Chemistry 270
Quantitative Chemical Analysis
Laboratory Manual

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Gustavus Adolphus College
Department of Chemistry

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*Indicates Spring Break  
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Introduction to the Quantitative Analysis Laboratory

The laboratory component of your Quantitative Analysis course serves two main purposes:

1. The laboratory experiments should deepen your understanding of the principles of quantitative measurements and chemical equilibria covered in the lecture and discussion component of the course.
2. A unique set of laboratory skills will be developed as the laboratory experiments are completed. Developing the ability to produce accurate and precise results in any quantitative measurement (whether you are studying a biological, chemical, or physical system) requires not only an understanding of the principles of the measurement, but also and perhaps more importantly the ability to properly manipulate the instruments (whether manual or machine) required to make the measurement. This skill in using instruments of measurement (things like volumetric glassware are especially important here) is developed only through hard work and attention to detail, and by learning from your own mistakes; there are no shortcuts.

In the interest of time, most experiments in this course are focused on the determination of the concentration of one component in a relatively pure solution or a simple mixture. You should realize that the techniques and mindset you learn here will apply to more complex problems in all areas of the physical sciences. This introductory section of the laboratory manual will help you understand what is expected of you in laboratory and how your work will be evaluated.

Laboratory Notebook

You must purchase a permanently bound lab notebook at the beginning of the semester. This notebook should be dedicated to your activities in the laboratory for this course, and will be your record of what you have done in lab and must be kept as neat and orderly as possible. Do not copy your notes into a second book simply for the sake of appearance. When you make a mistake, simply strike the mistake with a single line and continue. Do not erase entries in your notebook; they may turn out to have value later. Notebook format will be discussed briefly in class. Notebooks will be submitted at the end of laboratory for grading the results of previous experiments, and will be returned promptly. Calculations and results are due ONE WEEK after the completion of an experiment. A penalty of ten points per week after the due date will be assessed for late work. Results not reported by the end of "reading day" (the day before final exams begin) will be graded 0.

Your notebook should contain the following information for each experiment. The notebook grade will be assigned largely based on adherence to these guidelines.

Statement of Purpose
Each experiment should be preceded with a statement of purpose. This need not be elaborate, but it should be clear. Ask yourself – What is the goal of this experiment? Why am I doing it the way I am? Answering these questions will provide the content needed to write an effective statement of purpose.

Observations
This is where you record your raw data. In this laboratory this will be a series of recorded masses or volumes.
For some experiments, such as the HPLC lab, your raw data will be a chromatogram, which can be printed out from the instrument software, and pasted into your notebook.

**Results and Discussion**
This section should contain the calculations used in analyzing your data, as well as any notes on observations you make about your experiment or the data as you process your data. For lengthy calculations you are encouraged to use Microsoft Excel or some other software that is built for the purpose of analyzing data. However, you must include at least one example of each calculation; this is often useful for troubleshooting purposes when things go wrong.

**Conclusions**
Aside from a record of the raw data you collect, this is the most important part of the experiment and your record of your work. Here you should discuss whether or not the experiment was successful in fulfilling the purpose of the experiment as you laid it out in your statement of purpose. In discussing the success of an experiment, you should be quantitative; for example, if you find that the %RSD of your determinations of calcium in the gravimetric calcium experiment is 0.2%, you should say so in the conclusion, and point out that this is pretty good precision. If the experiment failed, this is the place to explain why, in what way, and how you might prevent similar failures in the future.

**Preparation**
Success in this laboratory requires that your work efficiently and effectively. You will need to prepare yourself to work efficiently and smoothly in lab. Come to lab knowing what you will be doing: know the reactions, the sequence of operations, and the reagents you will need. It is important that these things all be clear before you start work. Learn to anticipate which steps in your experiment will be ‘rate limiting’, such as prolonged heating or cooling steps; these things cannot be rushed, so you will need to plan your work carefully. If you are unsure of what you are doing, ask the instructor or the lab assistant for help.

**Safety**
Safety will be the foremost priority in the laboratory. Safety goggles are required when you work in laboratory. Be aware and considerate of those around you; it is unfortunate but true that often injuries occur not because of a mistake you have made, but because of a mistake made by someone else in the room. Learn the location of the safety showers, eyewash fountains, and fire extinguishers. Clean up acid spills immediately. Never leave broken glass on the floor or bench top. If you wear your hair long, tie it up to keep it out of the way. Never take your shoes off in lab, regardless of how sore your feet become. Report all chemical or flame burns to your instructor, no matter how insignificant they may seem to you. Clean, organized laboratories are safer than messy laboratories; please do your part to keep things clean and organized, and in doing so keep everyone safe.

**Grading**
For each experiment your laboratory notebook will be graded on a scale of 0 to 10, with 10 points awarded for a complete, organized notebook with correct calculations. Five points will be awarded if there are moderate problems with completeness, organization, or calculation errors, and zero points will be awarded if there are major problems in one or more of these areas. In the case of calculation errors, up to five points can be recovered by correcting the problem and submitting the notebook for a re-grade.
The total point number for Experiment 1 is 25 (including 10 for the notebook), and the total for Experiment 2 is 50 (including the notebook score).

For most experiments (3 through 9) your laboratory grade will be determined primarily by the accuracy and precision of your results. Accuracy is how well your result agrees with the true, known value, and will be evaluated on a relative basis. For example, you may receive an unknown containing 20.00% calcium. If you report the composition as 20.10% chloride, you will have a 0.5% error relative to the true value. Your numerical grade for accuracy is then determined by multiplying the relative error by a factor in the range of 5 to 30 and subtracting the product from 90. The multiplying factor varies from experiment to experiment, depending on the inherent difficulty of a procedure. The grading of inherently more difficult experiments will be more lenient than for relatively easier ones.

The precision of a result has to do with how well replicate determinations agree with one another. Generally, you will run three replicates for each analysis, although you may run four or more at your discretion. You will report precision as relative standard deviation (RSD). For example, in the titrimetric determination of calcium with EDTA, a r.s.d. of 0.1% (or 1 part per thousand) for triplicates is considered excellent work. A RSD of 0.5% is acceptable precision for beginners and 0.7% or greater is poor. Excellent precision will earn 5 points to be added to the numerical grade for accuracy, while poor precision will result in 5 points being subtracted. If the precision of your work is acceptable, your grade for accuracy will be unaffected.

The minimum grade you will receive for a reported result is 30 (plus the notebook score). If you report a single result (for which there can be no measure of precision) 10 points will be subtracted from your accuracy grade.

Please summarize your results in a clearly labeled table in your laboratory notebook. For unknowns in particular you should report the mean value of your determination along with the percent relative standard deviation of your replicates.
Experiment 1: Check-in and Weighing Experiment

Check-in:
Be sure to bring your notebook to lab on the first day. When you arrive in lab, look for a vacant lab desk. There will be a fresh equipment check-in list waiting for you, with the combination of the lock for the lab desk. Write the combination in your lab notebook before you do anything else. Open the lab desk and go down the equipment list, making sure that you have everything on the list and that there is no chipped or broken glassware. Replacement equipment can be obtained at the stockroom window. When you are satisfied that you have everything, give your check-in sheet to your instructor and begin work on the weighing experiment, Pennies, described below.

Before lab starts read the instructions for notebook format discussed in the introductory section of this manual. Leave the first two pages of your notebook open for a Table of Contents. Read about analytical balances and weighing techniques in this manual.

Pennies
Weighing a sample is a fundamental step in many types of quantitative determination. You must be comfortable with proper weighing techniques before you do an experiment that will be graded. This laboratory exercise will give you practice in using an analytical balance, one of the most delicate and sensitive instruments you will ever use. Your instructor will give you a brief demonstration of how to use an analytical balance before you begin.

You will find a bowl of pennies on the front bench of the laboratory. Pick out any five pennies, take them into the balance room (and bring your notebook to record your data), and find an available balance. Check that the balance reads 0.0000 g when the pan is empty. Place an empty weighing boat on the balance pan and weigh it to the nearest 0.1 mg (0.0001 g). Record the weight. Place a penny in the weighing boat, and weigh again. Record this weight, again to the nearest 0.1 mg, and then calculate the weight of the penny. Repeat these steps until you have added a total of five pennies to the weighing boat, and you have recorded all of the masses at intermediate steps so you can calculate the mass of each penny when you are done. The steps you have just taken amount to what is referred to as ‘weighing by addition’. Weighing by addition is probably the most common method of weighing. With modern electronic balances, a button marked ‘Tare’ is pressed to ‘zero’ the balance when a weighing boat is placed on it. The sample is weighed when it is added to the tared weighing boat.

Next, place all five pennies in the boat, record the weight, remove one penny, record the new weight, remove one penny, and so-on, until you record the weight of the empty boat. Calculate the weight of each penny by finding the difference in weight at each step. This is an example of ‘weighing by difference’. If you were weighing a salt by difference, you would place some salt in a clean, dry weighing bottle. You would weigh the bottle containing salt, then transfer some of the salt to a vessel such as a volumetric flask where measurements would be made, and then weigh the bottle again. The difference between the two weights recorded would be the weight of salt you transferred. Finally, calculate the mean and standard deviation of each set of mass measurements.

Discuss your results with your instructor before you leave for the day.
Experiment 2: Calibration of Glassware
(Developed by LWP, 1998, Adapted by DRS, 2009)

1.1 Introduction
This experiment will serve three useful purposes. First, you will learn how to use a volumetric pipette, a volumetric flask, a buret, and a mechanical micropipette for making accurate and precise measurements of volume. Second, you will use an analytical balance to measure weights. Finally, when the experiment is done you will have some calibrated glassware for use in subsequent experiments later on in the course.

1.2 Safety
Your instructor will demonstrate the use of Alcohol/KOH solution for cleaning glassware. Be very careful with this solution as it is extremely caustic and will cause burns. Wear gloves whenever handling this solution, and deposit the used solution in an appropriately labeled container.

1.3 Preparation
Chapter 2 of the Harris textbook contains much useful information about the care, cleaning, and use of various measurement devices you will encounter in the analytical laboratory. You should be familiar with this essential information before starting this experiment.

Before beginning the experiment you will be asked to report the water level in a buret, and dispense water from a 25-mL volumetric pipet and record the mass of water. These will be done using specific, well characterized glassware, and this will allow your instructor to make sure you are using the glassware correctly before you begin.

1.4 The Water Supply
You will need a supply of deionized (DI) water at room temperature for the calibration procedures outlined below. At the beginning of the lab period, fill a one-liter beaker with room temperature DI water and cover it with a watch glass. Your instructor will provide one NIST-certified digital thermometer for the group to share in measuring the temperature of your water. You will measure volumes of your glassware at whatever the room and water temperature happen to be, and then correct the observed values to 20 °C. Water densities are given in Table 2-7 of the Harris textbook.

2. Calibration of a 25-mL Volumetric Pipette
Begin by weighing a clean, dry 125-mL Erlenmeyer flask (with stopper) to the nearest 0.1 mg on an analytical balance. Using your clean 25-mL pipette, carefully transfer its volume of DI water (for which you have measured the temperature) to the Erlenmeyer flask. Weigh the flask and its contents to the nearest 0.1 mg. Repeat the transfer and weighing process several times. To save time add several samples to the receiving flask without emptying the contents between additions. Use the final weight of the first sample and the flask as the initial weight for the second determination, and so-on. The capacity of most macroanalytical balances is about 160 g, and this will limit the number of replicates you can measure in a single series without emptying the flask. Complete a total of five replicates before moving on.

Convert the measured weights to volumes using the density of water at the experimental temperature, and
a buoyancy correction (see Stoll/Secor handout for guidance). Calculate the mean, standard deviation, and relative standard deviation of your replicate measurements. Finally, convert the mean volume measured at room temperature to a mean volume at 20 °C, and compare this value to the value specified for the pipette. You should be able to achieve a precision of 1 part-per-thousand in your replicate measurements for the 25-mL pipette; it is more difficult to achieve this level of precision using smaller pipettes. Be sure you label the pipette to identify it as the one you have calibrated.

3 Calibration of a 100-mL Volumetric Flask

Your 100-mL volumetric flask must be clean and dry before it is calibrated. It will be especially important to make sure the neck of the flask is clean enough that water droplets do not cling to it. Use a 120 °C drying oven to dry volumetric flasks; never heat a flask with a burner flame, lest you distort the shape of the glass and change its volume. Weigh the clean, dry volumetric flask on a top-loading balance (capacity = 300 g). Record the weight of the flask to the nearest 0.01 g, and return to your desk to fill the flask with room-temperature DI water. Fill the flask to the mark, taking careful note of any droplets remaining on the neck of the flask above the mark. If you see some drops, you should clean the flask again before moving ahead. Weigh the filled volumetric flask again to the nearest 0.01 g, record the weight and the water temperature. Calculate the weight of water and the volume corrected to 20 °C. To repeat the process, empty the volumetric flask, rinse it with acetone, and dry it in the oven (make sure all of the visible liquid acetone is drained before placing it in the oven). Calculate the mean, standard deviation, and relative standard deviation of your replicate data, and use the mean volume for calculations in subsequent experiments. Be sure you label the flask to identify it as the one you have calibrated.

4. Calibration of a Buret

You will check out a 50-mL buret and calibrate it at 10-mL intervals between 10 and 50 mL. There will be a unique number on the buret. Be sure to record this number so that you can use the same buret every week, as your calibration data will be specific to the particular buret you use. The buret must be clean, but need not be dry for calibration. Attach a buret clamp to a ring stand, and place your buret in the clamp. Pour DI water at room temperature into the buret until the water level is a few centimeters above the top demarcation (0 mL). Open the stopcock fully to force air bubbles from the buret tip. If necessary, add more water to the buret, then add more water until the top line is reached. Make sure that there are no bubbles in the buret tip and that the stopcock does not leak. If bubbles remain in the tip, consult with your instructor for some tips on removing them. If there is a leak, gently tighten the Teflon nut on the stopcock. Do not apply excessive force to the nut; the threads on the Teflon nut will strip easily. When the buret is finally ready, read the volume to the nearest 0.01 mL. If the initial reading is more than 0.00 mL, do not try to add more water to reach exactly 0.00 mL, rather record the actual level as your starting point. Record the initial level in your notebook. Weigh a clean, dry, 125-mL Erlenmeyer flask to at least the nearest 5 mg, and record this weight. Place the flask under the buret and open the stopcock slowly. Let out about 10 mL of water over a period of about 30 sec. Wait another 5 sec. for water to drain down the walls of the buret, and record the level on the buret. Touch the wall of the flask to the tip of the buret to remove any excess liquid from the tip, remove the flask and weigh it to the same precision as before. Record the weight that you measure at this point. Return to your buret and transfer the next 10-mL portion to the Erlenmeyer flask. Use the weight of the first 10-mL portion and flask as the initial value in calculating the weight of the second 10-mL portion. Dispense water at about the same rate (about 5 sec./mL), and wait 30 sec. to read the level of the buret. Continue this process for measurements at 30, 40, and 50-mL levels on the buret.
Calculate the corrected volumes at 20 °C for 10, 20, 30, 40, and 50 mL levels of the buret. Finally, plot the cumulative error for your buret in the range of 10 to 50 mL.

5. Calibration of an Adjustable Mechanical Micropipette

Several adjustable micropipettes will be available in the lab. As a lab group you will evaluate both the precision of the set of micropipettes, as well as the accuracy of each pipette at regular volume intervals. Your instructor will assign specific roles to each member of the group; the calibration data will be collected and organized for reference in subsequent experiments.

6. Report

You may do the calculations for this experiment outside of the lab. You must submit your results one week after completing the work. Report individual values, means, and relative standard deviations for the glassware and micropipet you calibrate. Carefully consider what your data tell you about the performance of your devices, and address this in your conclusion statement.
Introduction

The determination of sodium carbonate by titration with standard acid solution is of interest for several reasons. Carbonate is an important component of limestone, natural waters, and the commercial material "soda ash", and is frequently encountered in commercial analyses. In addition, some experience with primary standard bases is important to students of analytical chemistry because they are widely used for preparing standard acid solutions.

Carbonate ion is a weak base ($K_b = 2.1 \times 10^{-4}$) and can accept a proton to form bicarbonate ion:

$$CO_3^{2-} + H^+ \rightleftharpoons HCO_3^{-}$$

Bicarbonate ion is an even weaker base than carbonate ion ($K_b = 2.2 \times 10^{-8}$) and can combine with another proton to form carbonic acid:

$$HCO_3^{-} + H^+ \rightleftharpoons H_2CO_3$$

In water, carbonic acid is in equilibrium with CO$_2$ gas:

$$H_2CO_3 \rightleftharpoons H_2O + CO_2$$

In the titration of sodium carbonate with hydrochloric acid, carbonate is titrated until it is completely converted to CO$_2$ (2 protons per carbonate). You will use the indicator bromcresol green, which is blue at pH levels greater than 5.5, yellow at pH levels below 4.0, and green in the region of pH 4.8. Direct titration to the yellow color would be possible if it were not for unfortunate fact that the solubility reaction for CO$_2$ in water is very slow to reach equilibrium, and a fading endpoint color change is observed along with bubbles of CO$_2$. Equilibration is hastened by heating samples to boiling just before the yellow color is reached. Carbon dioxide boils out, the color reverts to blue, and the subsequent blue-yellow color change is quite sharp. The change is so sharp that the intermediate green color may not be seen. A carbonate titration curve monitored with a pH electrode (see Figure 4.1) shows the effect of dissolved carbon dioxide on solution pH near the equivalence point.

In this experiment you will use the primary standard base, tris-(hydroxymethyl)amino methane (THAM). In water THAM is present in both the protonated and deprotonated forms of the amine:

$$H_3NC(CH_2OH)_3 + H^+ \rightleftharpoons H_3NC(CH_2OH)_3^+$$

The gram formula weight of THAM is 121.14 g/mol. Do not dry the THAM provided; it has already been dried in a vacuum oven.
Procedure

Place a little less than one liter of deionized water into a clean glass-stoppered one-liter bottle. Add enough concentrated hydrochloric acid (12 \text{ M} \text{ HCl}, stored in the fume hood) to produce a solution that is roughly 0.1 \text{ M} using a small graduated cylinder and mix the solution thoroughly.

Standardize your hydrochloric acid solution with THAM. Calculate the weight of THAM that you must take to require 30-40 mL of 0.1 \text{ M} hydrochloric acid solution for titration. Weigh and titrate three samples of THAM to the bromcresol green endpoint. Is boiling necessary?

Your sample of unknown contains sodium carbonate and some inert but water-soluble salt. When sodium carbonate gets moist, it will react with water and carbon dioxide to form sodium bicarbonate. Since your sample might have undergone this reaction, you must undo the possible damage by heating the sample to convert any sodium bicarbonate to sodium carbonate:

$$2 \text{NaHCO}_3(s) \xrightleftharpoons[140\degree C]{\text{\Large $\Rightarrow$}} \text{Na}_2\text{CO}_3(s) + \text{H}_2\text{O}(g) + \text{CO}_2(g)$$

Dry your unknown sample for two hours at 140 \degree C. Weigh out three or more samples, each containing 0.25 to 0.35 g of the unknown, into 250-mL flasks, and dilute as before. Titrate with standard acid to the endpoint as before.

Report the results as percent sodium carbonate, \% \text{Na}_2\text{CO}_3 (Range 20 - 50\% \text{Na}_2\text{CO}_3).

(Hint: do not forget that the reaction of THAM and HCl is a 1:1 reaction and that the reaction of sodium carbonate with HCl is a 1:2 reaction. Double-check your calculations.)
Figure 4.1 Titration of sodium carbonate with hydrochloric acid (Figure 12.14 from Potts and Evans, Quantitative Chemical Analysis).
Introduction:

A weak acid is characterized by two important properties, its equivalent weight and dissociation constant. Both properties can be determined from a titration in which solution pH is monitored as a function of the volume of standard base titrant added to the solution. A hydrogen-ion selective glass electrode is used to determine the pH of the solution during titration. The electrical potential developed between a glass electrode and a reference electrode (housed in the same body, referred to as a "combination electrode") is translated to a pH value by a pH meter, and displayed to three significant figures. In this experiment incremental volumes of standard base solution are added to a solution of an unknown weak acid. The pH of the solution is measured and recorded after each increment of base is added. A plot is made of measured pH versus volume of base added, and the equivalence point and dissociation constant are evaluated from the plot. Before coming to lab you will be told about the care and feeding of glass electrodes. Please use them with care; they are quite expensive, and not immune to abuse.

The equivalent weight of an acid is found from the volume of base required to reach the equivalence point in the titration. This point lies in the region of the titration curve where the pH changes most rapidly, and is approximated by the inflection point in the curve (20.00 mL in the figure below). The equivalent weight is related to the molecular weight of the acid in a very simple way: The equivalent weight is equal to the molecular weight divided by the number of hydrogen ions involved in the titration reaction. Since you will be working with an unknown weak acid, you will not know if it has one, two, or three reacting protons. Therefore the only real information you can obtain from this particular titration is the equivalent weight. If you are given an unknown sample that is a diprotic weak acid, its dissociation constants will be so close that only one equivalence point break is observed.
The dissociation constant of the weak acid can be determined from the pH at the midpoint of the titration (the 50% point, or the pH at 10.0 mL of base added in Fig. 3.1). At this point the acid ("HA") and its conjugate base (A\textsuperscript{-}) will be present at the same concentration (50% of the total concentration of weak acid species). Using the expression for the thermodynamic dissociation constant, we can substitute concentrations for activities as explained in your text:

\[
K_a^o = \frac{a_{H^+} \times a_{A^-}}{a_{HA}} = a_{H^+} \times \left( \frac{[A^-] \gamma_{A^-}}{[HA] \gamma_{HA}} \right)
\]

At any point in the titration, the mass-balance expression must apply:

\[
c_t = [A^-] + [HA]
\]

As base is added, the concentration of HA decreases and that of A\textsuperscript{-} increases; the sum must be constant. At the half-way point in the titration, [A\textsuperscript{-}] = 0.50 \(c_t\) and [HA] = 0.50 \(c_t\), and the concentration terms divide out of the \(K_a^o\) expression, leaving a ratio of activity coefficients,

\[
K_a^o = a_{H^+} \times \left( \frac{\gamma_{A^-}}{\gamma_{HA}} \right)
\]

or (taking negative logs of both sides of the equation),
We will calibrate the pH electrode and meter to measure hydrogen ion activity. Therefore, if we can find a way to approximate the activity coefficients of $A^-$ and $HA$, we can evaluate the thermodynamic equilibrium constant.

There will be two main tasks in performing the experiment: 1) standardizing the titrant (sodium hydroxide), and 2) titrating the samples of unknown weak acid. Prepare your base as described below, then titrate samples of your unknown while the primary standard potassium biphthalate dries in the oven. Near the end of the lab period (or the next week) standardize the sodium hydroxide solution.

**Procedure:**

1. **Prepare and standardize a 0.1 M solution of sodium hydroxide.** Boil about one liter of deionized water in a large glass beaker to remove dissolved carbon dioxide. Carefully pour the hot water into a one-liter polyethylene bottle. Cap the bottle and chill the water to room temperature with ice. At some point during the cooling process add about 7 mL of clear 50% NaOH (available in the hood) to the water using a 10 mL graduated cylinder. Be very careful, as 50%NaOH is quite caustic and will cause burns. Mix the resulting solution well and keep the bottle capped. Do not use this titrant solution until it is at room temperature.

2. Dry about 5 grams of primary standard grade potassium biphthalate ("KHP", M.W. 204.21 g/mol) in a weighing bottle at 110 °C for two hours. Cool and store the KHP in your desiccator. Weigh out as carefully as possible three samples of KHP (800 -900 milligrams each) into 250 mL Erlenmeyer flasks. When you are ready to standardize, dissolve each KHP sample in about 50 mL of deionized water. Add about two or three drops of 0.1% (w/w) phenolphthalein indicator, and titrate to the first persistent (for more than 15 sec.) pink color with your NaOH titrant. Be sure to rinse your buret thoroughly with standard base solution when you begin, and check to see that the buret dispensing tip is free of air bubbles. Read your buret to the nearest 0.01 mL.

3. If you find yourself waiting for something to dry or cool, look up the $pK_a$ of KHP and the color range of phenolphthalein, and check the wisdom of choosing phenolphthalein as the indicator.

4. **Calibrate a pH meter:** Use two buffer solutions (pH 7 and pH 4) available in the lab. The meters you will use have computer chips that calibrate the electrode and meter when the electrode is placed in the proper sequence of buffer solutions. Directions are provided for these computerized meters. Be sure to rinse off the electrode with deionized water whenever you move it from one solution to another.

5. **Trial titration:** Get an unknown weak acid from your instructor. Be sure to write down the number of the unknown, as this will be the only way to determine the accuracy of your results. **DO NOT DRY THE UNKNOWN.** Do a trial titration to find out what weight of weak acid sample will be needed to consume
between 30 and 40 mL of your titrant. To do this start by weighing out about 100 milligrams of your unknown and dissolving it in about 100 mL of deionized water. Place the freshly rinsed pH electrode in the solution, and fill the buret to the 0.00 mL mark with standard base solution. Titrate using 0.5-mL volume increments until you see the sudden jump in pH that signals your trial endpoint. Read the volume delivered from the buret, and use it to calculate the approximate weight of weak acid samples you need.

(4) The Unknown: Weigh by difference three samples of unknown weak acid into 250-mL Erlenmeyer flasks and dissolve each in 100 mL of deionized water. The volume of water used to dissolve each sample should be measured with a graduated cylinder; it will be important to know that the volume is 100 mL. Titrate each sample by adding base in 1-mL increments until you begin to see the pH begin to move rather rapidly with each addition (about pH 6 for most samples). Near the equivalence point add smaller increments, close to 0.1 mL. Once you are 1 to 2 mL past the equivalence point you may add base in 1 mL increments again. Stop titrating when you are about 5 mL past the equivalence point. The object is to collect many data points close to the equivalence point. Fewer data points are needed away from the equivalence point. Read your buret to the nearest 0.01 mL.

(5) Plot your titration curves with the help of a spreadsheet, and attach the curves permanently to your notebook. Calculate the equivalent weight of your unknown weak acid from the equivalence point volume and the concentration of your standard base solution. Report the average, standard deviation, and percent relative standard deviation (%RSD) for the replicate determinations of the equivalent weight of your unknown acid in your summary table.

Locate the points on the titration curves where exactly half the equivalence point volume has been added. Calculate the ionic strength of each solution at these midpoints using the millimoles of sodium ion, the mmoles of conjugate base formed (these are the same), and the total volume of the solution at each midpoint. Use the extended Debye-Huckel activity coefficient equation to calculate $\gamma_A$. Assume a Kielland diameter of 0.6 nm for the anion. You may assume that the weak acid is monoprotic, and that the anion is singly charged. Calculate the thermodynamic dissociation constant, $K_a^\circ$, and then its negative log, or $pK_a^\circ$. Report the average $pK_a^\circ$ value in your summary table.

Note: You will earn two separate grades on this lab, one for the $pK_a$ and one for the equivalent weight, which will then be averaged to arrive at your grade for the experiment.
Experiment 5: The Gravimetric Determination of Calcium as Calcium Oxalate Monohydrate

Introduction
Calcium is an important component of a number of minerals. In the form of lime (CaO) it is a valuable industrial commodity. Samples containing about 50 mg or more of calcium can be assayed conveniently by titration with EDTA or (after appropriate dilution) by atomic absorption spectroscopy. A less convenient but quite accurate and precise method involves the precipitation of a slightly soluble salt formed by calcium with oxalate ion. In this experiment, the salt is precipitated, purified, collected, dried, and weighed, in a process called gravimetry (literally measuring mass). Briefly, dried and weighed samples of an unknown containing calcium carbonate are dissolved with hydrochloric acid. Solutions are then filtered to remove silica. Solid urea and an acidified solution of ammonium oxalate are added to the solutions of sample, and the urea slowly hydrolyzes to generate oxalate ions homogeneously. Calcium oxalate precipitates gradually, forming large, pure crystals. When precipitation is complete the precipitate is collected by filtration with carefully cleaned and weighed sintered glass crucibles, washed with cold water, then acetone, and dried for one hour at 100-105 °C.

The chemistry of the overall process is described by the following equations.

\[
\text{CaCO}_3 + 2\text{H}^+ \rightleftharpoons \text{Ca}^{2+} + \text{CO}_2(\text{g}) + \text{H}_2\text{O}
\]

\[
(\text{NH}_2)_2\text{CO} \text{ (urea)} + \text{H}_2\text{O} \rightleftharpoons 2\text{NH}_3 + \text{CO}_2(\text{g}) \text{ (slow)}
\]

\[
2\text{NH}_3 + \text{H}_2\text{C}_2\text{O}_4 \rightleftharpoons 2\text{NH}_4^+ + \text{C}_2\text{O}_4^{2-}
\]

\[
\text{Ca}^{2+} + \text{C}_2\text{O}_4^{2-} \rightleftharpoons \text{CaC}_2\text{O}_4\text{H}_2\text{O} \text{ (s)}
\]

The reaction of urea with water (hydrolysis) is slow at room temperature, but can be accelerated by heating the solution. The rate at which ammonia forms determines the rate at which oxalate is produced from oxalic acid, and this in turn determines the rate at which calcium oxalate precipitates. Generation of the precipitating agent \textit{in situ} makes this a \textit{homogeneous precipitation}.

\textbf{CAUTION: Oxalate salts are poisonous}

This experiment will require two laboratory periods for completion. During the first period you will clean and dry to constant weight three glass filter crucibles, dry your unknown sample, weigh out and dissolve three portions of the unknown sample, and finally add urea and acidic ammonium oxalate to all three. Gently heat the solution so that it is hot, but not boiling. Cover each beaker with a watch glass to prevent loss of sample. Heat each sample gently (not boiling) until the end of the lab period, and then set your samples aside in your lab drawer (or in a designated safe place) and let the urea hydrolyze slowly at room temperature until the next lab period. During the second lab period you will check for completeness of
Precipitation, heat the solutions, filter, wash, dry, and weigh the precipitates.

Detailed directions for cleaning and drying filter crucibles will be given in class.

**Detailed Procedure:**
(Use check-off boxes if you wish)

1) Obtain an unknown sample from your instructor, place it in a clean, dry weighing bottle, and dry it in a drying oven at 110 – 120 °C for one hour to remove adsorbed water.

2) Clean and label three sintered glass filter crucibles. Place the crucibles in a 400-mL beaker labeled with your name, cover the beaker with a watch glass, and place it in an oven at 110 – 120 °C. After one hour remove the beaker and crucibles from the oven with beaker tongs. Using crucible tongs transfer the glass filters to your desiccator. Allow them to cool for 30 minutes before weighing them. Record their weights, return them to the beaker and the oven, and repeat the process until weights you measure agree within +/- 0.2 mg (that is, they are at constant weight).

3) While the filter crucibles are heating, weigh by difference three 0.3 to 0.4 g samples of unknown into separate 150-mL beakers. Record each sample weight to the nearest 0.1 mg.

4) Add about 75 mL of deionized water to each sample. Using a 10-mL graduated cylinder add about 5 mL of 6 M HCl, gently stirring the solution as you add acid. Do not let solutions spatter out of the beakers! You will see wisps of white powder (silica, insoluble in acid) remaining after adding hydrochloric acid, so heat your samples gently (not boiling) and filter them through number 42 or 44 filter paper into clean and labeled 250-mL beakers. Be careful not to mix up samples (or switch stirring rods) at this stage. After filtering, wash each filter paper with five 5-mL portions of hot deionized water. Be sure that these washings remain with their proper filtered sample solutions.

5) Add five drops of methyl orange indicator to each solution and stir. The solutions should be pink at this point. If they are not, ask your instructor for help.

6) Add to the contents of each beaker about 20 mL of a saturated solution of ammonium oxalate in 0.3 M hydrochloric acid (provided by instructor). A precipitation may start at this point if the sample is a little dilute in acid; this is not a problem, and you may proceed.

7) Dissolve about 15 g of solid urea in each solution. Even though the hydrolysis of urea is slow at room temperature, your solutions may start to get turbid in a few minutes. Cover each beaker with a watch glass and store the solutions in your lower lab desk compartment until the next lab period. You may speed up hydrolysis by heating the covered solutions *below boiling temperatures* until the indicator color changes from pink to yellow. This should take more than 30 min. Rinse any condensate on the watch glasses into the beakers. Boiling will break apart the crystals that you are trying to coagulate by heating, and should be avoided. Do not leave solutions on the burners unattended.

8) After storing solutions in your desk for several days, the indicator should be yellow or a yellowish-pink (*pH* of about 5), and the precipitation should be complete. If your solutions are still orange-pink, add dilute
ammonium hydroxide dropwise until the indicator color is yellow or until you see no more calcium oxalate precipitating.

9) Heat the three solutions nearly to boiling while you set up a vacuum pump and flask for your filter crucibles. Filter the warm solutions through the appropriate filter crucibles (already at constant weight) by vacuum. The excess oxalate ion in the warm solutions will suppress solubility of calcium oxalate.

10) Work with one beaker of precipitate and one filter crucible at a time. First transfer as much liquid as you can from the beaker containing precipitate into the filter crucible. After most of the liquid has been transferred, transfer the precipitate with the help of a rubber policeman. When nearly all the precipitate has been transferred to a filter crucible, use the rubber policeman and small volumes of ice-chilled (i.e., really 0 °C, not tepid) deionized water to transfer the last remaining precipitate from the beaker. Deionized water will dissolve some calcium oxalate unless it is ice cold.

11) Wash the calcium oxalate crystals in each filter crucible with two 10-mL portions of ice-cold acetone. Draw air through the crystals for several minutes to remove the last traces of acetone.

12) Place all three crucibles in your 400-mL labeled beaker, cover the beaker with a watch glass, and place it in an oven at 100 to 105 °C for one hour. The temperature of the oven must not be allowed to go above 105 °C.

13) Remove your crucibles from the oven and transfer them to your desiccator to cool for 30 minutes. Weigh the three crucibles to the nearest 0.1 mg. Return them to the 105 degree oven, heat for another hour, and check for constant weight.

Report percent calcium in the original unknown sample.

Introduction: The Reaction

Iron can be determined directly by titration of Fe(II) with dichromate in acidic solutions containing phosphate.

\[ 6 \text{Fe}^{2+} + \text{Cr}_2\text{O}_7^{2-} + 14 \text{H}^+ \rightleftharpoons 6 \text{Fe}^{3+} + 2 \text{Cr}^{3+} + 7 \text{H}_2\text{O} \]

The equivalence point is signaled by a change in color of the redox indicator, sodium diphenylamine sulfonate, from colorless to violet. The presence of phosphoric acid stabilizes the Fe(III) product and lowers the standard potential of the Fe(III)/Fe(II) couple sufficiently to guarantee that the redox reaction is quantitative and that the endpoint matches the reaction equivalence point. This experiment also illustrates the use of ferrous ethylenediammonium sulfate tetrahydrate (Oesper’s Reagent) as a primary standard. The equivalence point is sufficiently sharp to justify the use of a gravimetric titration procedure, which is presented as an alternative.

The Titrant

In many applications we can treat potassium dichromate as a primary standard. Unfortunately in this experiment, we use an indicator that requires the presence of iron to catalyze the endpoint color change. This “indicator blank” cancels out when both standards and unknowns are titrated. We will weigh out approximately what we need to dissolve to make a 0.007 M solution of reagent grade potassium dichromate, and standardize the solution with samples of Fe(II) prepared from primary standard grade ferrous ethylenediammonium sulfate tetrahydrate.

Caution: Postassium dichromate is quite toxic. Familiarize yourself with proper handling precautions before beginning work with this material.

Weigh out about 1.2 g of reagent grade K$_2$Cr$_2$O$_7$ (M.W. 294.19 g/mol) on a top-loading balance, record the weight and then transfer the salt to a clean glass bottle. Dissolve the salt in about 500 mL of deionized water. This is not a primary standard solution, and does not require the use of a volumetric flask. Make sure all the crystals are dissolved before you use this solution in a titration!

Before proceeding, you must do some calculations. Based on the weight of potassium dichromate you obtained, calculate the nominal concentration of the potassium dichromate solution. It should be about 0.007 M. The balanced chemical reaction between Fe(II) and dichromate shows that each mole of dichromate oxidizes six moles of Fe(II). Use this fact to calculate the number of moles of iron that will react with each milliliter of dichromate titrant. From that number, calculate the weight in grams of primary standard ferrous diethylammonium sulfate tetrahydrate (GFW 382.16) you must weigh out for each
replicate sample, to consume between 20 and 40 mL of dichromate titrant. The average atomic mass of iron is 55.847 Da.

**Standardization of Dichromate Titrant**

Estimate the amount of primary standard ferrous ethylenediammonium sulfate tetrahydrate, Fe(C$_2$H$_4$(NH$_3$)$_2$)(SO$_4$)$_2$*4H$_2$O (M.W. 382.16 g/mol), you must transfer to a clean, dry weighing bottle. Plan to use enough for triplicates and a reality check titration. DO NOT DRY THIS SALT IN AN OVEN!

Perform a reality-check titration. Weigh by difference on an analytical macro balance ca. 100 mg of the primary standard into a 250-mL Erlenmeyer flask. Take the flask back to your desk and add ca. 50 mL of deionized water (graduated cylinder) to dissolve the salt. Add 10 mL of 1M sulfuric acid, and then 10 mL of concentrated (syrupy) phosphoric acid using your 10-mL graduated cylinder. Add 3-6 drops of diphenylamine sulfonate indicator, and titrate quickly to the first permanent violet color. The product of the redox reaction is Cr$^{3+}$, which has a green color. Despite the green cast to the solution, the color change of the indicator is obvious, however the permanent dark color may take a few seconds to develop, so be patient when you approach the endpoint.

Based on the outcome of your reality check, determine the amount of primary standard salt you must weigh out for each of three standardization titrations. Run three replicates, but weigh out and titrate each sample before weighing the next; do not leave moist Fe(II) samples exposed to the air for more than a few minutes, as they will be oxidized by oxygen. This is especially true once phosphoric acid has been added, as Fe(III) - phosphate complexes are quite stable.

A disposal vessel will be provided for your solutions.

Do not flush titrated samples or standards down the sink! Do not flush leftover dichromate solution down the sink!

**The Unknown**

Your instructor will issue you an unknown sample that contains between 5% and 20% (w/w) Fe. DO NOT DRY THE UNKNOWN IN A DRYING OVEN! Transfer your unknown sample into a clean, dry, and labeled weighing bottle. Be careful not to confuse your unknown with the primary standard!

Assuming that your unknown sample contains between 5 and 20% iron, calculate a range of weights for your sample that will bring you into the range of 20 to 40 mL of dichromate titrant. Do a quick titration to check what sample weights will work best before actually titrating your unknown carefully. To do this, weigh out (analytical macrobalance) a 100-mg portion of your unknown into a clean (but not necessarily dry) 250-mL Erlenmeyer flask. Dissolve the sample in about 50 mL of deionized water (graduated cylinder), add 10 mL of 1 M sulfuric acid and then 10 mL of concentrated phosphoric acid (also graduated cylinder), 3-6 drops of indicator solution (same amount as you used in the standardization), and titrate with standard dichromate immediately to the violet endpoint.
Evaluate the situation based on your reality check titration, and estimate the mass of unknown needed in each titration to consume between 30 and 40 mL of titrant. Run three replicate titrations of your unknown (one at a time, without letting moist samples sit around).

Report percent iron in your sample. (Range: 5-20%)

Additional Notes
You may use either a volumetric technique or a gravimetric technique for this analysis. The volumetric method involves the use of a buret, just as you have in many other experiments. Be sure to use all the standard precautions. Be especially careful adding titrant close to the endpoint, be sure to wash down the walls of the flask and the tip of the buret at the endpoint, and add an extra drop of titrant, if needed.

Alternatively, you may transfer some of your dichromate stock solution to a 125-mL plastic bottle which is either in your lab desk or available from the stockroom. Weigh the plastic bottle and solution on an analytical balance or a top-loader. Squeeze the bottle to deliver dichromate to the sample solution. Be careful to transfer all the dichromate to the flask and none to the counter top. When the endpoint is reached, weigh the plastic bottle and its contents again. Calculate the concentration of dichromate as a "titre", in units "milligrams of iron per gram of dichromate solution". This simple ratio lets you ignore the 6:1 stoichiometry of the reaction, and greatly simplifies calculations. You will find that the precision of the gravimetric process (± 10 mg in 140 grams on a top loader for each weighing, two readings) is as good as that of the buret (± 0.02 mL in 40 mL, two readings). Remember that another factor is also the precision of your weighing of the iron salt samples, ± 0.2 mg.

Safety Notes: No special precautions are needed for handling either the primary standard iron compound or the unknown samples. Potassium dichromate contains Cr in the +6 oxidation state, and is a suspected cancer-causing agent. The lab procedure has been designed to minimize the creation of hazardous waste. Dispose of all chromium-containing solutions in the specially provided plastic bottle. Dichromate will be reduced to Cr$^{3+}$ with mossy zinc before disposal.
Experiment 7: Buffer Preparation  
(Developed by DRS, 2009)

Objective
The primary objective of this experiment is to apply your theoretical knowledge pertaining to buffers to actually prepare a buffer solution in the laboratory, and become familiar with some of the practical complications associated with this work. In this context, you are asked to work with a partner toward two goals:

1. Prepare 500 mL of a buffer solution with a measured pH within 0.1 pH units of the specified pH, containing the buffering agent at the 0.050 $F$ level.
2. If the initial goal is not met, troubleshoot the buffer preparation process to figure out why the measured pH is not the same as the specified pH, and correct the buffer preparation procedure to achieve goal 1.

Buffer Targets (all 50 $mF$ in the buffering agent)

1. Phosphate, pH 2.5
2. Phosphate, pH 3.0
3. Citrate, pH 3.5
4. Formate, pH 4.0
5. Acetate, pH 5.0
6. Phthalate, pH 5.0
7. Phthalate, pH 5.5
8. Phosphate, pH 6.5
9. TRIS, pH 7.0
10. TRIS, pH 7.5
11. Phosphate, pH 8.0
12. Ammonia, pH 8.5
13. Ammonia, pH 9.0
14. Carbonate, pH 9.5
15. Carbonate, pH 10.0

Materials
All of the materials in the laboratory and stockrooms are available to you, at your request. Let your instructor know what you would like to use, and roughly what quantity of each chemical. A pH meter will be available for each team.

Reporting your Results
You are asked to prepare, with your partner, a single report (not to exceed ten pages) describing your experiment and results. The report should include the following sections:
1. Introductory statement of the goals of your experiment
2. A concise experimental section describing the steps of your procedure, with the aim of providing enough detail that one of your peers could replicate the experiment (and understand what they are doing) without performing additional calculations.
3. A results section that summarizes your experimental results
4. A conclusion statement

Please submit your report electronically by emailing to dstoll@gustavus.edu.

Grading

As usual, the point total for the experiment is 100 points, and 10% of this total is dedicated to the evaluation of your laboratory notebook. The same score will be assigned to each person in your team. Of the remaining 90 points, 45 points will be based on your success in achieving goal #1, and 45 points will be based on the quality of a short team report of your experiments and results, as described above.
Objective

The primary objective of this experiment is to apply your theoretical knowledge of spectroscopy, calibration methods, and solution preparation techniques to the determination of an unknown solution of methylene blue dye in water. There is a significant focus on the quantitative aspects of this type of experiment, especially the impact of laboratory technique on quality of calibration data that result from the preparation of a series of calibration standards, and the subsequent estimation of the uncertainty associated with the determination of an unknown concentration based on those calibration data. You and a partner will share the same unknown sample, but each of you will prepare your own set of external standards. At the end you will compare the two datasets and discuss the advantages and disadvantages of each.

You are asked to work toward the three goals of the experiment:
1. Prepare a series of six calibration standards using either a volumetric or gravimetric approach (see below for guidelines).
2. Measure the absorbances of the samples and your unknown solution using a Spec-20 UV/Visible spectrophotometer set at 575 nm.
3. Estimate the concentration of the unknown dye solution to three significant figures, and the uncertainty associated with this estimate.

Materials and Guidance

Methylene blue dye is available to you as a dry solid. All solutions can be made up using DI water.

You should constrain your use of volumetric glassware to 5, 10, 15, 20, and 25 mL volumetric pipettes, 25, 50, 100, and 250 mL, and 1 L volumetric flasks.

Do not weigh out more than 300 mg of methylene blue for the experiment. The lower end of the mass you can reliably work with should be driven by the number of significant figures you would like to have in your final determination of the unknown.

Methylene blue dye is REALLY, REALLY blue. Be careful not to spill it on yourself or your clothing, otherwise your clothing will be stained irreversibly.

The molar extinction coefficient of methylene blue at 575 nm is roughly $2 \times 10^4$ L/cm$^1$*mol$^1$. Before preparing your standards you should obtain a rough estimate of the unknown concentration; this will inform your choice of external standard concentrations and standard addition levels.

You are encouraged to consider both volumetric and gravimetric approaches to the preparation of the
calibration standards. If you choose to use the gravimetric approach, you MUST report the density of the
most concentrated of your methylene blue solutions. Please do not hesitate to ask questions about these
approaches. In either case you are advised to think carefully about the precision and accuracy of the
devices involved to ensure you can ultimately legitimately report your estimate of the concentration of the
unknown to three significant figures.

Finally, you are encouraged to consult with your instructor to check to be sure that your scheme for
preparing your calibration solutions is reasonable before you actually start preparing any solutions.

**Making the Measurements**

Instructions for operating the Spec-20 can be found in the laboratory. Do not hesitate to ask your
instructor if you have questions about calibrating the instrument. Be sure to let the instrument warm up
for at least 15 minutes before making any measurements.

You are asked to make two replicate measurements of each calibration solution, and three replicates of at
least one of the calibration standards and the unknown. The following general scheme is prescribed to help
you catch problems with sample carryover and drift in the instrument over the course of an experiment;
you are encouraged to modify this scheme to fit your specific needs. You should rinse the cuvette a few
times with each new sample to prevent dilution or carryover.

```
Blank with water
Calibrant 1
Calibrant 2
Calibrant 3
Water (should produce zero absorbance again)
Calibrant 4
Calibrant 5
Calibrant 6
Water
Unknown
Calibrant 6
Calibrant 5
Calibrant 4
Water
Calibrant 3
Calibrant 2
Unknown
Calibrant 1
Water
```
Analyzing the Data

You are asked to use the Regression tool in the Data Analysis package in Microsoft Excel to analyze your calibration data, and ultimately estimate the concentration of the unknown and the uncertainty associated with this result. Be sure to calculate the residuals for your data as a means of evaluating the linearity of the data, and possibly support the exclusion of some data points from your analysis.

Reporting the Results

Prepare a report (less than ten pages of text, one report per team) summarizing your results; a plot of your calibration data, and a residuals plot should accompany the text. The plot of the calibration data should contain symbols ONLY for the raw data, and a line ONLY for the calibration line; please ask questions if you are unsure about how to do this. Send a spreadsheet containing your data and regression analysis results, and an electronic copy of your report to dstoll@gustavus.edu. At a minimum, include the following information in your summary:

1. Explanations of your approach to the preparation of calibration and standard addition solutions.
2. A table summarizing your raw data.
3. Estimates of the slope and intercept of your calibration line, and the standard deviations associated with these estimates; use appropriate numbers of significant figures in reporting these values.
4. An estimate of the concentration of the unknown and an estimate of the uncertainty associated with this estimate. Use the number of significant figures warranted by the quality of your data.

Grading

As usual, the point total for the experiment is 100 points, and 10% of this total is dedicated to the evaluation of your laboratory notebook. In this case 60 points will be awarded individually based on the accuracy and precision of your results. The remaining 30 points will be based on the quality of your report, and each member of the team will share the same score on the report. You will be allowed to revise the report once after the initial grade is assigned.
Experiment 9: Determination of an Active Ingredient in Cough Syrup by HPLC
(Developed by DRS, 2009)

Objective
The primary objective of your work in this experiment is to determine whether or not the concentration of the antihistamine doxylamine in an over-the-counter cough syrup is consistent with the value reported by the manufacturer on the label.

Materials and Guidance
You will be provided with (nominally) pure doxylamine (Sigma Aldrich), HPLC grade methanol, HPLC grade water, and a sample of over-the-counter cough syrup.

To quantitate the doxylamine in the cough syrup you ultimately will need to construct a calibration curve. You can use the dosage information on the cough syrup bottle to help make initial estimates for the range of your calibration standards. The number of standards you choose to use is up to you, but keep in mind that this is one factor that affects the precision of your estimate of the doxylamine concentration in the cough syrup.

It is recommended that you first prepare a stock solution of doxylamine in 10/90 (v/v) methanol/water at a concentration of about 1 mg/mL, and then prepare your calibration solutions by diluting this stock solution with water. **Doxylamine is expensive; please do not use more than 10 mg for your entire experiment.** The final calibration solutions should not contain more than 5% methanol (v/v). To ensure that the doxylamine fully dissolves, first weigh out the doxylamine solid, then add the volume of methanol needed, and then add the amount of water needed. Make sure the solid is fully dissolved before moving ahead.

The cough syrup sample must be treated before injecting it into the HPLC for analysis:
1. Filter roughly 1 mL of cough syrup through a 0.2 µm syringe filter
2. Dilute the filtered syrup 1:5 with water (4 parts water, 1 part syrup, by volume)

Finally, the dosage of doxylamine in the cough syrup is specified to two significant figures, so your results ultimately need to be precise to at least two significant figures as well.

Keep in mind that each HPLC analysis will take about 3 minutes to run.

Making the Measurements
1. Work with your instructor to set up the HPLC instrument using the operating parameters listed below. The HPLC column we will use for this work is an Restek DB C8, with dimensions of 50 mm x 3.0 mm i.d.
   - Stop time – 2.5 minutes
- Flow rate – 2.5 mL/min.
- Temperature – 60 °C
- Injection volume – 10 µL
- Detection by UV absorbance at 220 nm (make sure the detector is set to collect ‘All’ spectra)
- Solvent A – 0.1% (w/w) trifluoroacetic acid in water
- Solvent B – acetonitrile

Set up the following program to control the pump; the initial solvent should be set at 2.0% B.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>2.0</td>
</tr>
<tr>
<td>1.00</td>
<td>2.0</td>
</tr>
<tr>
<td>2.00</td>
<td>100</td>
</tr>
<tr>
<td>2.01</td>
<td>2.0</td>
</tr>
</tbody>
</table>

2. Setup a sequence in the instrument software to perform two ‘dummy’ injections (no sample injected, but the instrument still executes the method), followed by two injections of your most concentrated calibration standard, followed by two injections of the cough syrup sample. Use the retention time of the doxylamine peak in the chromatogram for the standard to identify which peak in the cough syrup chromatogram is doxylamine (consult with your instructor at this point if needed).

3. Work with your instructor to choose a wavelength for quantitation of doxylamine based upon the UV spectrum obtained in your initial runs. Before running the rest of your samples, be sure to change the wavelength in your analysis method.

4. If it looks like the doxylamine peak in the cough syrup will be within your calibration range, then set up a sequence to analyze the calibration samples and the cough syrup sample in duplicate (with two dummy runs at the beginning).

**Analyzing the Data**

Work with your instructor to integrate the doxylamine peak in each chromatogram and collect the peak areas for all of the samples in an Excel spreadsheet. Use your knowledge about calibration curves and regression to estimate the concentration of doxylamine in the cough syrup, along with an estimate of the uncertainty associated with this number. Finally, use a statistical test to judge whether or not the actual doxylamine concentration is as specified on the label.

**Reporting the Results**

Prepare a short (less than ten pages of text) report (one report per team) summarizing results; a plot of your calibration data, a residuals plot, and a representative chromatogram for the cough syrup sample with the doxylamine peak clearly indicated should accompany the text on separate pages. The plot of the calibration data should contain symbols ONLY for the raw data, and a line ONLY for the calibration line.
Send a copy of the report and the spreadsheet containing your data and regression analysis results to dstoll@gustavus.edu. The report should be detailed enough that a person that has completed the Quantitative Analysis course, but not necessarily this experiment, could complete the experiment by following the description of your approach outlined in your report. At a minimum, include the following information in your summary:

1. An explanation of your approach to the preparation of calibration solutions.
2. A table summarizing your raw data.
3. Estimates of the slope and intercept of your calibration line, and the standard deviations associated with these estimates; use appropriate numbers of significant figures in reporting these values.
4. An estimate of the concentration of the unknown and an estimate of the uncertainty associated with this estimate. Use the number of significant figures warranted by the quality of your data.
5. Using an appropriate statistical test, make a judgement about whether or not the dosage of doxylamine specified on the label accurately reflects what is in the bottle. If you find that the label is not accurate, you should suggest possible problems with your experiment that may have resulted in inaccurate results.