask available to compare with your endpoint color. Since H_2CO_3 or CO_2 is not a product in this reaction, you do not have to boil the solution to expel CO_2 like you did in the previous reaction. The TRIS solution will, however, pick up CO_2 from the air if it stands overnight, so do not make up your TRIS solution unless you can titrate it the same day.

Notes on indicator
Bromocreosol green is blue in the basic form and yellow in the acid form. It is only green at ~pH 4.5. In this titration it will start off as a pale blue. You are trying to get it to the green intermediate form. If you overshoot the endpoint you will get the indicator to a yellow form which is so pale it is hard to see. Bottom line: Titrate blue to green- OK, Titrate blue to clear - Overshot!

Name:_____

Report Sheet
Direct titration of Tris Base

I. Standardization

A. Standardization of HCl solution.

Blank

mls of titrant required to obtain green indicator blank _____

Titrations: Enter all data from the good runs you want to be graded on

	Run 1	Run 2	Run 3
Mass Na_2CO_3	_____	_____	_____
Volume of HCl to equivalence point	_____	_____	_____
Volume of HCl to get green color in blank	- _____	- _____	- _____
Net volume of HCl to equivalence point	_____	_____	_____
Molarity of HCl	_____	_____	_____

Average Molarity HCl: _____

Relative standard deviation of Molarity _____

II. Tris Titration

Unknown number _____

Blank

mls of titrant required to obtain green indicator blank _____

Titration

	Run 1	Run 2	Run 3
Volume of HCl	_____	_____	_____
Volume of HCl in Blank	-_____	-_____	-_____
Net volume of HCl	_____	_____	_____
Moles of HCl to equivalence point	_____	_____	_____
Molarity of Tris Solution	_____	_____	_____

Average molarity of Tris _____

Relative standard deviation of Tris molarity _____

Questions (Show all calculations)

Results

1. Using the data in part I and part II calculate the grams of Tris in your sample.

Error Analysis - Experimental

2. Using the relative standard deviations reported for your titrations in section I and II, calculate the experimental uncertainty in your answer for 1.

Error Analysis - Theoretical

3. Considering the uncertainties in all weight and volume measurements, what is the expected uncertainty for your HCl molarity in I? (Use the weights and volumes from a single run for this calculation.)

4. Considering the uncertainties in all weight and volumes measurements, what is the expected uncertainty for your Tris molarity in II? (Use the weights and volumes from a single run for this calculation.)

5. Considering the uncertainties in all weight and volume measurements, what is the expected uncertainty for your grams of Tris in question 1?

6. How does your experimental uncertainty in grams of Tris (Question 2) compare to your theoretical uncertainty (Question 5)?

Points

- 40 for how close your gram of Tris is to actual value.
- 6 for relative standard deviation of HCl titrations.
- 6 for relative standard deviation of Tris titrations.
- 8 points for each question, 1-6

Experiment 5: **SPECTROPHOTOMETRIC DETERMINATION OF THE K_A OF AN ACID-BASE INDICATOR**

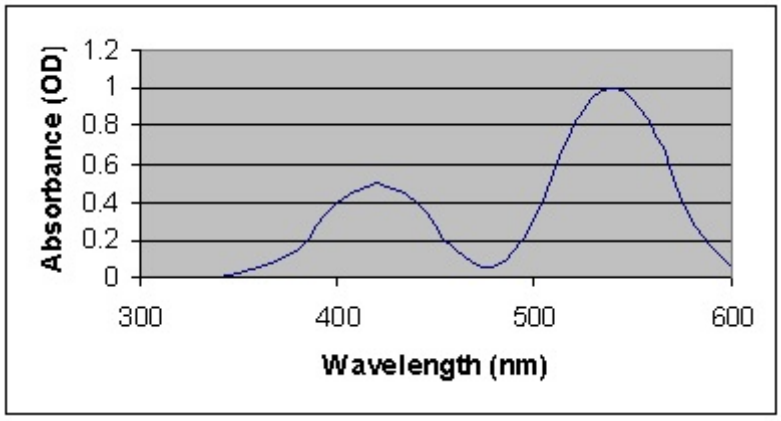
Purpose

To determine the acid equilibrium constant for an indicator, Bromocreosol Green, using photometric methods.

Background

Spectrophotometry is a method of analysis which uses optical properties of substances. For the chemist, the main interest in spectrophotometry is the ability of chemical species to absorb light energy.

Substances absorb some wavelengths of light much more than other wavelengths. In quantitative analysis of a particular light-absorbing species, the chemist chooses the wavelength where absorption is greatest or, if more than one absorbing species is present in the sample, the wavelength where the largest difference in absorption occurs. One must experimentally locate this wavelength by measuring the amount of light energy absorbed at each wavelength. This information is collected, presented graphically, and then the wavelength at which maximum absorption occurs, λ_{max} , is chosen. An example is shown below.



The absorbance (A) of light by a sample is proportional to:

1. The length of the path through the sample (b)
2. The number of absorbing molecules, which can be measured as molar concentration (c).

That is, A $\propto$bc. A is measured using a spectrophotometer and is the log of the ratio of light intensity entering the sample (I_o) to that of the light leaving the sample (I).

$$A = \log \frac{I}{I_0} \propto bc$$

The proportionality constant is called the molar absorptivity coefficient, ε, if b is measured in cm and c in moles/liter.

$$A = \log \frac{I}{I_0} = \varepsilon bc$$

This is known as the Beer-Lambert Law. If we measure A at a constant wavelength (λ constant) for a constant path length (b) at two different concentrations of the same species,

$A_1 = \varepsilon_1 bc_1$ and $A_2 = \varepsilon_2 bc_2$

then

$A_1 / A_2 = \varepsilon_1 c_1 / \varepsilon_2 c_2$

If one concentration is known, the other can be calculated after measuring A_1 and A_2.

In this experiment we use spectrophotometry in the visible region of the spectrum. We will use the spectrophotometer to determine the absorbances of an acid-base indicator at several different pH values. From data obtained we should be able to determine the equilibrium constant for the indicator.

Acid-base indicators are generally organic compounds that function as weak acids. If HIn designates the weak acid form of the indicator, then

$$HIn + H_2O \rightleftarrows H_3O^+ + In^-$$

The only difference between an indicator and a weak acid is that the acid form (HIn) is one color while the base form (In^-) is a different color. Hence the color of a solution containing an indicator will depend upon the relative concentrations of In-and Hin, *i.e.* upon the ratio
[In-]/[HIn]. Note that the total concentration of indicator is equal to [HIn] + [In^-]. In a solution where the hydronium ion concentration is high, the equilibrium will lie to the left, ([In-]/[HIn] « 1), and the solution will be the color of the acid form of the Indicator. In a solution where the hydronium ion concentration is low, the equilibrium will lie to the right, ([In-J/[HIn] » 1), and the solution will be the color of the base form of the Indicator. Since both the acid and base forms of the Indicator absorb light in the visible region of the spectrum, we can use the spectrophotometer to determine the [In-]/[HIn] ratio at a given pH. This information enables us to calculate Ka for the indicator.

Experimental Procedure

A. Preparation of solutions

Each group should obtain 150 mL of Bromocreosol Green Indicator solution,
20 mL of this solution should be placed in each of seven 100 mL volumetric flasks. Label these flasks 1 thru 7.

In flask 1 place 50 mL of 0.02 M HCl
In flask 7 place 50 mL of 0.002 M NaOH

Add 1.0 M acetic acid and 1.0M sodium acetate to flasks 2 thru 6 according to the following table

Flask	mL of Acetic Acid	mL of Sodium acetate
2	46	4
3	38	12
4	25	25
5	12	38
6	4	46

Fill each flask to the mark with deionized water and mix well.

B. Spectrophotometry
Using a 1 cm cell and a water blank in the Lambda2 spectrophotometer, determine the absorbance spectrum of all solutions from 400-700 nm. Plot this data and determine λ_{max} (wavelength with the greatest absorbance).

Analysis of data

1. Flasks 1 and 7 represent extremes of pH.
   - What is the color of these two flasks?
   - What it the pH of these two flasks?
   - What is the form of the indicatior (HIn or In^-) in these two flasks?
   - Plot the absorbance spectrum of Bromocresol Green in the HIn form.
   - Plot the absorbance spectrum of Bromocresol Green in the In^- form.
   - At what wavelength the is the absorbance maxima for HIn? for In^- forms of Bromocresol Green?

2. Flasks 2-6 represent intermediate pH values.
   - (Show sample calculations, then place all this data in a single table)
   - What is the concentration of acetic acid and sodium acetate in each flask?
   - What is the pH in each flask?
   - What is the absorbance of each solution at the wavelength that is the maximum for HIn?
   - What is the absorbance of each solution at the wavelength that is the maximum for In^- ?

3. Plot absorbance vs pH for your two wavelength maxima.(See diagram on board) From this plot determine the ratio of Hin:In^- for solutions 1-7. You can also use the equation:

$$\frac{[In^-]}{[HIn]} = \frac{A_{solution} - A_{low\ pH}}{A_{high\ pH} - A_{solution}}$$

4. Now plot log($[In^-]/[HIn]$) vs pH. What does this tell you? Well let's go back to the Henderson- Haselbach equation, and use HIn for HA and In^- for A^-

   $pH = pK_a + \log [In^-]/[Hin]$

   $\log [In^-]/[HIn] = pH - pK_a$

   Think of this as Y = mX + b with $\log [In^-]/[HIn]$=Y and pH=X The slope should be 1, and the Y intercept is $-pK_a$! (Actually the X intercept is + pK_a)

5. What is the pK_a of your indicator?

Experiment 6: **POTENTIOMETRIC TITRATION OF AMINO ACIDS**

In this experiment group you will learn how to use a pH meter. The pH meter will then be used to do a potentiometric titration of an unknown amino acid. From this data you should be able to identify the amino acid, and be reminded of important concepts like pI, pH free bases and free acids. Remember many drugs behave just the same way these amino acids do.

Reading Assignment

Read Chapter 11 of your text.

Experimental Procedure

Part I A. Preparation of 0.1 N NaOH

Prepare standard 0.1M NaOH using the procedure given on page 214 of your text. Make sure you boil your water before making this standard. When standardizing the NaOH do three runs with KHP and phenolphthalein indicator. Your book asks you to calculate the amount of KHP you need for your titrations. Just so you get off to the right start, let's just say that you should be using about .7-.8 g of KHP for each titration. Your fourth run will be with the pH meter as given below. Because this run may not be as accurate, you do not need to use it in your determination of NaOH Molarity.

Part I B. Potentiometric determination of KHP

Accurately weigh a final .7-.9 gram sample of KHP. From the above standardization, calculate where the equivalence point of this titration should be. Add 50 mls of water to the KHP and determine the initial pH. Also add 3 drops of phenolphthalein indicator. Now add NaOH to the KHP in 2 -3 ml increments until you are 2 mls before your expected equivalence point. Record the pH after each of these additions of NaOH. When you are 2 mls before the equivalence point change the amount of NaOH added to about 0.1 ml increments. Continue the titration recording your pH until you are 2 mls past your expected equivalence point. Make sure you note down the pH at which the indicator changes color as well.

Part II. Determination of pK's and identity of unknown Amino acid

Obtain an unknown sample from Dr. Z. Accurately weigh about 3.5 g of this sample and dissolve in about 200 mLs of water. Transfer the solution to a 250 mL volumetric and fill to the line with water. Remove a 25 mL aliquot and place in a 100 mL beaker. Place a stir bar and pH electrode in the beaker determine the pH of the solution. Titrate your amino acid with base. by adding 5 mL aliquots of titrant and recording the pH of the solution. Roughly sketch the titrations curve. Decide where the endpoint (or points) of the titrations are. **If you have any doubts, check with the TA. This titration is far less dramatic than the one you had for KHP. Why is that?** Repeat the titration with another 25 ml aliquot of the unknown solution. Again start off

recording the pH at 5 ml intervals, but when you are near the endpoint, slow down and use 0.25 ml increments. Repeat this slower titration two more times time. Note that for the amino acid you will probably get better results if you use a Gran plot to find the equivalence point. Gran plots work best using the data from the last 10-20% of the titration curve, so if your equivalence point is at 20 mL, make sure you have data from 16 mL to 20 mL.

From the buffer regions of the graphs determine what the pK's of the amino acid are. From the number of mLs required to reach each endpoint you know how many moles of amino acid there are. From this and the initial weight, determine the molecular weight of the amino acid, and the identity of the amino acid.

Graphical analysis

(Use Excel, it will save you lots of time and grief)

KHP

1. Plot pH vs. Vol.of Base for the KHP run done with the pH meter
2. Plots of both the first derivative and the second derivative. (Text section 10-4 pages 202-206)

From this data determine your endpoint and K_a of KHP

Amino Acid

For the 'best' run

1. Plot pH vs. Vol. Base for your best run with your amino acid
2. 1st and 2nd derivative plots
3. A Gran plot (Not in text, see class notes)

For the other two runs

1. Select the type of plot worked the best for your amino acid and repeat for the other two sets of data.

From the above three sets of data you should have obtain an average molecular weight and an average K_a

Notes on experiment and calculations:

1. The best way to find your unknown is from the molecular weight, <u>not</u> the pK_a

2. Here is a list of amino acids that are readily available for use in this lab, sorted by molecular weight:

ACID	MW
Gly(Free Base)	75.07
Ala	89.09
Gly.Na	97.05
Ser	105.1
Gly.HCl	111.5
Pro	115.1
Val	117.1
Thr	119.1
Cys(free base)	121.2
Ile	131.2
Leu	131.2
Asn	132.1
Asp(Free acid)	133.1
Gln	146.1
Lys(Free Base)	146.2
Glu(Free Acid)	147.1
Met	149.2
Asn.H2O	150.1
Asp.Na	155.1
His(Free Base)	155.2
Cys.HCl	156.7
Phe	165.2
Glu.Na	169.1
Arg(Free Base)	174.2
Cys.HCl.H2O	175.6
Tyr(Free Base)	181.2
Lys.HCl	182.6
Glu.HCl	183.6
Trp	204.2
His.HCl.H2O	209.6
Arg.HCl	210.7
Tyr.HCl	217.7
Lys.2HCl	219.1
Tyr.2Na	225.2

pK_a's are available in Table 11-1 of your text, or in most Biochemistry texts. Note that the pK_a's vary from text to text. This is one reason they aren't a very good indicator of your unknown.

3. Notice that in the above list many amino acids are available in different forms, such as: the free acid or base, the Na^+ salt, the HCl Salt, or with different amounts of water of hydration. You should clearly understand what the difference between these forms is, and be able to make a guess as to the initial pH of any of these compounds when dissolved in water. You should further be able to predict the titration curve for the compound in acid or base.

Many of the compounds are in the free form where there is no complexing salt. In this case the compound is in its zwitterionic form, and it will have a base titration curve that resembles figure 7.3 on page 183 of your text. Notice how weak and indistinct the equivalence point is in this titration. If you try to titrate this with acid your results will be even worse. Your acid standard is not strong enough to titrate the acid functionality.

There is also a chance that your amino acid will not be in its zwitterionic form. In this case you will have a different titration curve, or you may have to titrate with acid instead of base. Your initial pH should give you a good clue as to what to expect. If you are uncertain be sure to ask Dr. Z. before you waste too much time and titrant.

4. Also notice the range of molecular weights. A 3.5 gram sample of glycine (MW 75) has .047 Moles, when diluted to 250 mls and a 25 ml aliquot is removed will yield .0047 moles of amino acid and will require 47 mls of .1M NaOH to titrate. On the other hand your lab partner may have Tyr·2Na with a molecular weight of 225.2. In this case it will take only 15.54 mls to titrate. So don't worry if these amounts vary widely. Also feel free to compensate. If you need 47 mls to titrate a 25 ml aliquot of glycine cut the aliquot back to only 10 mls. Your new endpoint can be calculated from the simple proportionality 47/25 = X/10 ; X= 18.8 mls. This will save you lots of titrant.

5. Some amino acids <u>cannot</u> be completely identified in this experiment. Leucine and isoleucine, for example, have exactly the same molecular weight. If this happens to you DON'T PANIC. Simply write down the range of molecular weights that you calculate for your unknown, identify all amino acids in this range, and if your unknown is in there you are safe. You might try to look at some of the possible amino acids in the list and rule them out based on some observed or predicted properties.

Experiment 7: **EDTA TITRATION OF Ca^{2+} AND Mg^{2+} IN NATURAL WATERS**

Note: This procedure was originally found in the 2nd edition of your text, but since Harris hasn't put it in your current edition I have copied it here for your use.

Note for Steps 2 and 3. The pH 10 buffer is an ammonia buffer, so it is pretty stinky. Add the buffer in the hood and then titrate the sample right away, then flush the sample down the drain with plenty of water. Please do not set up all your samples at one time and have them sitting on the lab bench stinking up the lab.

1. Dry $Na_2H_2EDTA{\cdot}2H_2O$ (Molar mass 372.24) at 80^0C for one hour and cool in a desiccator. Accurately weigh out ~1.2 g and dissolve it with heating in 800 ml of distilled water in a 1 liter volumetric flask. Cool to room temperature, dilute to the mark, and mix well.

2. *Preparation of a blank for the first titration*. Place 40 mls of distilled water, 3 ml of pH 10 buffer and 6 drops of Eriochrome Black T indicator in a flask. This should be a blue solution (not reddish blue or purple). If it isn't blue, add a drop of two of EDTA to make it blue, and note the volume required for this indicator error in your notebook. Keep this solution on the lab bench for your next titration so you can try to match this color at every end point. You will also have to subtract this volume from the total volumes of all your titrations to correct for this indicator error.

3. *Determination of total Mg^{2+} and Ca^{2+} .* Pipet 20 ml of Dr. Z's well water into a 250 ml flask. To this flask add 10 ml of distilled water, 3 ml of pH 10 buffer and 6 drops of Eriochrome Black T. Titrate this same until you have roughly the same shade of blue you saw in your blank in step 2. Do this sample once for each group. Now, for each individual in a group find a different simulated water sample, and do this titration at least 3 times on your simulated water sample.

4. *Preparation of a blank for the second titration.* Place 100 mls of distilled water in a flask. Add 30 drops of 50% NaOH and ~0.1g of solid hydroxynapthol blue indicator. This should be a different blue solution. If it isn't blue add a drop of two of EDTA and see if the color changes. Again mark down the volume required for this indicator error in your notebook, and keep the solution for visual comparison to your other endpoints. Be aware that this color comparison is a bit trickier. With a real sample you will have suspended $Mg(OH)_2$ particles that will make the titration solution look a bit different.

5. *Determination of Ca^{2+} only.* Place 10 ml of Dr. Z's well water in a flask and add ~65 ml of deionized water. Add 30 drops of 50% NaOH and swirl for 2 minutes. You will probably see a $Mg(OH)_2$ precipitate form. Add ~0.1g of solid hydroxynapthol blue indicator and titration with your EDTA solution to an endpoint similar to that seen in Step 4. Again your group only has to do the titration once with Dr. Z's well water one, but each individual in the group must do this titration at least three times with his or her simulated water sample to get accurate results on an unknown.

Name:_____

Report Sheet
EDTA Titration of Ca^{2+} and Mg^{2+} In Dr. Z's drinking water

I. EDTA

Grams of EDTA used: _____

Molarity of EDTA solution: _____

II. Data MZ well water sample (1 titration per group)

1. Total Molarity of Ca^{2+} and Mg^{2+}

Total volume of EDTA to endpoint in Ca^{2+} Mg^{2+} Titration _____

Volume of EDTA in Ca^{2+} Mg^{2+} Blank _____

Actual volume of EDTA to endpoint in Ca^{2+} Mg^{2+} Titration _____

Total Molarity of Ca^{2+} and Mg^{2+} _____

2. Molarity of Ca^{2+} ONLY

Total volume of EDTA to endpoint in Ca^{2+} only Titration _____

Volume of EDTA in Ca^{2+} only Blank _____

Actual volume of EDTA to endpoint in Ca^{2+} only Titration _____

Total Molarity of Ca^{2+} _____

6. Convert from Moles/liter to g/liter

Ca^{2+}(g/l) _____

Mg^{2+} (g/l) _____

7. Convert from g/liter to ppm

Ca^{2+} (ppm)_____

Mg^{2+}(ppm) _____

III. Simulated water data

Simulated water sample # _____

	Run I	Run II	Run III
1. Total Molarity of Ca^{2+} and Mg^{2+}			
Total volume of EDTA to endpoint in Ca^{2+} Mg^{2+} Titration	____	____	_____
Volume of EDTA in Ca^{2+} Mg^{2+} Blank	____	____	____
Final Volume of EDTA to endpoint in Ca^{2+} Mg^{2+} Titration	____	____	____
2. Total Molarity of Ca^{2+} and Mg^{2+}	____	____	____

Average Molarity _____

Relative Standard Deviation of molarity _____

3. Molarity of Ca^{2+} ONLY			
Total volume of EDTA to endpoint in Ca^{2+} only Titration	____	____	____
Volume of EDTA in Ca^{2+} only Blank	____	____	____
Actual volume of EDTA to endpoint in Ca^{2+} only Titration	____	____	____
4. Molarity of Ca^{2+} ONLY	____	____	____

Average Molarity _____

Relative Standard Deviation of molarity _____

5. Molarity of Mg^{2+} only (total-moles Ca^{2+}) _____

6. Convert from Moles/liter to g/liter

Ca^{2+}(g/l) _____

Mg^{2+} (g/l) _____

7. Convert from g/liter to ppm

Ca^{2+} (ppm)_____

Mg^{2+}(ppm) _____

Now for the comparisons

Milk	≈ 30 mM Ca^{2+} (10 mM free ion, 20 mM bound to casein)
Soft Water	0-17.1 ppm
Slightly Hard Water	17.1-60
Moderately Hard	60-120
Hard	120-180
Very Hard	>180

(Water hardness numbers taken from www.hardwater.org)
Note that water hardness does not differentiate between Ca^{2+} and Mg^{2+} so you should add the Ca^{2+} and Mg^{2+} ppm together before you decide if I have hard water.

II. Error Analysis

Uncertainty in Molarity of EDTA

Using the uncertainty of the mass of EDTA and the uncertainty of the volumetric flask, and the uncertainty in the purity of EDTA, calculate the uncertainty you should have in the molarity of EDTA.

Uncertainty in total molarity

Assume you used a 10 ml type A volumetric pipet twice to deliver the 20 mls volume of MZ water, and this took 25.00 mls of EDTA (measured in a buret) to titrate, what is the total metal molarity and the uncertainty in this molarity?

Uncertainty in Ca^{2+} molarity

Assume you used a 10 ml type A volumetric pipet twice to deliver the 20 mls volume of MZ water, and this took 16.00 mls of EDTA (measured in a buret) to titrate, what is the Ca^{+2} molarity and the uncertainty in this molarity?

Uncertainty in Mg^{2+} Molarity

Based on the above numbers, what is the Mg^{2+} molarity and the uncertainty in this number?

Experiment 8. **POTENTIOMETRIC HALIDE TITRATION WITH Ag^+**

This procedure also comes from the 2nd edition of your text

Earlier in the semester you did a precipitation titration of Cl^- with Ag^+. This titration will be similar, but this time you will have a mixture of both Cl^- and I^- to deal with. How do you determine two different precipitates at once? You make an electrode that senses the Ag^+ concentration, and watch the the potential of the Ag^+ ion change as the different precipitates form.

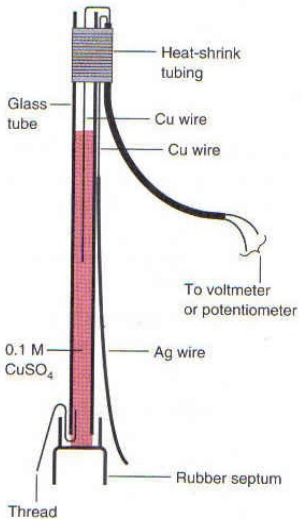
Unknown: Each student will receive an unknown containing between 0.22-0.44g of KCl plus 0.5-1.0g of KI

Procedure

1. Pour your unknown into a 100ml volumetric flask and dissolve with 50 ml of distilled water. Fill to mark and mix well.

2. Dry ~3 g of $AgNO_3$ (FM 169.87) at 105°C for one hour and cool in a desiccator for 30 min with minimal exposure to light. Accurately weigh 3.0 g and dissolve in a 250ml volumetric flask. Keep flask covered if the solution will not be used the same day it is made.

3. Set up the combination electrode shown in the figure below. If it is too difficult to keep the copper an silver wires from touching, the silver wire may be taken off the electrode an simply dangles in the solution separately.



4. Pipet 25.00 ml of unknown into the beaker. Add 3 ml of pH 2 buffer. And begin magnetic stirring. Record the initial level of $AgNO_3$ in the buret and the initial voltage of the electrode.

5. Titrate the solution using 1.5 ml amounts of $AgNO_3$ and recording the potential after each addition. Continue the titration until you have observed 2 sharp voltage changes. This homemade electrode will be a bit noisy and will have some drift, so don't take more than 30 seconds to record each point.

6. Plot this data and note the exact position you're your two equivalence points.

7. Do this titration two more times. You can continue to use 1.5 ml aliquots of titrant until you are 75% of the volume of the first equivalence point. At this point slow down to 4 drops (~.2 ml) until you have past the 1st equivalence point. You can then use 1.5 ml aliquots of titrant until you are 75% of the

way to the second equivalence point. The slow down to 4 drops per point until you have obtained your second equivalence point**.**

Notes on analysis of titration curve

Ordinarily the equivalence point with the steepest slope on the titration curve. The first equivalence point on the this titration curve is the exception to the rule. Look at figure 6-5 on page 121 of your text. As shown in curve b, if you were titrating only KI in your sample, the potential near the equivalence point would swing about 600 mV as the Ag^+ concentration swings from very low because it is being precipitated out by the KI to very high as you add excess Ag^+. In this case the endpoint would be where it normally is, at the point of steepest slope.

When you have KCl in the sample things change. Instead of having the Ag^+ swing from very low to very high, the Cl^- starts making the excess Ag^+ precipitate out, so you get the curve shown in line a of the graph. Essentially the Cl^- starts making some of the Ag^+ precipitate out, even before you get to the 1^{st} equivalence point, so by taking the steepest part of the curve as the first equivalence point you are underestimating the volume of Ag^+ needed to precipitate the I^- because you haven't actually gotten to the I^- equivalence point yet. The proper way to find the first equivalence point is shown in the insert on the graph. You must extrapolate the steep curve and the flat part of the Cl^- titration curve and see where those two lines meet for to find the equivalence point.

This interference effect will also be seen in your potential calculations that are based on the Ag^+ concentration. If you estimate the Ag^+ concentration at this first equivalence point based on the Ksp of AgI you get one silver concentration and one potential. If you use the Ksp of AgCl you get a different concentration and potential. In reality this is one of those places where you should be using both equilibria and solving Ag^+, Cl^- and I^- concentrations all simultaneously using the tools you were taught in the previous chapters.

Name:

Write-up for Analytical Chem lab
Potentiometric Halide titration with Ag^+

1. What is the molarity of your $AgNO_3$ solution? _____

2. Attach a plot of your best titration curve

3. Your unknown contained both Cl^- and I^-. Which precipitated first?

4.	Titration 1	Titration 2	Titration 3
Volume to 1st equivalence point	_____	_____	_____
Volume to 2nd equivalence point	_____	_____	_____
Moles of I^- in 25 ml aliquot	_____	_____	_____
Moles of Cl^- in 25 ml aliquot	_____	_____	_____

5. Average moles of I^- in 25 ml aliquot_____ in total sample_____

6. Average moles of Cl^- in 25 ml aliquot _____ in total sample _____

7. Grams of KI in total sample _____

8. Grams of KCl in total sample _____

Analysis

Calculate the Ag^+ concentration in your sample at the following points:
(Use a separate sheet for calculations)

½ way to 1st equivalence point_____

1st equivalence point _____

midway between 1st and 2nd equivalence points _____

2nd equivalence point _____

Using the above [Ag^+] concentrations calculate the voltage of your cell at the same points:

½ way to 1st equivalence point_____

1st equivalence point _____

midway between 1st and 2nd equivalence points _____

2nd equivalence point _____

What were your experimental voltages at these point? Are experimental and theoretical values close?

Experiment 9. **BACK TITRATION OF TRIS**

Part I - Preparation of standard acid and base solution

Part II - Titration of unknown quantity of Tris

Tris (Tris [hydroxymethyl]aminomethane, $NH_2C(CH_2OH)_3$) is a common reagent used for making buffers with a pH of around 7.5. Earlier in this manual there is a procedure for the direct titration of this base. Here we explore a back titration of the same material..

Experimental Procedure

Part I - Preparation and standardization of solutions (Week 1)

KHP: Place about 4-5 grams of potassium acid phthalate (KHP) in the oven and dry at 110° for 1 hour. Get this sample into the oven FIRST, and then proceed to make up the next two solutions

H_2SO_4 : Measure approximately 1 liter of distilled water into a **glass** stoppered bottle. With your 5 ml graduated pipet transfer 2.8 mLs of H_2SO_4 into this bottle. Mix well and label the bottle. BE CAREFUL WITH THE SULFURIC ACID. CLEAN UP AND SPILLS IMMEDIATELY. NO MOUTH PIPETTING.

NaOH: Measure approximately 1 liter of distilled water into a **plastic** bottle. Carefully transfer 6 to 7 mls of the 1:1 NaOH stock solution (Provided by TA's) into the bottle. Mix well and label.

Standardization of NaOH with KHP:
Cool you KHP sample in the desiccators provided. When cool weigh accurately 0.7-0.9 grams of KHP into each of three Erlenmeyer flasks. Label flasks and record the appropriate weights in your lab notebook. To each flask add 50 mls of distilled water and dissolve the KHP. Titrate the KHP with NaOH using phenolphthalein indicator. NOTE: in this titration, since there is a different amount of KHP in each sample, the endpoints will be different, so you won't be able to go as quickly as you did in the HCl titration.

Determination of Relative Concentrations of H_2SO_4 and NaOH Solutions:
Using your 25 ml volumetric pipet place 25.0 mls of your acid solution into a 125 ml erlenmeyer flask. Add two drops of PHENOLPHTHALEIN indicator. Fill your buret with the NaOH solution and titrate the acid with the NaOH. In this procedure use a buret reader. Also make sure there are no bubbles in the buret tip. Occasionally rinse the sides of the flask with distilled water to wash any sprayed drops back into solution. As you get close to the endpoint you will see a pink color that persists for a longer and longer period of time. The endpoint is achieved when you get a pink color that persists at least 15 seconds during mixing. (This color may disappear over long periods of time

as the solution picks up CO_2 from the atmosphere.) Record your final volume. Repeat at least 2 more times. Note: Since you titrate exactly the same amount of acid every time your NaOH volume should be exactly the same every time. Thus, on your second and third titrations you can add NaOH to within .2 mls in one big shot and save yourself lots of time.

Save all solutions for Week II!

Part II - Titration of Tris (Week 2)

A weighing boat containing an unknown quantity of Tris will be distributed to each person. You want to transfer ALL of this material into your 250 ml volumetric flask and add to it exactly 150mLs of your H_2SO_4 solution. The best way to do this is to transfer the Tris into your 250 ml volumetric flask, and then use about 50 ml of deionized water to rinse the remaining Tris from the weighing boat into the flask. Then fill your 50 mL volumetric pipet with acid solution and pour the acid into the volumetric flask. Swirl the Tris solution around until it is dissolved, then REPEAT 2 MORE TIMES for a total volume of 150 mls of acid. Fill the volumetric to the line with distilled water and mix thoroughly.

Use your volumetric pipet to remove a 50 mL aliquot out of the flask. Place the aliquot in a 150 ml erlenmeyer, add 2 drops METHYL RED / BROMOCRESOL GREEN INDICATOR. Titrate with NaOH. The solution should start off an orangy-pink. It turns clear at the endpoint. If it turns green you overshot. Do this 3 times total.

Notes on experiment:

1. Tris has a formula weight of 121.1 g/mol. It is a weak base, pK_b=8.075. It is so weak that it can't be directly titrated with strong acid in aqueous solution (Try to figure out why, when we get to acids and bases in lecture). That is why we use an indirect procedure here. A known amount of a strong acid (H_2SO_4) is initially reacted with an unknown amount of lidocaine. The excess strong acid reacts with the weak base, and we then titrate the remaining acid with NaOH, a strong base.

In this titration we use a mixture of methyl red (that changes from red to yellow between pH 3.1 and 4.4) and bromocresol green (that changes from yellow to blue between pH 3.8 and 5.4). Neither of these yellows is very strong so you should first see your red solution turning clear, and then your clear solution turning green. The endpoint you want is when the solution first turns clear. If it turns green you have overshot and are beginning to titrate the conjugate acid form of the lidocaine back into the base form.

Notes on Calculations:

Standardization of NaOH from KHP

Let's say it takes 39.64 mls of NaOH to titrate .8543 grams of KHP. The molecular weight of KHP is 204.233 so our sample contained .8543 grams / 204.223 (grams/mole) or 4.183×10^{-3} moles of KHP. We don't know the molarity of the NaOH, but we do know that each mole of NaOH reacts with a mole of KHP. Thus our 39.64 mls of NaOH also contained 4.183×10^{-3} moles of NaOH. If Molarity = Moles/volume, then molarity = $4.183 \times 10^{-3} / 39.64 \times 10^{-3}$ = .1055 M. Note that for NaOH, Molarity = Normality

Standardization of H_2SO_4 with NaOH.

Here it is convenient to work in normality because at the equivalence point we have $N_1V_1=N_2V_2$. Let's say our volumetric pipette delivers exactly 25 mls of solution, and that it took 26.02 mls of NaOH to titrate this solution. Since Normality = Molarity for NaOH we have: 26.02(.1055)=25.00(X); and X=.1098 N.

Determination of Tris

Here I will calculate how much of the above NaOH solution is needed to titrate a 0.7 gram sample of Tris made up in 150 mls of the above H_2SO_4 solution. You will have to turn the equations around to find out how much Tris is in your unknown sample.

Let's start with how many moles of Tris is in our 0.7 grams sample:
$0.7/121.1=5.780 \times 10^{-3}$ moles or equivalents (1:1 reaction with acid)

We react this with 150 mls of .1098 equivalents of H_2SO_4 so we have
Equivalents of H_2SO_4 remaining= Total equivalents ofH_2SO_4 - equivalents of Tris

$150 \times 10^{-3}(.1098)-5.780 \times 10^{-3} = 10.69 \times 10^{-3}$

This 10.69×10^{-3} moles is then placed in a 250 mls volumetric and a 50 ml aliquot is removed. This aliquot represents 50/250 or 1/5 of the total volume. 1/5 of the total volume is that same as 1/5 of the number of moles, so the number of moles of H_2SO_4 in the aliquot is 10.69×10^{-3} x (1/5) = 2.138×10^{-3}. This is the total number of equivalents. If the NaOH is .1098 Normal then : Equivalents= Normality X Volume and we have 2.138×10^{-3}=.1055(Volume) ; and it would take 20.27 mls of .1055 N NaOH to reach our endpoint.

Grading:
70 points on relative error between your value and the unknowns actual value.
(Actual value- your value)/actual value.
<6 ppt 70 points
- 0.75 points for each additional ppt

10 points each for the coefficient of variation for your standard acid and base
<1 ppt 10 points
-1 point for each additional ppt

10 points for the coefficient of variation of the unknown.
<2 ppt 10 points
-1 point for each additional %

Tris Titration
Report Sheet

Name:_____

_____

_____ Unknown Number

A. Standardization of NaOH

Molarity of NaOH

(Run 1) _____
(Run 2) _____
(Run 3) _____
Average _____
Standard Deviation _____

B. Standardization of H_2SO_4

Molarity of H_2SO_4

(Run 1) _____
(Run 2) _____
(Run 3) _____
Average _____
Standard Deviation _____

C. Determination of Lidocaine

Grams of Lidocaine in sample

(Run 1) _____
(Run 2) _____
(Run 3) _____
Average _____
Standard Deviation _____

D. Error Analysis (Attach a separate sheet with all calculations)

1. Estimated error in concentration of NaOH based on weights and volumes used in this experiment?

2. Estimated error in concentration of H_2SO_4 based on weights, volumes and concentrations used in this experiment?

3. Estimated error in g of Tris based on weights, volumes and concentrations used in this experiment?

Experiment 10. REDOX TITRATION OF VITAMIN C

Reading Assignment

Read section 16-9 of your text.

Experimental Procedure

Part I A. Preparation of 0.05 N Iodine

Obtain a 250 ml Erlenmeyer flask with a rubber stopper and cover the stopper with a piece of Al foil. **In the hood, using a triple beam balance,** weigh out 20 gms of KI (you need not be very accurate) and dissolve it in 50 ml of distilled water. Weigh out approx 6.4 gms of I_2 and dissolve it in the KI solution. **Do not take iodine crystals back to your lab bench because they give off a corrosive vapor!** Once you have the iodine in solution, then you can take it back to your lab bench.

Once your I_2 and I^- are dissolved, pour the solution into a 1000 ml volumetric, add water to bring the volume up to the 1000 ml mark, then mix the solution and store it in your cabinet, out of direct sunlight. It may not be possible to completely dissolve the I_2. If you have some undissolved I_2 simply decant your solution into a 1 L glass bottle and dilute it to 1 L with distilled water. When doing this make sure that no undissolved I_2 is transferred into your solution because this will dissolve over a period of time and change the normality of your solution. Standardize this solution in the next step, but Do not throw away because this solution is needed in the second part of the lab.

Part 1 B. Preparation of 0.05 N Sodium thiosulfate

Accurately weigh about 2 g of $Na_2S_2O_3$ (Primary Standard). This will be found in the lab desiccator. Place this sample in a 250 volumetric and fill to the mark. Note: this solution is not stable for long periods of time. If won't be using this solution within the next 24 hours, see your instructor or your text for instructions for making a stable standard solution

Part 1 C. Standardization of I_2

Pipette out a 25 mL aliquot of your sodium thiosulfate standard using a volumetric pipette and add about 2 mL of the starch indicator solution. Titrate this solution against the I_2 solution. The end point is indicated by the appearance of a deep blue color which should persist for at least 1 min. Unlike the earlier titrations which you have done, the endpoint in this reaction does not come instantly. Instead, as you near the end point you will see the appearance of a blue color which disappears when swirling the solution. So when you reach this point in your titration add the I_2 solution one drop at a time and swirl your flask vigorously. Do this until you reach the endpoint. The dropwise addition is very important if you want to get accurate results. When you have standardized your Iodine solution you may dispose of the Thiosulfate solution.

Part II. Determination of Vitamin C

Vitamin C spontaneously oxidizes when exposed to air. If you don't keep Vitamin

C tablets tightly closed they will slowly lose their potency. You can see that if you ever wanted to determine the potency of Vitamin C that has sat on the shelf for a while, this would be a reasonable procedure. You will do two determinations of Vitamin C. In the first you will be given a standard of known potency from Dr. Z., so we can see how your technique is, and in the second you will be given some off the shelf pills, so you can see for yourself how good the manufacture of these pills is.

Part II A. Determination of an unknown (One unknown for each person)

You will be given an unknown containing approximately 1 gm of Vitamin C. Quantitatively transfer this to your 250 ml volumetric (Make sure is was well cleaned and rinsed since it just had Thiosulfate in it) and dissolve the Vitamin C in 250 mls of water. Since this will slowly air oxidize, keep this volumetric capped and place it in a beaker of ice to slow the oxidation rate down. Do all titrations of your unknown on the same day and DO NOT store the solution overnight.

To titrate this solution simply remove a 25 ml aliquot with a volumetric pipette and transfer the aliquot to a 250 ml Erlenmeyer and add 2 mL drops of starch indicator. Titrate directly with your Iodine solution. Do at least three titrations

Part II B. Determination of % purity in an vitamin pill (One set of 3 for each group)

Obtain 3 Vitamin C pills. For EACH pill, weigh pill, grind to a fine powder, accurately weigh about 100 mg of the powder into a 250 ml Erlenmeyer, dissolve in 25 mls of water, add 2 mL of starch indicator, and titrate with the Iodine solution.

Notes on Experiment:

1. I_2 sublimes (goes directly into a vapor form) at room temperature. This vapor form is caustic and a potential health threat. That is why you must weigh it in the hood, and add the KI and put it into solution as quickly as possible. DO NOT HAVE SOLID I_2 SITTING ON YOUR LAB BENCH FOR ANY LENGTH OF TIME. It is also a good idea to dispose of the Iodine solution in a sink in the hood, rather than the sinks in open part of the lab.

Notes on Calculations**:**
1. Notice that the concentration of the stock solution is in NORMALITY. Normality = Molarity * n, where n is a coefficient that takes care of reaction stoichiometries. In redox reaction n = number of electrons in the reagent's ½ reaction. The nice thing is that N*V=N*V. No matter what the reaction stoichiometry is. So if we had 50 mls of .1007 N stock solution and added to it 50 mls of H_2O, our final normality is .05035N. If 25 mls of this is titrated with 26 mls of our Iodine solution we then have 25(.05035)=26(X), and X=.05144N .

2. What is the Normality of our thiosulfate solution? In making this solution we have 2 g or $Na_2S_2O_3$ (FW=158.09) in 250 mL of water so the Molarity is 2/158.09/.25 = .05061M The thiosulfate ½ reaction is: $2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2e^-$ In this equation we see that each mole of thiosulfate makes 1 mole of electrons so N=M and N= .05061

2. Let's assume you have a 1.0 gram sample that was 95% Vitamin C. 1.0 grams X .95= 0.95 grams. The equivalent weight of Vitamin C is 88.07 in this reaction so .95 grams represents .95/88.07 or 10.787 mequivalents of vitamin C. You take a 25 ml aliquot from a 250 ml volumetric, so you are actually titrating 1.079 meq. This will require 1.079 meq of I_2 solution to titrate it, so the equivalence point should be at $1.079x10^{-3}$/.05144 or 20.98 mls.

3. We are titrating the vitamin C in the individual tablets so you can see how much variability there is in the manufacturing. For each tablet multiply the % purity times the weight of the tablet to find out how many milligrams of vitamin C was actually in the pill. Note that the tablets can be in many different sizes, but will probably have a nominal dosase like 100, 250, 500, or 1000 mg.

Grading:
75 points on the relative error in your unknown or relative error in your % purity.
<3 ppt 75 points
-1.5 point for each additional ppt.

10 points each for the coefficient of variation for your standardization of the Iodine.
<1ppt 10 points
-1 point for each additional ppt.

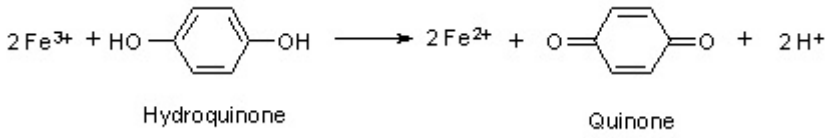
10 points each for the coefficient of variation in your unknown determination.
<1ppt 10 points
-1 point for each additional ppt.
10 points of the variability of the unknown

5 points for your determination of % purity in the Vitamin Pills. Note: Experience has shown that there will be MUCH more of variability in these answers!

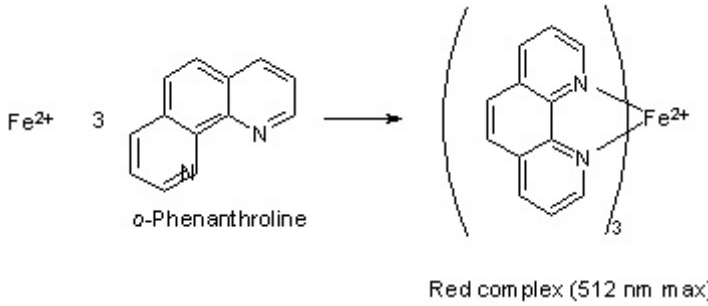
Experiment 11: **SPECTROPHOTOMETRIC DETERMINATION OF IRON IN DIETARY TABLETS**

This is another experiment that is taken almost verbatim from the second edition of your text.

In this experiment, we will analyze iron from two different sources, a dietary supplement tablet, and an unknown that I give you. In the iron supplement pills the iron is in the Fe^{+3} form and the first step is to dissolve in in acid and reduce it to Fe^{+2} using hydroquinone"



Once in the +3 state the iron forms an intensely colored complex with *o*-Phenanthroline



FYI. The tablets say that the iron is 'carbonyl iron'. Since I have never heard of this form of iron, I spent a bit of time trying to figure it out. As near as I can tell, carbonyl iron is iron that is derived form the decomposition of iron pentacarbonyl, $Fe(CO)_5$. This decomposition process makes the 'carbonyl iron' almost pure iron in microspheres a few micrometers in diameter. In this form the iron has a high surface area so it reacts in the acid of the stomach to make Fe^{+2}. ($2H^+ + Fe \rightarrow Fe^{2+} + H_2$) My guess is that since it is fairly easy to oxidize Fe^{+2} to Fe^{+3}, the first reaction is used to insure that all the iron is in the +2 state so it makes the correct complex with the *o*-phenanthroline**.**

Procedure:

Iron Tablet (1 per group)

In the Fume hood. Place one tablet in a 125-ml flask or 100-ml beaker, add 25 ml of 6M HCl. Boil the solution gently for 15 minutes. Filter the solution into a 100-ml volumetric flask. Wash the beaker and the filter with several aliquots of distilled water to be certain that all the iron has been transferred to the flask, then cool the flask, fill to the

mark, and mix well. Make a more dilute iron solution by using a 5.00 ml volumetric pipet to transfer 5.00 ml of your original iron solution into a second 100-ml volumetric flask. Fill this second flask to the line with distilled water and mix. This is your iron tablet sample.

Pipet 10.00 ml of the final iron tablet solution into a beaker and measure the pH with pH paper. Add sodium citrate solution one drop at a time until the pH is about 3.5 Record the number of drops in your notebook and discard this solution. (It may take as much as 3.5 ml of the citrate solution to get to the proper pH)

Using the appropriate volumetric pipet, transfer 10.00 of the iron tablet solution into a 100-ml volumetric flask. Add the appropriate amount of citrate solution to bring the pH to 3.5. Do NOT fill this flask to the mark. This will happen later in the procedure

Unknown (1 for each student)

You will be given a 50 ml volumetric flask containing an iron unknown. Fill the volumetric to the mark with distilled water and mix.

Pipet 10.00 ml of the unknown solution into a beaker and measure the pH with pH paper. Add sodium citrate solution one drop at a time until the pH is about 3.5 Record the number of drops in your notebook and discard this solution. (This should take less than 30 drops)

Using the appropriate volumetric pipet, transfer 10.00 of the iron unknown solution into a 100-ml volumetric flask. Add the appropriate amount of citrate solution to bring the pH to 3.5. Do NOT fill this flask to the mark. This will happen later in the procedure. If you have enough glassware you may want to make up one or two additional unknown samples to you have multiple determinations for added accuracy.

Preparation of standards (1 set for each group)

1. Pipet 10.00 ml of the standard Fe solution into a beaker and measure the pH with pH paper. Add sodium citrate solution one drop at a time until the pH is about 3.5 Record the number of drops in your notebook and discard this solution. (It should have taken around 30 drops)

2. Using the appropriate volumetric pipets, transfer 10.00, 5.00, 2.00 and 1.00 mls of the standard iron solution into four 100-ml volumetric flasks. Make a fifth volumetric flask to be used as a blank by adding 10.00 ml of distilled water to the flask.

3. To <u>ALL</u> the volumetrics containing iron samples at pH 3.5 (4 standards, 1 blank, 1 iron tablet, 1 or more unknowns) add:

2.00 ml of hydroquinone solution
3.00 ml of *o*-phenanthroline solution
Add water to the mark and mix well

Let the solutions stand for 15 minutes to react. Using distilled water as a blank, measure the absorbance of all the solution at 512 nm using the same spectrophotometer.

Analysis

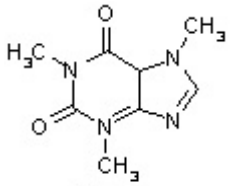
Use the least squares spreadsheet that you prepared at the beginning of the semester to determine the line of best fit for the standards. From this line of best fit you can determine the concentration of iron in your unknown.

To be turned in

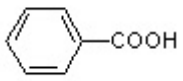
1. Fe calibration curve. Both the plot, and the spread sheet analysis
2. Amount of Fe in dietary tablet
3. Amount of iron in the 50 ml volumetric that I gave you
   Uncertainty in this number based on uncertainty in calibration curve
   Uncertainty in the number based on sample repetition.

Experiment 12: **SPECTROPHOTOMETRIC ANALYSIS OF A MIXTURE: CAFFEINE AND BENZOIC ACID IN A SOFT DRINK**

Two majors chemical species found in soft drinks and 'energy' drinks are caffeine, added as a stimulant, and sodium benzoate, added as a preservative. The structures of these species are shown below:



Caffiene



Benzoic Acid

As you can see both compounds have an aromatic ring system, so you might guess that they should absorb light energy in the ultraviolet range. Caffeine has UV absorbances at about 205 and 275 nm, while benzoic acid absorbs at about 230 nm. These absorbances are just far enough apart that we can determine the concentrations of these two species simultaneously using their UV absorption. To make this lab a little more interesting we are going to try to compare some of the soft drinks and energy drinks that are found on campus, and compare the caffeine ingested to the caffeine found in a 'No-Doz' tablet.

In doing this analysis we have to avoid both drinks that have a dark color and those with artificial sweeteners, because these compounds have additional absorbances in the UV range. Even our lighter colored, sugar sweetened drinks still contain some interfering compounds, so our results will not be absolutely correct. Next semester, in Instrumental Analysis, we will try this experiment a second time using the HPLC. This instrument will separate each compound before it is quantitated, so the effect of interfering compounds will be eliminated, and we will be able to get more accurate data to compare our results to.

Procedure:

Soft or Energy Drink (1 for each group)

Record the volume of the container of soft drink or energy drink you are analyzing. In your notebook. Warm about 20 mls of the drink in a beaker on a hot plate to expel CO_2.

After cooling to room temperature, filter the drink to remove any particulate matter.

Pipet 4.00 ml of the drink into a 100-ml volumetric flask.

Prepare a second, more dilute sample, by pipeting 2.00 ml of the drink into a 100-ml volumetric flask.

Do NOT fill the flask to the mark just yet.

Unknown (1 for each person)

You will be given an unknown in a 100-ml volumetric flask. Write down the number of the flask in your notebook.

Do NOT fill the flask to the mark just yet.

Standards

Benzoic acid

Add 2.00, 4.00, 6.00, 8.00 and 10.00 mls of 100 mg/L benzoic acid standard to each of 5 100-ml volumetric flasks.

Caffeine

Add 2.00, 4.00, 6.00, 8.00 and 10.00 mls of 200 mg/L caffeine standard to each of 5 *differen*t 100-ml volumetric flasks.

Blank

Prepare one blank 100 ml volumetric with 10 mls of distilled water

Solution Preparation

To all solutions (1 blank, 5 benzoic standards, 5 caffeine standards, 2 drink, and all unknowns) add 10.0 mls of 0.1M HCl.

Fill all volumetric to the mark with distilled water.

Spectrophotometric Measurement

Using your blank sample as a reference, determine the UV absorbance curve of Caffeine from 200 to 350 nm.

Using your blank sample as a reference, determine the UV absorbance curve of Benzoic Acid from 200 to 350 nm.

From the above two absorbance curves determine wavelengths at which both these compounds have their absorbance maxima. Check with the instructor that you have properly found these wavelengths before proceeding further.

Using the blank sample as the reference, determine the absorbance for all samples (5 benzoic standards, 5 caffeine standards, 2 drink, and all unknowns) at BOTH of the wavelength found above.

Analysis

Using the equations found in section 19-2 (page 404) of your text, determine the concentration of caffeine and benzoic acid in your unknowns and your drinks.
Once you have determined the concentration of these species in the soft drink aliquot, use the original volume of th drink container to calculate the total number of mg of caffeine you would have ingested if you had drunk the entire container.

For a reference point, a NO-DOZ tablet contains 100 mg of caffeine, and you are not supposed to take more than 2 tablets every 3-4 hours.

What to hand in:

1. Absorption spectrum of benzoic acid with chosen absorption peak marked.
2. Absorption spectrum of caffeine with chosen absorption peak marked.
3. Plot of Absorbance vs concentration for benzoic acid at chosen wavelengths.
4. Plot of Absorbance vs concentration for caffeine at chosen wavelengths.
5. Molar absorptivity of benzoic acid at chosen wavelengths.
6. Molar absorptivity of caffeine at chosen wavelengths.
7. Absorption spectra of the drinks your group prepared and analyzed.
   Comment on the above spectra.
8. The Concentration of caffeine and benzoic acid in all drinks your group analyzed.
9. Using the above concentrations, calculate the total amount of caffeine in each of the original drink cans. How does this compare to the 200 mg dose of caffeine you would get from consuming two No-Doz tablets.
10. Number of your unknown.
11. Absorption spectrum of your unknown
12. Concentration of Benzoic acid and Caffeine in your unknown.

Experiment 13: **MICROSCALE SPECTROPHOTOMETRIC MEASUREMENT OF IRON IN FOOD BY STANDARD ADDITION**

Taken from 2nd edition of Harris text. In this experiment we will again do a determination of iron using hydroquinone and *o*-phenanthroline as we did in Experiment 11, but the preparation of iron from a solid food material makes this an interesting lab procedure, as well as reinforcing the concept that iron is found in all living organisms.

1. Fill a porcelain crucible with 6M HCl in the hood and allow it to stand for 1h to remove all traces of iron from previous use. Rinse well with distilled water and dry. After weighing the empty crucible, add 5-6g of finely chopped food sample.

2. This step could require 3 h, during this time you can be doing other work as needed. Carefully heat the crucible with a Bunsen burner in a hood. Start with a low flame to dry the sample, being careful to avoid spattering. Next increase the flame temperature to char the sample. Keep the crucible lid and tongs nearby. If the sample bursts into flames, use the tongs to place the lid on the crucible to smother the flame. After charring, use the hottest possible flame to ignite the black solid and turn it into a white ash. (At this point the crucible should be glowing a cherry red!) Continue the ignition until all traces of black have disappeared.

3. After cooling the crucible to room temperature, add 12 ml of 2.0M HCl by pipet and swirl gently for 5 min to dissolve the ash. Filter the solution and collect the filtrate in a small flask. You will need 8 ml for a complete analysis

4. Obtain 4test tubes and fill them shown in the table below:

Sample Table

Name	0	1	2	3	Blank
4 ml Sodium Citrate	+	+	+	+	+
2 mL sample	+	+	+	+	-
2 mL 2M HCl	-	-	-	-	+
0.2 mL hydroquinone	+	+	+	+	+
0.3 ml phenanthroline	+	+	+	+	+
0.25 ml Iron Std	-	1x	2x	3x	-
water	3.5	3.25	3.0	2.75	3.5

5. Allow 15 minutes for the flasks to develop their full color, then measure the abosrbance of each solution at 512nm in a 1 cm cell. Make sure the cells are throughly rinsed between each measurment.

6. Subtract the absorbance of the blank from each reading and make a graph like that shown in Figure 5-4, page 101 of your text. Read this section (5-3) of the text on how to interpret this graph.

What to hand in:
1. Table of data showing absorbance for each sample

2. Plot of Absorbance vs. concentration for your samples

3. Determination of Iron in your sample, expressed at mg iron/ g sample.

Experiment 14: **MULTI-COMPONENT ANALYSIS OF HOPS**

Background

The addition of the lupin glands or cones, of the hop plant (*Humulus lupus*) is an important step in the beer brewing process. This plant material contains the compounds humulone, adhumulone and cohumulone (Figure 1)

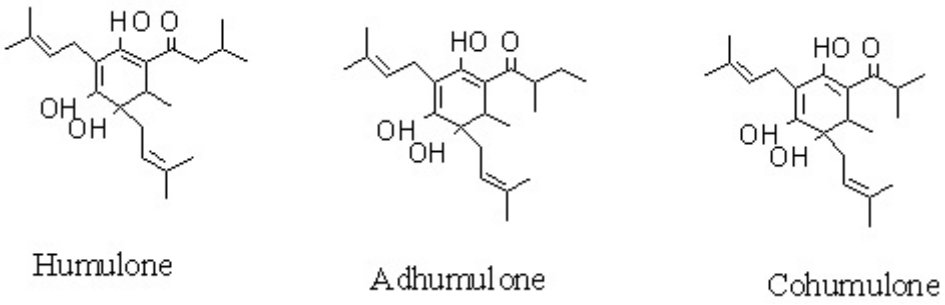


Figure 1.
Alpha Acids from Hops

These compounds by themselves do not have much flavor, but in the brewing process they are heated for several hours to perform an isomerization reaction to become the respective iso-alpha acids.

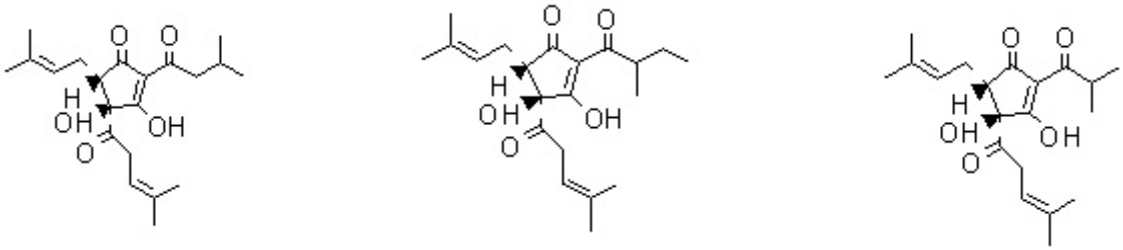


Figure 2. Iso-alpha acids

It is these iso-alpha acids that gives beer its bitter taste and counterbalances the sweetness introduced by the malted (germinated) grain in the beer. The hop plant extract also contains the beta acids lupulone, colupulone and adlupulone (See Figure 3.) In general the beta acids do not make a significant contribution to the taste of beer, so when a brewer starts adding hops to a brew, he is looking for a source that is rich in the alpha acids, and has low amounts of the beta acids.

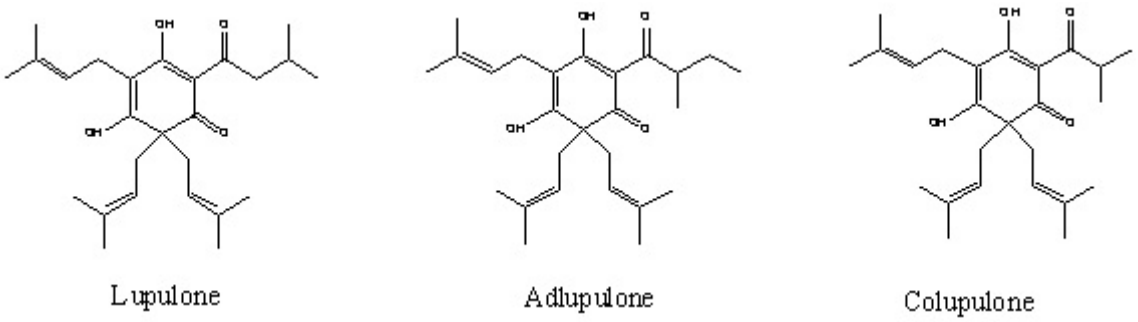


Figure 3. Beta Acids from Hops

There is sizable variability in the quality of hops based the strain of the plant, on its ripeness when picked, how long it has been stored, and the conditions in which it is stored. Thus one other parameter that is looked and in evaluating the quality of hops is a measure called the Hops Storage Index (HSI)

Procedure

Solution 1 : Methanolic NaOH

Pipette 0.5 ml of 6 M NaOH into a 250 ml volumetric flask

Fill to the line with spectrophotometric grade Methanol

(This solution must be made fresh daily - Note: Parafilm is slightly sensitive to methanol, so its is best to use a ground glass stopper with parafilm wrapped around the outside to stopper this solution.)

Preparation of samples (each group will prepare 2 samples)

Grind approximately 3 g of hops in coffee grinder.

Using an analytical balance exactly weigh exactly 2.500 (+/-.005) grams of ground hops directly into a dry 100 ml beaker.

Use a dry 50 ml volumetric pipet to add exactly 50 ml of toluene to the beaker (Note: toluene eats plastic weighing boats)

Place a magnetic stir bar in the bottom of the beaker and cover with a watch glass. Stir the toluene/hops mixture for 30 minutes.

Let settle for 10 minutes (Do NOT let sit for more than 1 hour, the sample will degrade)

Use a pipetman to remove 50 µl aliquot and place in a 25 ml volumetric.

Fill the volumetric to the line with the Methanolic NaOH solution.

Prepare 2 volumetrics for each hops sample

Preparation of a Blank

Pipet 50 ul of toluene into a 25 ml volumetric flask

Fill to line with methanolic NaOH

Acquisition of data

Using the blank to zero the spectrophotometer

Obtain a spectrum for all samples between 520 and 270 nm.

Record the absorbancies for all solutions at 355 nm, 325nm an 275 nm

Disposal

All methanol solutions can be poured down the drain. The toluene solutions, including the toluene/hops mixtures should be poured in a waste beaker in the hood for disposal by the instructor.

Analysis

Plot one spectrum for each sample between 400 and 275 nm

Make a data table containing the absorbance values for all solutions at 355nm, 325 nm, and 275 nm.

The spectra for mixtures of pure alpha and beta acids is shown in Figure 4. (Data taken from Alderton *et. al.* 1954)

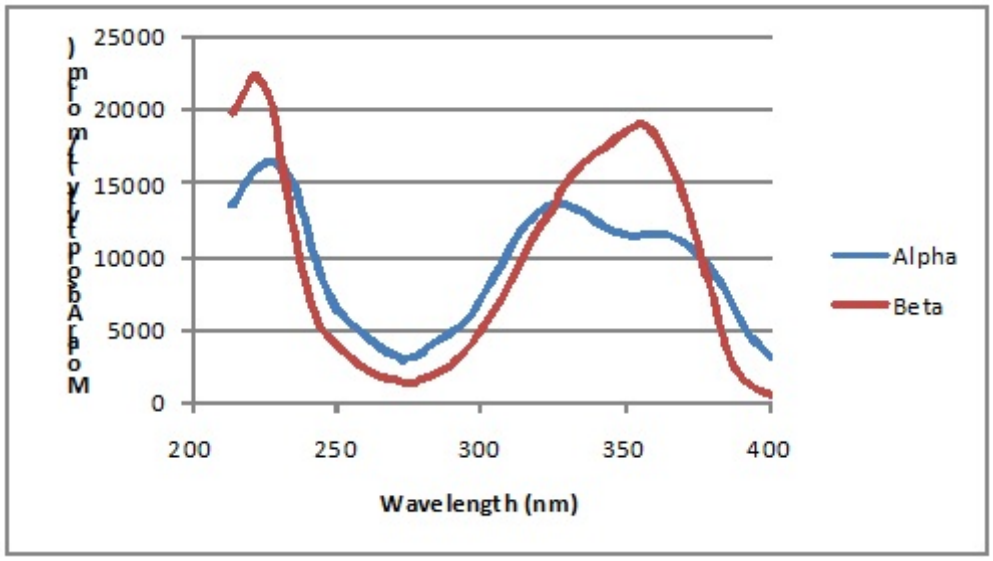


Figure 4. Spectra of Alpha and Beta Acids

Note that your data only goes down to 275 nm. This is because your did your extraction in toluene, and toluene has strong absorbance band at 260nm that obscures any data below 275 nm. It can be seen that the alpha acids have an absorbance maximum ar 325 nm and the maximum for the beta acids is at 355. Assuming the alpha acids have a molar mass of 358 g/mol and the beta acids have a molar mass of 410 g/mol, the molar absorptivities for at these maxima are:

	ε_{355}	ε_{325}
Alpha Acids	11360	13600
Beta Acids	13360	18720

Using the above data, do a two component analysis of your spectra and determine the grams of alpha and beta acids in your extract and the % alpha and beta acids in your original hops sample.

The number your just obtained are actually not correct because this is actually a three component system, not a two component system. The third component is not well characterized, but is thought to be decomposition product of the alpha and beta acids. While not isolated, a spectral analysis of hops systems has been done, and the proposed spectrum of this component is shown in Figure 5.

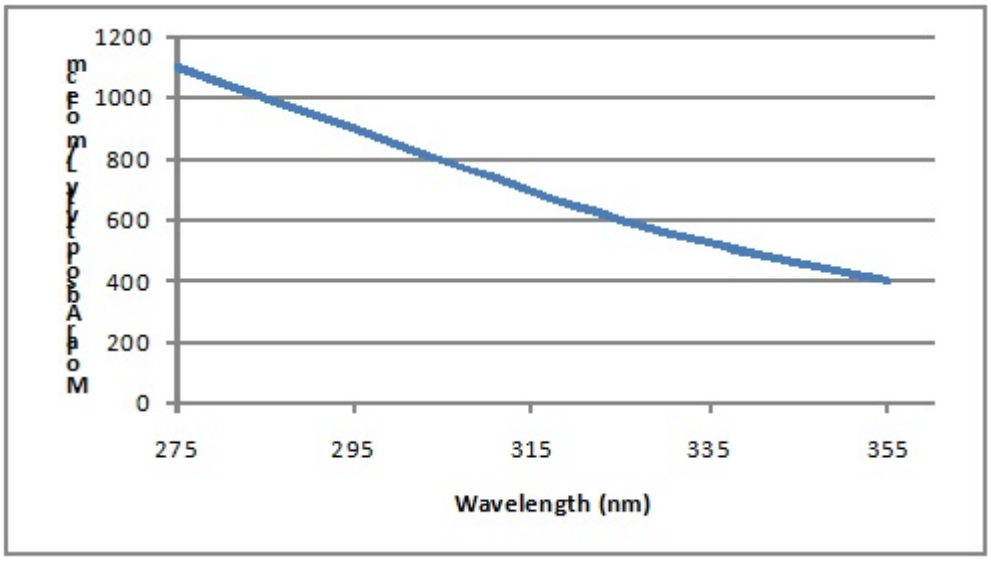


Figure 5. Spectrum of third Hops component

Assuming the molar mass of this component is 400 g/mol, the pertinent molar absorptivites of this component are: 1240 @ 275nm, 600 @ 325 and 400@ 355. If we add in the molar absorptivities of alpha and beta acids of 3110 and 1510 respectively, we can actually solve this system of three equations and three unknowns:

A_{355} = 11360 [Alpha] + 13360 [Beta] + 400[Decomp]
A_{325} = 1360 [Alpha] + 18720 [Beta] + 600 [Decomp]
A_{275} = 3110 [Alpha] + 1510 [Beta] + 1240 [Decomp]

Solving three equations for three unknowns is not trivial, and requires the use of matrix methods from linear algebra. Students who have taken linear algebra are encouraged to try this, and will be given bonus points for this analysis.

For everybody else, especially the home brewers, there is a short cut. If you started your procedure by extracting exactly 2.5 grams of hops then you can use the 'Black Box' equations:

$$\% \text{ Alpha} = -(A_{355} \times 51.56) + (A_{325} \times 73.79) - (A_{275} \times 19.07)$$
$$\% \text{ Beta} = +(A_{355} \times 55.57) - (A_{325} \times 47.59) + (A_{275} \times 5.10)$$

These equations were found by solving the three component system, and then making coefficients from the solution that could be used to find the % Alpha and % Beta directly from the absorbance values. **Calculate your %Alpha and % Beta using these equations. How to they compare to your two component analysis?**

Since the decomposition product has an absorbance at 325 where both Alpha and Beta acids have a minimum, if your sample has a strong absorbance at this wavelength this would indicate that your sample had started to decompose. This is the basis for a measure of hops quality called the HSI or Hops Storage Index.

$$HSI = \frac{A_{275}}{A_{325}}$$

Calculate the HSI for your sample.

In ordinary usage, the HSI is simply a number that can be used to tell the quality of the hops. If your hops is fresh, or it has been properly stored, the HSI should be .28 +/- .02. As the HSI gets > .3, this indicates the build up of the decomposition product, and poorer quality hops.

It can be used in a more analytical way to determine the amount of hops decomposition. Likens *et. al.* (1970) has shown that there is a linear relationship between the log of the HSI parameter and % of alpha acid that has been lost. This relationship is found in the equation:

% Hops acid lost = 61.8 + 102 log(HSI)

Use this equation to calculate the % of hops acid that have been lost in your sample.

References

Alderton, G., Bailey, G.F., Lewis, C.J., and Stitt,F. *Anal. Chem* 26:983-992 (1954).

Likens, S.T. & Nickerson, G.B., *A.S.B.C Proc.* 68-74 (1970).