Laboratory Manual
Chem-221
Analytical Chemical Methods

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INTRODUCTION

What is This Course All About?

The objective of this course is to teach the students the science of chemical measurements. Often, scientists and engineers are faced with the question, what is in a given sample, and how much? Chemical measurement or analytical chemistry is the systematic study of methods which can be used to answer these questions.

This course has been designed for students taking their first laboratory course in quantitative methods of chemical analysis. The topics covered fall under the general category of Analytical Chemistry. By the end of the semester, the students are expected to have acquired fundamental knowledge to prepare them to embark upon a rational approach to qualitative and quantitative analysis. After reviewing the large number of topics and experiments that could have been included here, we have selected a small set of diverse experiments that we believe will provide fundamental knowledge of various popular and powerful analytical techniques. These experiments include data evaluation, gravimetric, complexometric/volumetric/potentiometric titration, gas chromatography, UV-Vis molecular spectrophotometry and atomic absorption spectrometry.

It will be to the student's advantage to continue to read and reread the chapters in their textbooks on laboratory techniques throughout the semester. There are also some practical aspects of chemical analysis that are best learnt during the experiments. For example, you will learn that one should not use a graduated cylinder for accurate volumetric measurements, and that solids are best dissolved in a beaker covered with a watch glass. The laboratory professor or teaching assistant (TA) will usually explain and/or demonstrate these and many more techniques which have been used successfully over the years.

Students are encouraged to ask questions before it is too late and the **mistakes have already been committed.**

"Every scientific development has been preceded by a development in analytical methodology"

1. ***Dr. Milos Novotny, Pittsburgh Conference, 1990.***

COURSE OUTLINE

(Read the relevant chapters and sections in your textbook in order to become familiar with the analytical principles of all the experiments)

1. **INTRODUCTION TO ANALYTICAL CHEMISTRY –** Fundamentals of analytical chemistry, classical and instrumental methods, units of concentration,

2. **STATISTICAL HANDLING OF DATA** – Errors in chemical analysis, concepts of accuracy and precision, normal distribution, statistical tests for comparison of mean, rejection of outliers.

3. **GRAVIMETRIC METHODS OF ANALYSIS** – Properties of precipitates and precipitating agents, drying and ignition of precipitates, gravimetric calculations.

4. **TITRIMETRIC METHODS OF ANALYSIS –** Volumetric calculations, buffer solutions, titration curves for simple and complex acid base systems, Concept of pKa, potentiometric titration, complexometric titration.

5. **ELECTROCHEMICAL METHODS, PH METER** – Introduction to electrochemistry and electrochemical methods, Ph meter and its application to potentiometric titration.

6. **SPECTROSCOPY** – Introduction to spectrochemical methods, absorption and emission spectroscopy, instrumentation for optical spectroscopy, details of UV-Visible spectrophotometry, Beer's law calculations.

7. **ATOMIC SPECTROSCOPY FOR METALS** – Detemination of metals by atomic absorption, brief description of instrumentation, sample preparation for analysis of metals.

8. **CHROMATOGRAPHY** – Introduction to chromatographic separation, gas liquid chromatography, HPLC, brief description of instrumentation.

Listing of Experiments Based On Analytical Technique

Gravimetry

- Determination of sulfate as barium sulfate (2 weeks)

Titrimetry

- Determination of percentage of Na_2CO_3 in a sample (2 weeks)
- Determination of hardness of water. (1 week)
- Potentiometric titration (2 weeks)

Spectroscopic Determination Using a UV-Visible Spectrophotometer

- Quantitative determination of iron in a sample (1week)
- Simultaneous determination of Co and Ni (2 weeks)

Atomic Spectroscopy

- Determination of Na in potato chips (1 week)
- Determination of Pb in soil. (1 week)

Gas Chromatography and HPLC

- Separation and analysis of a mixture of unknown volatile liquids using a gas chromatograph and HPLC (1week)

SAFETY IN THE LABORATORY

SAFETY GLASSES MUST BE WORN AT ALL TIMES

1. If the fire alarm sounds, leave the building **immediately.**

2. Always conduct yourself in a professional manner. Have fun while working in the laboratory, but refrain from activities that might be dangerous to you or your neighbor.

3. You must learn where the safety equipment is and how to use each item during the first day in class. In the event of an emergency, you should use whatever you need to address the emergency. Again, you do not need to ask for permission to respond to an emergency. Usually, your response will be to advise your TA and instructor and then follow his/her instructions. As a general rule, and if time permits, students should not attempt to provide first aid but should concentrate on contacting a professional (x3111 for emergency) in that area.

4. No consumption of food (including gum) or beverages will be allowed.

5. You are not to perform any unassigned experiments.

6. Do not use your mouth to fill pipettes.

7. If something is spilled on you, wash it off immediately with lots and lots and lots of water, and then report to the TA. Clean up the spill later according to instructions from the TA.

8. Uncontrolled long hair or clothing (loose sleeves, ties, jewelry) that might come in contact with a flame or become entangled in mechanical equipment will not be permitted. You will not be permitted to work in the lab without protection for your feet (no sandals, for example).

9. Never heat a closed system. It may result in an explosion.

10. Never heat flammable materials with an open flame or near an ignition source.

11. Do not heat or mix any thing near your face (or anyone's face).

12. Review the hazards of all reagents for an experiment before you start, so you know how to respond to an emergency. The MATERIAL SAFETY DATA SHEETS (MSDS) for each reagent we use are available on the Internet (Consult Fischer Scientific Website www.fischersci.com). You are encouraged to review any MSDS any time you have a question. You should also note that a considerable amount of safety information is on the reagent labels. Read them before you use the reagent.

13. Do not rub your eyes with your hands. Your hands are frequently contaminated.

14. Protective clothing (lab coat) is not required, but highly recommended. You will not be permitted to work in the lab in shorts and without protection for your legs and feet.

15. You cannot tell when glass and other objects are hot by looking at them. Be careful and don't get burned by trying to pick up something that is hot.

16. Do not store reagents near a sink or leave them near the balance where they will be in the way and get knocked over. Return all reagents to their proper location as soon as possible after you have finished with them. Be sure everything is returned to its original location before you leave and that you have left nothing in the balance room, in a fume hood or at some other location.

17. Be sure you know where the safety equipment is located so you can find and use each item in an emergency (if the power fails, and the lab is dark, for example).

18. Be sure that, in an emergency, you know how to turn off all of the utilities (gas, water, electricity) you have been using.

19. Never attempt to identify an unknown by smelling or tasting it as recommended in some (especially old) textbooks.

20. Use the appropriate safety equipment (safety shield, gloves, fume hood, shower, eye wash, etc.) and supplies as needed. Be sure any supplies you use are promptly replaced so they are available for the next emergency. It may be you again.

21. Read all chemical labels prior to use. Be sure you know what you are using.

22. Do not store chemicals near non-compatible chemicals (acids with bases or oxidizers with f fuels, for example) even for short periods of time.

23. Transport and dispose of all chemicals properly. If you are not sure how to do so, ask your TA.

24. Do not use chipped or broken glassware. Broken glassware will not be accepted at the end of the course and should be replaced during check-in or as soon as it is broken.

25. Do not operate electrical equipment with wet hands.

26. If at all possible, do not wear contact lens to the laboratory. If you must, be sure the TA is informed so special precautions are used.

27. The EMERGENCY telephone number is x3111 for university security/safety, x3568 for the department office.

GENERAL LABORATORY RULES

1. Lab sections will meet only at the scheduled times. There will be no makeup sessions, so plan to use all of your available time effectively. If you do, you will have plenty of time to finish all of the required experiments.

2. Most experiments should be done in triplicate. You must record all data in your lab notebook using the correct number of significant figures. You may discard any of your values when you report your results. You must use the same balance for all weighing. (Balances will be assigned to you)

3. We will grade the results you turn in "as received" and will not permit you to make changes after the grade has been given. Therefore, be sure your report does not contain mistakes. **A mistake on the unknown number, for example, will probably result in a failing grade even though the analysis was done properly.**

4. Use distilled water from a wash bottle for the final rinse of glassware that has previously been washed or rinsed with tap water. Do not rinse glassware at the distilled water tap. Use distilled water to prepare all solutions requiring water even when the directions only specify water.

5. Never assume glassware or other materials are clean unless they are so marked.

6. Label all of your solutions and reagents to avoid mix up. The label should have the name (not the formula) of the material in the container, the date and your initials as a minimum.

7. Heat concentrated acids and bases in fume hoods. Dilute concentrated acids before disposal in the sink.

8. The equipment, glassware and supplies you will be using in these courses are expensive. Take care of them. You are responsible for all breakage of glassware and for any other damages above the normal wear and tear.

9. It is essential that you keep all common work areas (such as fume hoods, balance areas, etc.) and your work area clean. All spills should be cleaned up and reported to your TA/Instructor IMMEDIATELY

10. You should be THOROUGHLY FAMILIAR WITH EACH EXPERIMENT BEFORE YOU START IT. You should know all of the chemical reactions and why each reagent is being used. You should continuously observe each experiment for reasonable properties and consult your TA/Instructor if anything looks strange or is unexpected.

11. Grading – Your final grade will be composed of the laboratory report grades and one mid-term exam, as announced by your instructor. Weekly assignments will also be graded.

12. When you report on the analysis of an unknown, you must provide the following: (1) your name, (2) the date, (3) the experiment name, (4) all the analytical results and (5) the final value (average and relative standard deviation) (6**) your unknown number**. See sample cover sheet.

13. **This is a Lab Manual. In addition to this manual, you will need a Lab Notebook. Record all of your original data in ink directly into your Lab Notebook**. If you make an error, draw one line through the incorrect data, date and initial the line and record the correct data. Be sure someone else can still read the incorrect data. Record enough information so a trained chemist could reproduce your work and calculations, in your absence, using the format outlined in the section entitled Laboratory Reports.

COMMON ERRORS TO AVOID

a) Incorrect use of the balance

b) Inadequate mixing of solutions or samples

c) Reading and/or recording data incorrectly

d) Using contaminated glassware

e) Incorrect use of pipets, burets and volumetric flasks.

f) Failure to understand what an instrument is doing.

g) Failure to study and understand each experiment and to recognize significant information while doing the experiment.

h) Some modern balances have a “tare” option. If you elect to use the tare option, rather than the recommended weighing by difference option, be sure someone else doesn’t change the tare between your weighings. It is a good idea to use the same balance for all of your weighing on a given day.

LABORATORY REPORTS

A written report should be submitted on 8-1/2 x 11 paper and must be typed. Learn how to type sub and superscripts, equations, mathematical expressions and neat tables. Keep descriptions in the past tense and passive voice: (These samples were titrated with…. The objective of this experiment was to determine the concentration of….etc.) Avoid use of the personal pronoun, particularly in the Procedure Section. (I filled my buret with acid…. Should not be used). The report must be submitted to your instructor the week after you complete the experiment. Points will be deducted for late submission. The report must contain the following.

Cover Page- See the format on p.14

Title- Use the title in this manual. Include your name and the date that the experiment was performed.

Objective- Carefully study and record what you are trying to do. One or two sentences are usually sufficient to indicate the goals of the experiment. Refer to the manual.

Theory- This section should provide the chemical basis of the experiment with balanced equations and any mathematical equations needed to do the calculations. All terms used in equations should be defined.

Procedures – Do not make a list of steps. In paragraph form, give a brief synopsis of the process, and detail any deviations from the lab book directions.

Data – Use tables and graphs. Label them carefully and clearly, making sure that the units are included. Enough data should be given to be able to calculate answers, but not all raw data are necessary.

Calculations– You must take a set of your data and show a sample calculation in detail. As you do the calculations, be sure to carry all units through to be sure the results have the correct units. This also allows the instructor to identify any errors you have made.

Results with Statistical Analysis- Report the results along with your unknown number in bold or highlighted in some way. Results have to be in the recommended units (concentration, wt%, mg etc.). Statistical analysis of data in terms of mean, relative standard deviation etc. must be presented clearly. Results of Q-test for rejection of outliers also need to be included.

Conclusions- This section should summarize your main results and include some comments on possible sources of error.

Remember- ***Two thirds of your grade depends on the accuracy of your results. Precise and accurate work is the major requirement of this course.***

Useful References:

a) D. A. Skoog, D. M. West, F. J. Holler and S.R. Crouch, "Fundamentals of Analytical Chemistry", 7th edition, Saunders College Publishing Co., 1999.

b) D. C Harris, "Quantitative Chemical Analysis", 4th edition, W. H. Freeman and Company, New York, 1995, ISBN 0-7167-2508-8

c) J.F. Rubinson and K.A. Rubinson, "Contemporary Chemical Analysis" Prentice Hall, NJ, (1998)

COVER PAGE

COURSE – CHEM 221

NAME

EXPERIMENT NO. & TITLE

UNKNOWN NUMBER ______________________

DATE REPORT DUE______________________________

DATE REPORT SUBMITTED_______________________

GRADE ACCURACY_______________________

PRESENTATION____________________

TOTAL____________________________

WEIGHING TECHNIQUES

For accurate weighings an analytical balance will be used. This balance weighs to 4 decimal places. In order to obtain an approximate weight a Digital Top Loading balance (measuring to 2 decimal places) will be used. Solids and liquids should be weighed in weighing bottles. Do not use weighing paper. Never use your fingers to handle the object being weighed. Use tongs or a paper strip.

All weighings should be by difference. The analytical balance is accurate to ± 0.000 1g.

Example

Initial weight of bottle + sample = 24.5678g
Final weight of bottle minus sample = 24.1357g
Weight of sample = 0.4321g

VOLUMETRIC GLASSWARE

Beakers and flasks

Beakers and flasks are marked to indicate approximate measure only. They should never be used to measure a liquid accurately. Look at the beakers and flasks in your cabinet; most have 5% stamped near the top. This means each measure indicated on the beaker is 5% more or less than the actual volume. For example, if the top measure is 400 ml, the beaker, when filled with liquid to the 400 ml mark, may actually contain anywhere from 380 to 420 ml. This is not accurate enough for most experimental work.

GRADUATES (GRADUATED CYLINDERS)

The graduate is generally used for a close, approximate measure of volume. The volume needed dictates the size of the graduate to use. As a general rule, the graduate chosen should not have a total volume more than 10 times the volume to be measured. Look at your graduated cylinders. Most graduates have TC 20° stamped near the top. This means that each line on the scale was calibrated to **contain** that amount of liquid at 20°C (normal room temperature).

Liquids in glass cylinders have a curved surface due to adhesion of the liquid to the walls of the cylinder. This curved surface is called the meniscus. Fill one of your graduates about half full of water and note the meniscus.

If the liquid is clear (transparent) and has a concave meniscus the volume is read by placing your eyes level with the bottom of the meniscus and reading the volume with respect to the graduated scale on the side of the cylinder. When a liquid is cloudy (opaque) or has a convex meniscus the same procedure is used except that the top of the meniscus is read.

Pipets

Two types of pipets are commonly used. The Mohr pipet is marked with graduations to enable the user **to deliver** a definite volume of liquid. This type of pipet is normally stamped with a TD 20ºC on it. The transfer pipet delivers a fixed volume. Solutions are drawn up into both pipets in the same manner. A special pipet pump should be used. First of all insert the pipet into the collar of the pipet pump. Push gently past the stabilizing fingers at the end of the pump's collar. The stabilizing fingers will prevent the pipet from wobbling while in use. A gentle twisting motion will ease insertion of the pipet. Push the pipet in until it is well seated in the soft chuck.

To Pipet –

1. Depress the plunger
2. Insert the proper pipet
3. Rotate the wheel counter/clockwise and the liquid will rise in the pipet.
4. Stop when the required amount is reached.
5. To release the liquid drop by drop, turn the knurled wheel slowly in the opposite direction.
6. For fast release of the liquid, press the side lever until the pipet is empty.

Both types of pipets are calibrated to deliver, TD, at 20°C. Look at some pipets. You will see the TD 20° mark stamped near the wide, open end. Many Mohr pipets are also stamped with their standard deviation (± 0.05 ml). This is the same idea as the deviation marked on beakers, except that the pipet has a lesser deviation.

A Mohr pipet is chosen over a transfer pipet generally only when the volume required is an amount in which a transfer pipet is not made.

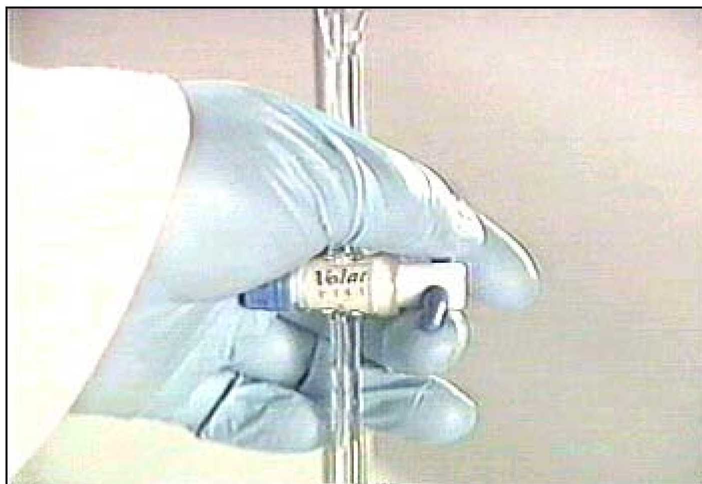
Burets

Burets deliver variable volumes of liquids. Burets consist of a small diameter graduated column, a capillary tip and a valve called a stopcock which controls the flow. The buret, like the pipets, is calibrated to deliver (TD) at 20°C. The most commonly used buret has total capacity of 50 ml and is calibrated in 0.1 ml intervals. The buret is supported by a clamp to a support stand.

A buret must be "Lined" before being used. Make sure that the stopcock is closed, then carefully pour some of the solution to be used in the experiment into the top of the buret, with the aid of a small funnel. Fill the buret only about a quarter of the total volume. The funnel is removed and the buret taken from its support and rotated along its length. The solution is rolled around, wetting the inside walls of the buret. Pour the solution out through the top. Repeat a second time, this time running the solution out through the capillary tip.

The buret, after being lined, is filled to over the zero mark. To flush air from the capillary, the stopcock is opened fully for an instant, then closed. If any air bubbles remain the flushing must be repeated. After removal of all air bubbles, the liquid level is adjusted to some mark (it should not be zero) and recorded. When the amount of liquid needed has been released from the buret, the stopcock is closed. Count to 15 to allow drainage along the sides, then touch off any drop which might be hanging from the tip.

The operation of the stopcock is illustrated below. With practice, the stopcock may be manipulated to allow fractions of a drop to form at the tip and be touched off into the receiving vessel.



The Buret and Volume Delivering

Volumetric methods of analysis depend on the volume of a standard solution. Usually, this solution is delivered from a buret. When writing volume data obtained from using a buret, it should be done in the following manner:

Titration of 24.95 Ml of HC1 0.09678 M vs. NaOH:

	#1	#2	#3
final buret reading (Ml)	35.87	35.45	35.90
initial buret reading (Ml)	0.74	0.34	0.78
volume delivered (Ml)	35.13	35.11	35.12

The initial buret reading should not be 0.00, it should be anywhere between 0.00 and 1.00. The last digit of the number, if you are reading the buret correctly, almost always is going to be different as in the example above.

All glassware such as burets, pipets, volumetric flasks have a certain tolerance as supplied by the manufacturers. The table below shows such tolerances for grade A glassware. If grade B glassware is used, then it is appropriate to multiply the tolerances by a factor of 2.

Sources of Error

The sources of error that lead to inaccuracy in a given measurement are categorized as either **statistical** or **systematic** errors. Statistical errors are also called **random errors** and they have an equal probability of making the reported measurement too high or too low. Statistical errors may be minimized by repeating a measurement several times.

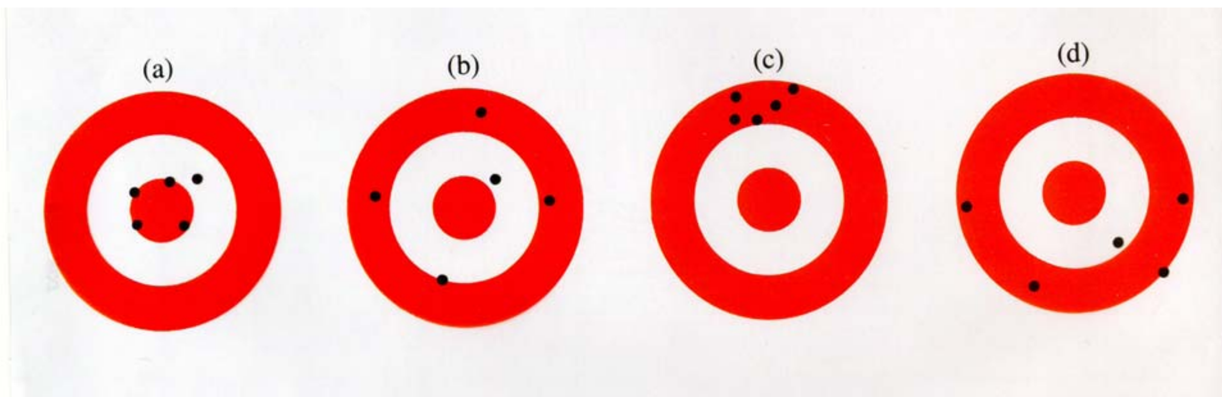
Systematic errors occur in the same direction each time. For example, if one weighs a series of samples and forgets to zero the balance at the start of the measurements, then a systematic error has been introduced. Impurities can lead to systematic errors.

Tolerances for Volumetric Glassware, Ml

CAPACITY, LESS THAN AND INCLUDING	VOLUMETRIC FLASKS	TRANSFER PIPETS	BURETS
2		0.006	
5		0.01	0.01
10		0.02	0.02
25	0.03	0.03	0.03
50	0.05	0.05	0.05
100	0.08	0.08	0.10
200	0.10	0.10	
500	0.15		
1000	0.30		

PRECISION AND ACCURACY

There are two types of uncertainty that must be considered, precision and accuracy. **Precision** reflects the reproducibility of measurements made by identical methods. **Accuracy** is a measure of how close a measurement is to the "actual" value.



The diagram above gives examples of precision and accuracy. (a) – high precision and accuracy; (b) low precision and high accuracy (using average); (c) high precision and low accuracy; (d) low precision and low accuracy

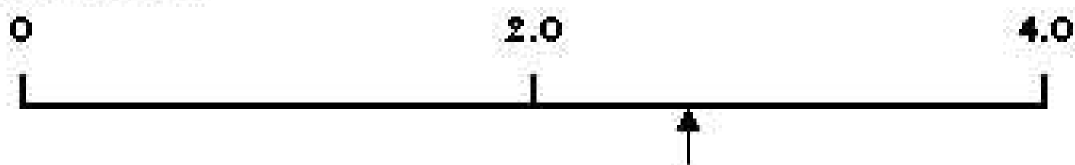
The accuracy of a measurement describes the difference between an experimental value and the accepted, correct value. The measurement has low or high accuracy depending on whether the experimental value is close to or away from the accepted value. Precision, however, describes the difference among the individual measurements themselves; if they are close together, precision is high; if they are scattered, the precision is low.

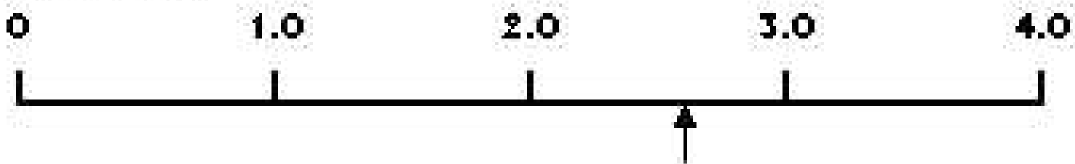
SIGNIFICANT FIGURES

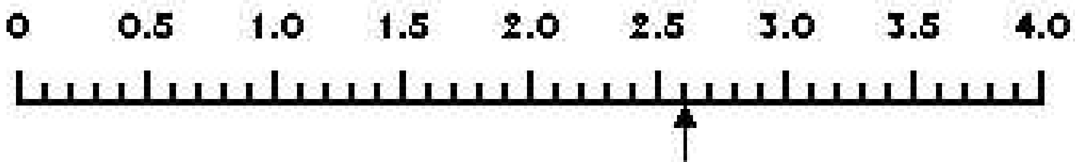
The significance of digits is a matter of accuracy (and therefore precision). Do not be led into believing that just because a number has eight decimal places that it is highly accurate. The accuracy of an answer is limited by the least accurate fact obtained.

SIGNIFICANT FIGURES AND MEASUREMENTS

When looking at significant figures the numbers can be divided into two general groups: exact numbers and inexact numbers. Exact numbers are obtained from counting (counting the number of people in the room) or from definitions (1.0 liters = 1,000 mls). The second group, inexact numbers, are obtained from measurement. The accuracy for measurement depends upon the device used.







In example a, the arrow is between 2 and 4 and can be estimated to be 3. A definite answer cannot be given. In example b, the scale is slightly more accurate and the arrow can be estimated to be between 2.5 and 3. Still no definite answer can be given.

Example c is highly accurate where the value for the arrow is definitely 2.6. On this scale a reasonable estimate of the second digit can be made.

The number of significant figures in exact numbers is infinite. Inexact numbers contain a limited number of digits which are determined by counting the number of reasonable digits. Reasonable digits are only those that are known accurately plus one, and only one, digit that is in doubt.

SIGNIFICANT FIGURES AND ZERO

When the measurement contains the digit zero some rules must be applied:

a.) zeros in between two significant figures are significant.

b.) zeros before a number are not significant and serve only to locate the decimal point.

c.) final zeros to the right of the decimal point are significant. If it was not a reasonable digit it should not have been written.

d.) final zeros to the left of the decimal point may or may not be significant. The zero may be used to locate the decimal point or may be a reasonable digit. The data from which the number was obtained must be looked at.

Examples:

0.00045	……….two	significant figures
0.0608	……….three	" "
47.90	……….four	" "
11.0000	……….six	" "
2.037	……….four	" "
4.1055	……….five	" "

SIGNIFICANT FIGURES AND CALCULATIONS

In addition and subtraction the result should have only as many decimal places as there are in the number in the calculation with the least number of decimal places.

Examples:

28.5	158.906
14.26	–145.26
9.378	13.646 = 13.65 (rounded off)
52.138 = 52.1	

In multiplication and division the answer should contain only as many significant figures as are contained in the number in the calculation with the least number of significant figures.

Examples:

	1.008	(4 significant figures)	458.1952	(7 significant figures)
x	5.67	(3 significant figures)	7.260	(4 significant figures)
5.71536 = 5.71			63.11228 = 63.11	

The Q Test

When there are only a few data points available (3 or 4), the best test for determining whether a data point should be rejected is the Q test:

$$Q_{exp} = \frac{[\text{suspect value - nearest value}]}{[\text{largest value - smallest value}]}$$

Where nearest value refers to the value numerically closest to the suspect value. The suspect number should be rejected if Q_{exp} exceeds the tabulated value Q_{tab} ($Q_{90\%}$)

N	3	4	5	6	7	8	9	10
$Q_{90\%}$	0.941	0.765	0.642	0.560	0.507	0.468	0.437	0.412

Where $Q_{90\%}$ refers to measurements at the 90% confidence level and N refers to the number of observations.

KNOWLEDGE AND ABILITY EXPECTATIONS

We expect you to have a certain level of understanding of basic scientific principles and chemistry. The following questions are to ensure that you are at this level. If you do not already know the following definitions and conversions, learn them now. You should learn them well enough that you are able to easily answer related questions. You should also be able to answer the chemistry questions. If you aren't comfortable with any of this material, we suggest that you put in some extra effort in order to catch up. Please feel free to discuss your concerns with your instructor.

Definitions and Conversion Factors:

1. **gram (g) –** there are 453.6 grams in a **pound** and 1000 g in a **kilogram**. It is a unit of mass.
2. **centimeter** (cm) – there are 2.54 cm in an inch and 100 cm in a **meter**. It is a unit of length.
3. **second (sec**) – there are 60 seconds in a minute. It is a unit of time.
4. **volume** – length cubed is volume. Common units are cm^3 which is a ml (There are 1000 milliliters in a liter) and gallons (there are 231 $inch^3$ in a gallon.). Therefore, there are $(2.54 \text{ cm/inch})^3 \times 231 \text{ in}^3\text{/gal} = 3784 \text{ cm}^3\text{/gal}$ or about 3.8 liters per gallon. In Europe, a liter of gasoline costs about the same as a gallon costs in the U.S.
5. **energy** – Work and heat are forms of energy. Energy is a force acting through a distance or force x length. The units of measure are the **erg** (cm x dyne or a cm times $\text{g cm/sec}^2 = \text{g cm}^2\text{/sec}^2$), the **joule** ($\text{kg m}^2\text{/sec}^2$), the **calorie** (there are 4.184 joules in a calorie) and the **British Thermal Unit or BTU** (one BTU will increase the temperature of one pound of water one Fahrenheit degree.). The student should also recall that energy may be related to the frequency of electromagnetic radiation via Planck's constant or **E = h** (ergs = 6.62×10^{-27}) υ where υ is the frequency in cycles per second.
6. **power** – power is the rate of doing work. Therefore, the units are work per unit time such as a joule/sec (which is one **watt)** and which is also equal to one **volt** times one **amp**.
7. **coulomb** – A coulomb is a unit of electrical charge. An **amp** is a unit of electrical current and is a flow of one coulomb per second. Since the charge on an electron is 1.6×10^{-19} coulombs then Avogadro's number of electrons will have a charge of (1.6×10^{-19} coulombs/electron x 6.023×10^{23} electrons/mole) = about **96485 coulombs/mole**. This number is one **Faraday**. It may be used as a conversion factor between chemical mass units and electrical units of measure.

In summary, using the definitions and the conversion factors given above, you can relate mass, length, and time to area, volume, velocity, energy and power used in electrical, mechanical and chemical systems.

General questions that you should be able to answer from memory:

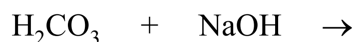
Define the following
gram
meter
square inch
joule
att
volt
frequency of electromagnetic radiation
mole
speed of light
Planck's constant

Name the following
HCl
NaBr
HNO_2
HNO_3
H_2SO_4
C_4H_{10}
H_2CO

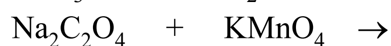
Give chemical formulae for the following
phosphoric acid
sodium bicarbonate
potassium hydroxide
potassium chromate
potassium dichromate
acetic acid
iron III chloride
manganese dioxide

Complete and balance the following equations

$HCl + NaOH \rightarrow$


$H_2CO_3 + NaOH \rightarrow$

$FeCl_3 + SnCl_2 \rightarrow$


$Na_2C_2O_4 + KMnO_4 \rightarrow$

SCHEDULE

Week 1

Check in
Lecture: Introduction to analytical chemistry, how to use the balance, statistical methods.
Experiment: Techniques in Preparing Solutions.

Weeks 2 and 3

Lecture: gravimetry.
Experiment: Determination of sulfate as barium sulfate (2 weeks)

Weeks 4 and 5

Lecture: Titrimetry, titration curves, and potentiometric titrations.
Experiment: Determination of percentage of Na_2CO_3 in a sample (2 weeks)

Week 6

Lecture: Titrimetry, theory of complex formation, complexometric titrations.
Experiment: Determination of hardness of water (1 week).

Weeks 7 and 8

Lecture: Analysis of acid mixture by titration; pK_a
Experiment: Potentiometric determination of an acid mixture (2 weeks)

Week 9

Lecture: Introduction to spectroscopy, UV-visible spectrophotometry.
Experiment: Quantitative determination of iron in a sample. (1 week)

Weeks 10 and 11

Lecture: Continuation of spectroscopy
Experiment: Simultaneous determination of Co and Ni using spectrophotometry (2 weeks)

Week 12

Lecture: Atomic spectroscopy.
Experiment: Determination of Na in potato chips or Pb in soil (1 week).

Week 13
Lecture: Chromatography
Experiment: Separation and analysis of mixtures using GC and HPLC (1 week)

Week 14

Check out and completion of experiments when needed

EXPERIMENT 1

TECHNIQUES IN PREPARING SOLUTIONS

INFORMATION AND TECHNIQUES

- Concentration Units
- Volumetric Techniques
- Use of Electronic Balance

INTRODUCTION

In this exercise you will become familiar with the following concentration units

MOLARITY	MOLALITY
WEIGHT PERCENT	PPM

You will learn the techniques required in the preparation of standard solutions. These include

1. Drying and storing of chemicals
2. Weighing with analytical balances
3. Use of volumetric flasks and pipets

Your instructor will demonstrate the proper technique to be used in weighing with the analytical balances, and the correct method for using the volumetric equipment.

You are to prepare a $KMn0_4$ solution of a concentration such that accurate preparation will require appropriate dilution with volumetric equipment. For example, suppose that you are to

prepare a solution which contains about (but known exactly) 0.0l0mg $KMn0_4$/ml. This could be done weighing out the following amounts of dry $KMn0_4$, and using the listed volumetric glassware:

1. 0.0005 grams, 50 ml volumetric flask.
2. 0.0010 grams, 100 ml volumetric flask.
3. 0.0500 grams, 50 ml volumetric; 1 ml pipet, 100 ml volumetric.
4. 0.1250 grams, 50 ml volumetric; 1 ml pipet, 250 ml volumetric.

There is obviously a large error in accurately weighing out amounts as small as 0.0005 grams. The weighing error can be reduced by preparing a more concentrated solution (e.g. 0.1250 grams in 50 ml) and carrying out an appropriate dilution (1 ml & 250 ml) to obtain the desired concentration. This method is very accurate assuming that correct volumetric glassware and proper pipeting techniques are used and that all the glassware is clean.

Now, your assignment is to prepare no less than 10 ml of a $KMn0_4$ solution of approximate (but known exactly) concentration _____mg/ml. You have available to you pipets of size 1,2,5,10,25, and 50 ml and volumetrics of size 10,25,50, and 100 ml. Using the appropriate size pipet and volumetric prepare a $KMn0_4$ solution of the concentration assigned to you. Your instructor will check the accuracy of your prepared solution using the Spectronic 20. Good technique is required to obtain the concentration assigned.

Report the assigned concentration of your solution in the following units (show method of calculation):

1. Molarity
2. Molality (assume the solution density is 0.99823 g/ml)
3. Weight percent
4. Parts per million (ppm)

EXPERIMENT 2

DETERMINATION OF SULFATE AS BARIUM SULFATE USING GRAVIMETRY

INFORMATION AND TECHNIQUES:

1. Learn proper use of an analytical balance.
2. Learn how to deal with a precipitate.
3. Quantitative gravimetry.

INTRODUCTION

In this experiment the sulfate in a sample is determined by precipitation as barium sulfate. The sulfate salt is dissolved in a dilute solution of hydrochloric acid and barium chloride is added slowly to it resulting in precipitation of the sulfate salt. The precipitate is digested, filtered, washed free of impurities and dried before being weighed accurately.

To obtain high accuracy, care should be taken that no precipitate (or no more than a tenth of a percent of the total precipitate) is lost. $BaSO_4$ is also susceptible to coprecipitation of foreign ions, so care should be taken to keep the supersaturation ratio as low as possible during precipitation.

There are several important steps and operations involved in a gravimetric analysis.
They can be summarized as follows:

- Preparation of solution
- Precipitation
- Digestion
- Filtration
- Washing
- Drying or igniting
- Weighing

- Calculation

It is also important that the precipitate be sufficiently insoluble that the amount lost to solubility will be negligible and also the precipitation should consist of large crystals so that they can be easily filtered.

PROCEDURE

Preparation of the crucibles: Prepare a set (three) of Gooch crucibles by putting two borosilicate-glass filter pads in each crucible. The pads should not have any holes and this can be examined by holding them in the light. Place the crucible in a suction filtration assembly, and pull a gentle vacuum, using the aspirator. First moisten the pad with a few drops of distilled water and then add a few ml of dilute nitric acid. This removes any soluble material that may have been on the filter pad. Place the crucible on the burner using a second crucible to place the Gooch crucible in and ignite for 10 to 15 min. Use an iron ring and clay triangle for support. The heating should be to the point that the crucible glows faintly red. Heating too intensely may result in melting of the pad. Allow the crucible to cool to room temperature (may take half an hour) in a desiccator and then weigh it. Repeat the HNO_3 rinsing and heating of the crucible till it reaches a constant weight (within 0.2 mg of the previous weight). NOTE: A glass Y-tube will permit you to run two burners off one gas outlet.

Sample preparation and precipitation**:** Weigh accurately about 0.5 g. of the dried sulfate unknown sample. Place it in a clean 400 ml beaker and dissolve in 250 ml of distilled water and 3 ml of 6M HCl. Heat the solution to near boiling. Slowly add an excess (approximately 70 – 80 ml) of 0.05 M $BaCl_2$ solution with efficient stirring. Use a separate stirring rod for each sample. Digest the precipitate for about 30 min near the boiling point, over a low flame. Completeness of the precipitation can be tested by letting the precipitate settle, and adding a few drops $BaCl_2$ to the clear liquid above the precipitate. If cloudiness appears, it means that some sulfate is still in solution. An additional 5-10 ml of $BaCl_2$ needs to be added and the digestion procedure repeated.

Filtration and ignition of the precipitate: Place the crucible on the filtration assembly. Decant the clear supernatant liquid through the filter. Transfer the precipitate completely to the crucible. Use a stream of distilled water from your wash bottle, and a rubber policeman to scrape off any remaining precipitate sticking to the beaker wall or the stirring rod. Wash the precipitate several times with a few ml of water. The filtrate needs to be checked for the presence of soluble salts by checking for chloride. This is done by collecting a 5-10 ml portion in a test tube and then adding 1 ml of 0.1 M HNO_3 and a few drops of $AgNO_3$. If Cl^- is present, solid AgCl will precipitate out indicating that the precipitate in the crucible needs further washing with water. Ignite the crucible so that a constant weight is reached by following the procedure mentioned before.

Report: % SO_3 by weight in your sample.

NOTES

- HCl is added to the sulfate solution to prevent the precipitation of barium salts of weak acids such as CO_3^{2-}, PO_4^{3-} etc., which would volatilize during ignition.
- Presence of excess of $BaCl_2$ reduces the amount of sulfate remaining in the solution by the common ion effect.
- Digestion of precipitate increases the average particle size and reduces co-precipitation.
- Relative supersaturation $= \frac{Q - S}{S}$

Where Q is the concentration of the solute at any instant and is the degree of supersaturation, and S is the solubility of the precipitate at equilibrium. The particle size is related to the relative supersaturation. A high relative supersaturation produces many small crystals, whereas a low relative supersaturation produces fewer, larger crystals. Precipitation reactions are often so slow that even with drop wise addition of a precipitating agent, some supersaturation is likely.

Co- Precipitation Errors

In this determination occlusion of other barium salts can occur. If the occluded contaminant is barium nitrate, a positive error is observed because this compound has a larger molar mass than

the barium sulfate that would have formed had no co-precipitation occurred. If barium chloride is the contaminant, the error is negative because it's molar mass is less than that of the sulfate salt.

The organization of a data table when performing an experiment is very important.
Below a typical data table for this experiment is given. For future experiments you should design your own data table. This also assists you with performing the experiment in the correct sequence.

Typical Data Page

Unknown Number____

Sample 1

Weight of weighing bottle + dried unknown __________________________

Weight of empty weighing bottle___________________________________

Weight of Sample____

Weight of dry Gooch Crucible (1st wgh)________________________________

Weight of Gooch Crucible (after second drying) (2nd wgh)_________________

Weight of Gooch Crucible + $BaSO_4$ (after drying) (1st wgh)_________________

Weight of Gooch Crucible + $BaSO_4$ (after the second drying) (2nd wgh)_________

Weight of $BaSO_4$ ____

% SO_3 ____

Repeat for each sample

	Sample 1	Sample 2	Sample 3	Average	Average Deviation
% SO_3					

CALCULATIONS

$$\% SO_3 = \frac{(\text{wt. } BaSO_4 \text{ precipitate})}{(\text{wt. Unknown sample}} \times \frac{(\text{mol. Wt } SO_3)}{(\text{mol. Wt. } BaSO_4)} \times 100$$

The relative range for the results should not exceed 0.4%.

EXPERIMENT 3

DETERMINATION OF THE PERCENTAGE OF Na_2CO_3 IN A SAMPLE

INFORMATION AND TECHNIQUES

- Titration using an indicator and a pH probe.
- Learn to use a pH probe with direct data acquisition into a spreadsheet.
- Determine the percent of Na_2CO_3 in an unknown sample.
- Develop a titration curve and perform a graphical analysis of a titration curve to determine the equivalence point of a titration.

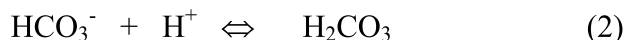
INTRODUCTION

The objective of this experiment is to determine the percentage of Na_2CO_3 in the given unknown sample. The technique used here is titration. First an HCl solution is prepared and standardized against pure Na_2CO_3. Then this solution is titrated against the unknown sample to determine the percentage of CO_3^{2-} in the unknown.

HCl completely dissociates to form H^+ (in reality, H_3O^+) and Cl^-. Carbonate in aqueous solution acts as a base. It accepts a proton to form bicarbonate:

$$CO_3^{2-} + H^+ \Leftrightarrow HCO_3^- \qquad (1)$$

Then the bicarbonate can combine with another H^+ to form H_2CO_3.



$$HCO_3^- + H^+ \Leftrightarrow H_2CO_3 \qquad (2)$$

The equilibrium expressions for the dissociation of bicarbonate and carbonate may be written as:

$$K_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]}$$

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3\ ]}$$

where, K_1 and K_2 are the first and the second dissociation constants for H_2CO_3. Published values of K_1 and K_2 are 3.5×10^{-7} and 5×10^{-11} respectively. The above equations can be used to calculate the pH at different points on the titration curve, a plot of pH versus the amount of titrant added.

The analytical chemistry textbook should be consulted to find out how pH titration curves are calculated.

As one would expect from the above discussion, a titration of CO_3^{2-} with HCl would yield two equivalence points according to equations 1 and 2.(two protons being added to the carbonate ion stepwise) A typical titration curve is shown in Figure 1, where A and B are equivalence points. Either of the equivalence points can be used in the determination of CO_3^{2-}. However, the second end point (B) tends to be a sharper end point than that obtained at the first end point (A). An uncertainty of 0.1 pH at an end point results in approximately 1% error. However, in both cases the pH change is not large in the region of the equivalence point.

Therefore the more accurate method is to carry out the titration at the second end point. At this point H_2CO_3 dissociates to CO_2 according to:

The CO_2 can be driven off by shaking or boiling and causes the above equilibrium to be driven to the right. So, the titration is carried out close to the end point and the solution is boiled to eliminate the CO_2. The pH rises rapidly to about 8, as the concentration of carbonic acid drops. As the carbonic acid is removed, the pH is controlled by the small concentration of HCO_3^- which is basic. At this point if the titration is resumed, the pH goes down rapidly because the amount of carbonic acid is small and the buffering effect is negligible. The indicator, bromocresol green gives a sharp color change under these conditions. (blue → yellow)

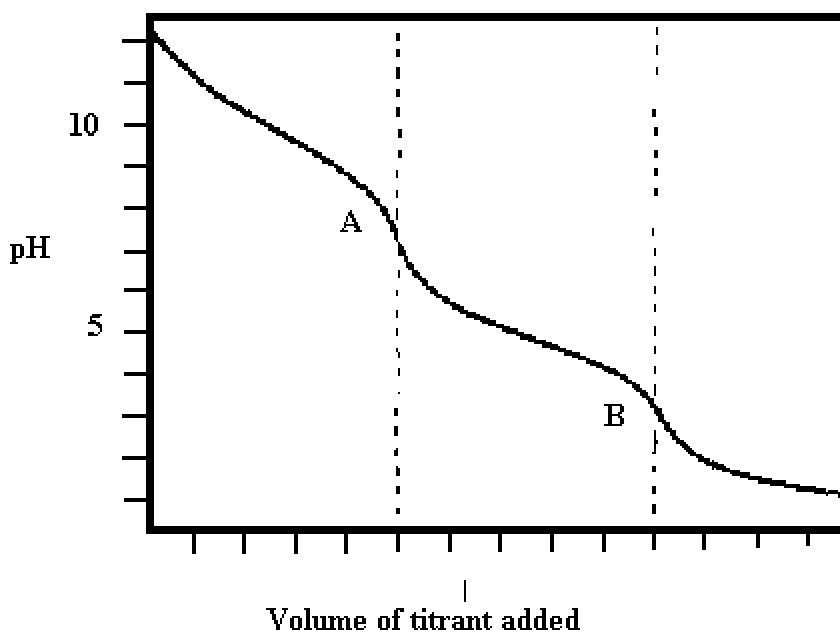


Figure 1: A titration curve for the titration of carbonate. The points marked A and B are the inflection points which indicate the two end points. Notice that it takes the same amount of acid to reach the first point as to go from the first to the second end point. Can you explain why this must be true?

The titration curve is the plot of pH versus the amount of titrant added. The end points can be obtained from the points of inflection.

PROCEDURE

During the first week of the experiment, you will standardize the HCl solution and determine the % of carbonate in the unknown using the visual indicator method. In the second week, you will determine the percentage of carbonate in the unknown using the pH sensor (a glass electrode). Note that enough HCl should be prepared during the first week for use in the second week. This will enable you to calculate the % of carbonate in the unknown sample using two different methods and allow you to compare the results. For the visual indicator method three titrations should be performed. Two titrations are required for the sensor method.

The first step in this experiment is to prepare a HCl standard. Since HCl as purchased varies in concentration, a solution of exactly known molarity cannot be prepared by dilution of the commercially available reagent. Thus, the HCl must be standardized indirectly. This is done by titrating the HCl solution against a primary Na_2CO_3 standard solution. It is necessary to prepare an approximately 0.2 M HCl standard. (Note the molarity (after the standardization) must be known precisely) Take a little less than a liter of distilled water and add the calculated volume of 6 M HCl to make approximately 0.2 M HCl. This solution needs to be titrated against a Na_2CO_3 primary standard to determine its concentration accurately.

Put approximately 1.5 to 2 g of pure Na_2CO_3 in a weighing bottle and dry in an oven at 150-160°C for approximately 2 hrs. (The drying has already been done) Cool it in the dessicator and weigh it. Weigh out three portions of approximately 0.25 g., each (with a precision of $\pm$ 0.1 mg) into 200 ml Erlenmeyer flasks. By weighing the sample bottle after each portion is poured out, you can weigh three portions with only four uses of the balance. This is called weighing by difference. Add 50 ml of H_2O to each sample, and swirl gently to dissolve the salt.

TITRATION USING AN INDICATOR:

Standardization of the HCl: Add 3 to 4 drops of the indicator ***bromocresol green***. Titrate with the HCl solution until a green color is formed. Stop the titration, boil the solution for a minute or two making sure no solution is lost. Cool the solution to room temperature, wash down the sides of the flask with distilled water from your wash bottle and continue titrating until the first appearance of a yellow color occurs. Record the buret reading. Calculate the molarity of the HCl solution. The titration should be repeated three times.

Analysis of the Sample: Dry the unknown sample in a weighing bottle by heating for 2 hours at 150 to 160 ^{o}C. (Make sure that you carry out the drying at the beginning of the experiment). Weigh samples of about 0.5 g. into a 200 ml Erlenmeyer flask to the nearest 0.1 mg. Dissolve the sample in distilled water and titrate using the indicator method . Use the Q-test as a criteria for rejection of suspect experimental data points.

POTENTIOMETRIC TITRATION USING THE pH SENSOR: Learn how to use the pH probe from your instructors. Instructions for operating and calibrating the pH probe

can be found at the end of this section. Further information on the pH Amplifier is given in the appendix. Titrate the first portion past the second end point, using the computer interfaced pH probe. Set up the software so that you enter the Buret readings after each addition of approximately (but known exactly) 1 ml, (less near the break points) and you specify when the computer is to read and record the pH (when the reading stabilizes after each addition). The pH probe can enter the data directly into the PC in a spreadsheet format. These data can be used to draw the titration curve. Use the pH sensor method only for the determination of the % carbonate in the unknown sample.

DETERMINATION OF THE END POINT FROM THE TITRATION CURVE

The end point is determined from the inflection point of the titration curve. To achieve this, first draw the steepest tangent to the curve in the end point region and then locate the points where the smoothed curve departs from the tangent. The end point is located midway between the two points of departure.

REPORT: % Na_2CO_3 in your unknown. Present the titration curve. As you will have at least five data points, the Q test must be applied to see whether any of the measurements should be discarded.

INSTRUCTIONS FOR USE OF pH SENSOR

The connections:

1. Plug the pH electrode into the pH Amplifier Interface box.

2. Connect the pH Amplifier Interface Box to Port 1 of the serial box Interface.
3. Connect the serial Box Interface to the COME or COM2 port of the computer using the cable provided. (Use the 9-pin to 25-pin adapter if necessary.)
4. Plug the Serial Box Interface into an electrical outlet using the AC adapter.

OPERATING PROCEDURE FOR THE pH SENSOR

1. Turn on computer.
2. CLICK ON START
3. CLICK ON PROGRAM; THEN CLICK ON VERNIER SOFTWARE; THEN CLICK ON LOGGER PRO
4. FILE →OPEN→pH system→open
5. For CALIBRATION pH 4, pH 7, and/or pH 10:

SET UP→Sensors
→Calibrate
→Perform now

Reading 1 (Place sensor)
in buffer, pH=4)
value: 4.0 enter →keep →ok

Reading 2 (Place sensor in buffer,
pH=10)
Value: 10.0 enter→keep→ok

As a check on the accuracy of the calibration place the sensor in a buffer of pH = 7 and the meter reading should be 7.0 (Also make sure that between readings the sensor is well washed with distilled water to avoid any contamination).

NOTE: Do not throw the buffer solutions away but return them to the original bottles after use.

6. For DATA COLLECTION:

   SET UP →DATA COLLECTION

   →MODE

   →EVENTS WITH ENTRY

   column label: volume (ml)

   abbreviated title: Vol

   Units mL

   →OK

7. Collect

8. The first line on the screen will then show **"STOP KEEP"**

   Click on KEEP, then enter the titration volume used, then ENTER

   EX: Keep 0.0 enter (that is if you start the buret at zero; otherwise subtract the first readings from each of the other readings to obtain the total volume added)

   Keep 0.5 enter

   Keep 1.0 enter

   Keep 1.5 enter

   The pH value will show up on the bottom line and right hand Column of the screen.

SAVING THE DATA

You must provide your own diskette. After completing each titration you will need to save the data by EXPORTING it to a TXT File. In order to do this, first click on FILE → EXPORT. Give the file a name such as pH TIT: Your last name. The data will be exported to a TXT File using the A drive. (if you use the diskette) You can then input the data to EXCEL by clicking on FILE → OPEN → EXCEL.

If you need to print a copy of the data you should PRINT at HP 4050 Printer.

FOR DISPLAY OF METER, GRAPH AND TABLE ON THE SAME SCREEN

In order to get all three items onto the screen, you will need to reduce the size and position of the graph i.e. create some space for the additional display.

Click on WINDOWS → NEW TALL WINDOWS

→ METER → pH Meter → OK

EXPERIMENT 4

DETERMINATION OF THE HARDNESS OF WATER USING EDTA

INFORMATION AND TECHNIQUES:

- Learn about ligands and chelates and the use of EDTA as a complexing agent
- Perform a titration using dilute reagents
- Gain experience working with buffers
- Determine the hardness of your water

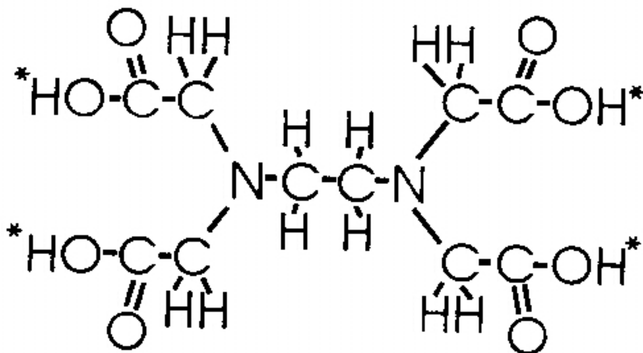
INTRODUCTION:

A ligand, such as ethylenediaminetetraacetic acid (EDTA) will react with a metal in a 1 to1 ratio (the oxidation state of the metal does not change the ratio) to form a hexadentate (six bonds between the ligand and the metal) chelate. A pair of unshared electrons capable of complexing with a metal ion is contained on each of the two nitrogens and each of the four carboxyl groups, thus forming six complexing groups. We can represent EDTA by the symbol H_4Y. It is a tetraprotic acid and the hydrogens in H_4Y refer to the four ionizable hydrogens. It is the unprotonated ligand Y^{4-} that forms the complexes with the metal ions, that is the protons are displaced by the metal ion upon complexation. Chelates are very stable and most are soluble.

$$M^{+n} + Y^{-4} \rightarrow MY^{n-4} \text{ and } K_f = \text{formation constant} = (MY^{n-4})/(M^{+n})(Y^{-4})$$

The formation constants are pH dependent (pH affects the concentration of Y^{-4}) and are usually above 10^{15}. The formation constants for Ca and Mg are less; Ca ($K_f = 5.01 \times 10^{10}$); Mg ($K_f = 4.9 \times 10^{8}$) By controlling the pH, it is frequently possible to use a titration to determine the concentration of one or more metals without obtaining significant interference from other metals in the solution. Water hardness is one such analysis. The major metals are usually Ca and Mg with lesser amounts of Fe and other metals. However, the formation constants of the EDTA

complexes of calcium and magnesium are too close to differentiate between them in an EDTA titration, even by pH adjustment, so they will titrate together. By



EDTA

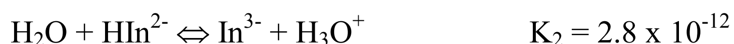
convention, the total of these is reported as ppm (mg/L) $CaCO_3$. Consult your textbook for more details on this method. In this experiment you will determine the concentration of an unknown and a sample of water which you have collected either at home or outside. The only metal in your unknown is Mg. Your "real" water sample may contain many metals at widely different concentrations.

Use of Eriochrome Black T as an Indicator

Eriochrome Black T contains three ionizable protons (a triprotic acid)and can be represented as H_3 In. It is used in the pH range 7 to 11, where the blue form of the indicator predominates. Its behavior as a weak acid is described by the equations

$H_2O + H_2In^- \Leftrightarrow HIn^{2-} + H_3O^+$ $\qquad K_1 = 5 \times 10^{-7}$

Red Blue



$H_2O + HIn^{2-} \Leftrightarrow In^{3-} + H_3O^+$ $\qquad K_2 = 2.8 \times 10^{-12}$

The metal complexes of Eriochrome Black T are generally red, as in H_2In^-. Thus for metal ion detection, it is necessary to adjust the pH to 7 or above so that the blue form of the species, HIn^{2-}

predominates in the absence of the metal ion. Until the equivalence point, the indicator complexes the excess metal ion so that the solution is red. With the first slight excess of EDTA, the solution turns blue as a consequence of the reaction.

$Mg\ In^{-} + HY^{3-} \rightarrow MgY^{2-} + HIn^{2-}$

Red Blue

The metal indicator complex must be less stable than the metal EDTA complex, or else the EDTA will not displace it from the metal. On the other hand, it must not be too weak, or the EDTA will start replacing it at the beginning of the titration and a diffuse end point will result. In general, the metal-indicator complex should be 10 to 100 times less stable than the metal titrant complex.

Procedure:

Prepare 250 ml of approximately 0.02M EDTA by weighing out the correct amount of the disodium salt of EDTA, $Na_2H_2Y{:}\ 2H_2O$(Mol wt = 372.24) transferring it quantitatively to a 250 ml volumetric flask, dissolving it, and diluting to volume.

Note that EDTA dissolves slowly and may take some time to dissolve. Unknown samples may be issued as liquid solutions or as solids. Follow the proper procedure below for your unknown.

If the sample is solid: Add 5.0 to 6.0 ml of pH 10 buffer to the unknown sample and enough distilled water to give a total volume of about 100 ml, and 5-10 drops of freshly prepared (see below) erichrome black T indicator and titrate to a permanent blue endpoint with 0.02 M EDTA. The color change tends to be slow in the vicinity of the end point. Care must be taken to avoid overtitration.

If the sample is a solution: ***Quantitatively*** transfer the entire sample to a 100 ml volumetric flask, and dilute to volume. Pipet a 25.00 ml aliquot into a 500 ml Erlenmeyer flask. Add 5-6 ml of pH 10 buffer, about 200 ml of distilled water, and 5-10 drops of eriochrome black T indicator. Titrate as above with EDTA. Do three replicates.

Water sample: After titrating your unknown samples, take a 250 ml portion of your home tap water in a 500 ml Erlenmeyer flask. Add buffer and indicator as above. Titrate to the same blue endpoint. Do 2-3 replicates.

Report:

Report the percentage of MgO in the solid sample, or ppm of Mg ***in the 100 ml solution of unknown*** which you made from the concentrated solution given to you. Report the hardness of the water sample. This is defined as ppm of $CaCO_3$. Remember to identify the source of your water sample, well or reservoir, water company who supplies it if you know, and whatever other pertinent information you can find. Remember to include the calculations and chemical equations in your report.

(Recall that ppm means grams of analyte per million grams of solution. Since these solutions are predominantly water, 1 ml = 1 gram. Therefore, in water, 1 ppm is equivalent to 1 g in 10^6 grams or 1 mg/liter.)

NOTES:

1. Ethylenediaminetetraacetate (EDTA) may be prepared by weighing out the hydrated disodium salt as a primary standard. Before preparing, check with the TA about using the bulk supply in the laboratory.
2. Be sure the buffer has adequate capacity to hold the pH at 10 during the titration.
3. Both calcium and magnesium are titrated using this procedure. The total concentration makes up the bulk of water hardness even though hardness is, by convention, reported as calcium carbonate.
4. Water is classified as follows according to the hardness data:

a) 0-75 ppm = soft water

b) 75-150 ppm = moderate water

c) 150-300 ppm = hard water

d) >300 ppm = very hard water

5. Although your data are not official, this is the test EPA uses to measure the hardness of water samples. It is an opportunity for you to determine the quality of your water. Feel free to analyze additional samples if you want to and have the time. When the U. S. EPA, Region VII in Kansas City did this analysis 20 times on a single water sample, the range of the values obtained was 207 to 218 ppm. The mean was 213 and the standard deviation was 0.6 ppm.

6. When you get ready to purchase a home, this is one of the items you might want to check. Generally, water from ground water "deep wells" (rather than cisterns which are really surface water supplies even though they are commonly called wells in New Jersey) will be hard or very hard water. The State Geologist can tell you in advance what kind of water you will get and how deep you will have to drill. Surface water supplies (lakes, streams, and reservoirs) will usually be moderate to soft. Rainwater (cisterns) is most often soft water. Treatment of very hard water (sometimes called copperas, sulfur or mineral water and formerly thought to have healing properties) is expensive and must be done on a continuing basis. If not treated, hard water requires more soap to be effective and leaves a residue or "film" after use with soap or detergents. Hard water also leaves a rust colored residue (Fe_2O_3) as the oxygen in the air oxidizes the soluble and colorless iron II in ground water to the insoluble and rust colored iron III oxide. However, hard water is not harmful for drinking..

7. Erichrome black T indicator is prepared by adding 0.1 g of the solid to 15 ml of triethanolamine and 5 ml of ethanol. The solution is stable for several weeks.

8. The kinetics are somewhat slow at the endpoint, so allow time (a few seconds between drops) for the color to develop. The endpoint will not be as sharp as those for the acid-base titration because you are using a more dilute titrant.

EXPERIMENT 5

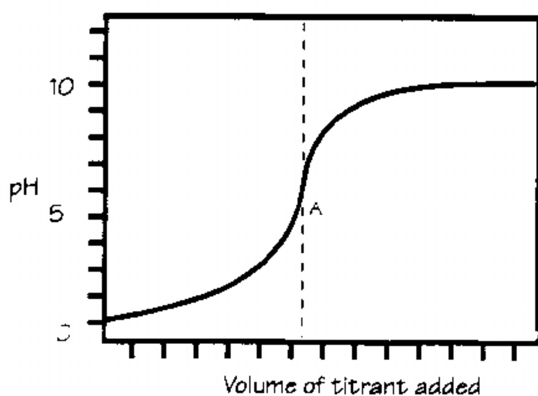
POTENTIOMETRIC TITRATION OF AN ACID MIXTURE

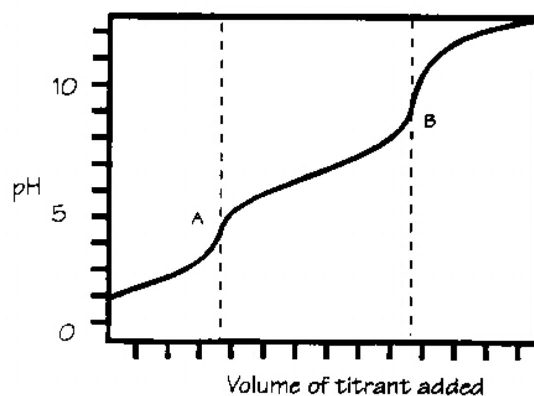
INFORMATION AND TECHNIQUES

1. Obtain a titration curve with a pH meter
2. Determine the concentration of an acid mixture.

INTRODUCTION

In this experiment, a mixture of two acids is titrated with a standard base. In this titration, the pH does not change with sufficient abruptness at the equivalence point to allow a sharp color change in an indicator. Therefore, a pH meter is used to measure the pH near the equivalence point. The titration curve which is the plot of pH as a function of volume of titrant added can be used to calculate the equivalence point. When the pK's of two acids are sufficiently different, then the concentrations of acids in a mixture can also be determined. A typical titration curve for a single acid and an acid mixture is shown in the figures below.





In general, the mixture of two acids can be analyzed with an accuracy of a few parts per thousand if the pK values differ by 10^3 or 10^4.

PH Meters and Use of the Glass Electrode

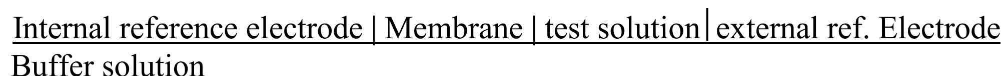
The measurement of pH involves the measurement of the potential between two electrodes, a glass electrode that responds to H^+ and a reference electrode which is often a standard calomel electrode (SCE). Often a combination electrode is used.

As the resistance of a glass electrode is very high ($10^7 – 10^9$ ohms) the meter used to measure the potential must be very sensitive (A current of 10^{-10} ampere causes a voltage drop across 10^8 ohms of 10 millivolts, which is equivalent to 0.2 pH unit.

The Glass Electrode

A glass electrode consists of a thin glass membrane sealed onto the end of a glass tube. A constant pH is maintained on the inside of the bulb by filling it with an internal buffer solution. Contact between the buffer and the lead to the meter is made through an internal reference electrode (calomel or silver/silver chloride).

Measurement of the pH of a solution involves immersion of the glass bulb, along with an external reference electrode, in the solution and a measurement of the potential developed between the two electrodes. The cell may be written as



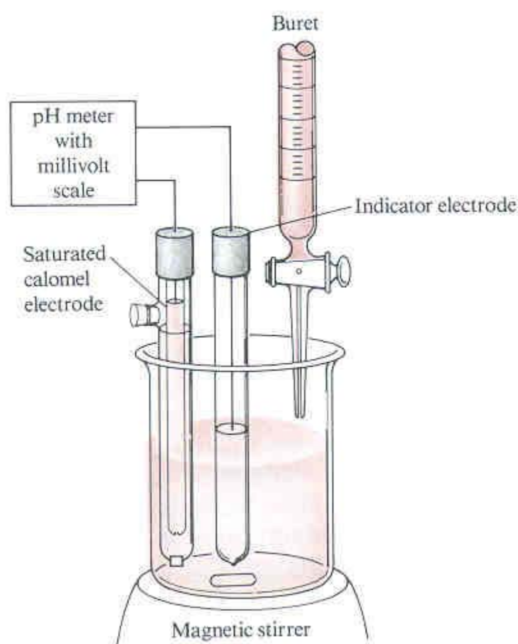
The potential varies with the hydrogen ion activity of the solution according to the Nernst equation.

$$E = E' - \frac{2.303RT}{F} \log\left(\frac{1}{a_{H^+}}\right)$$

Where E' depends on the nature of the reference electrode used as well as some other factors. The mechanism of the glass electrode involves an ion exchange equilibrium.

The surface of a glass membrane consists of a hydrated layer of glass about 0.1 μm thick. In this surface layer, lithium or sodium ions present at lattice sites in the glass can exchange with hydrogen ions in solution. This equilibration produces an electromotive force across the membrane that is a function of the pH of the solution.

A diagram of the typical set up for a pH measurement is shown below.



pH meter and pH measurements: The functioning of a pH meter will be demonstrated in class. When using the analog pH meter calibrate it against pH 4 and pH 10 buffers by first adjusting the CALIBRATE knob to give the correct pH4 reading and then adjusting the TEMPERATURE COMPENSATION knob to give the correct pH 10 reading. You will need to cycle through the calibration procedure several times. Also set the temperature knob to the temperature of the solution, using the slope indicator adjustment. Each time you change solutions, rinse the electrodes with water and blot off the excess to avoid cross contamination. When using the digital pH meter, it is best to use 3 buffers (pH = 4, 7 and 10) and to use the autocalibration method. Detailed procedures for operating both an analog and a digital pH meter are given at the end of this experiment.

PROCEDURE

The experiment consists of two separate parts. First, 0.2M NaOH solution is prepared and standardized by titrating against sulfamic acid. Then, the NaOH solution is titrated against the acid mixture.

Preparation of 0.2M NaOH:

Add sufficient NaOH to make 300 mL of a 0.2 M solution. Alternatively, a concentrated solution of NaOH may be supplied from which to prepare the 0.2 M solution.

Standardization of NaOH solution: The sulfamic acid has been pre-dried and is ready for use. Carefully weigh approximately 0.5 g. of sulfamic acid (NH_2SO_3H), formula weight 97.09 into a 250 ml beaker. Add 50ml of distilled water. Insert the pH electrode into the beaker and titrate with the NaOH solution. Stir while titrating, and record pH after each addition (1mL), and for every drop near the end point region. Plot the titration curve while the titration is being carried out and continue the titration until the plot begins to level off around pH 10. Repeat this procedure using another sample of sulfamic acid.

Titration of Unknown: If the unknown is a solid then weigh approximately a 0.5 to 0.6 g. sample of the acid mixture into 250 ml beakers. Dissolve the sample in water and titrate with the standardized NaOH using the method mentioned above. Run a duplicate titration.

If the unknown is a liquid in a vial, then transfer the entire liquid sample **quantitatively** into a 100 mL volumetric flask. Remember to rinse the cap, and vial several times with distilled water adding the rinsings to the volumetric flask, then dilute to 100 mL.

If on the other hand, the unknown is a small quantity of liquid in a volumetric flask, then fill the flask up to the 100 mL mark with distilled water.

Pipet 25 mL of this solution into 200 mL beaker, add 50 mL of water and titrate.

Data Analysis

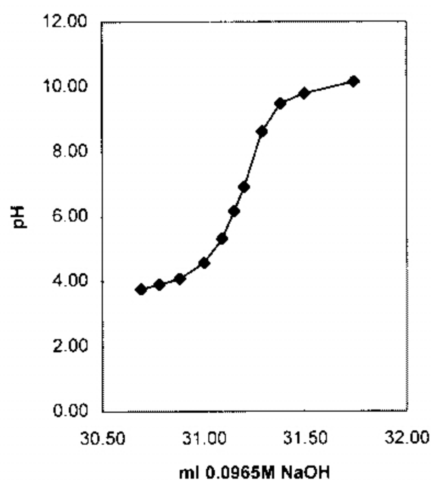
Draw the titration plot by drawing a smooth curve through the points. The end points are the points of inflection. To determine the point of inflection draw the steepest tangent to the curve in

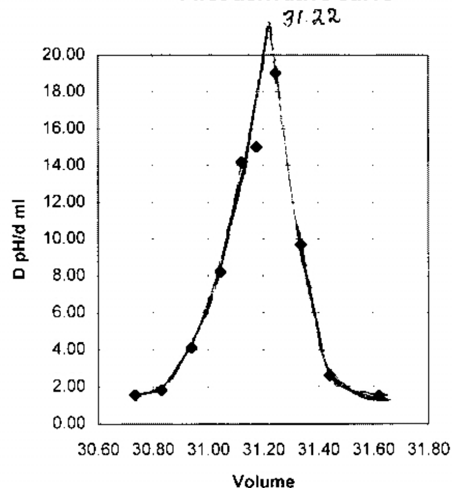
the end point region. Locate the points where the smooth curve departs from the tangent. The end point is located midway between two points of departure. Also, draw the first derivative curves. Typical titration curves for a titration of HCl with NaOH are shown below.

REPORT

Assume both acids to be monoprotic. For each acid report the millimoles per gram for a solid sample and the millimoles per ml (as diluted) for a liquid sample.

ml NaOH	pH	d pH	d ml	V(average)	d pH/d ml
30.69	3.75				
30.78	3.89	0.14	0.09	30.74	1.56
30.88	4.07	0.18	0.1	30.83	1.8
31	4.56	0.49	0.12	30.94	4.08
31.09	5.3	0.74	0.09	31.05	8.22
31.15	6.15	0.85	0.06	31.12	14.17
31.2	6.9	0.75	0.05	31.18	15
31.29	8.61	1.71	0.09	31.25	19
31.38	9.48	0.87	0.09	31.34	9.67
31.5	9.79	0.31	0.12	31.44	2.58
31.74	10.15	0.36	0.24	31.62	1.5





The first two columns in the table are the values taken from the original data obtained in the laboratory. The third column is obtained by taking the difference between two consecutive values of pH. The fourth column is the difference between two consecutive values of the buret reading. The last column is the quotient of the division of column 3 by column 4. The fifth column is the average of consecutive buret readings. The first derivative plot shows a maxima where the change in pH with respect to volume is the greatest. The average volume for this point is the equivalence point for the titration. As you will see from the Figure, the data may be extrapolated to obtain the peak.

OPERATION OF pH METERS

ANALOG pH METER

The pH scale is numbered from pH O to pH 14 in divisions of 0.1 pH. The meter also has a millivolt scale numbered from –700mV to +700mV in divisions of 10mV.

FUNCTION SWITCH

The FUNCTION SWITCH sets the instrument for the desired type of measurement (e.g. pH, MV, X^{++}, X^{-} etc.). The function switch should be in the OFF position when the meter is not in use.

OTHER CONTROLS AND CONNECTORS

TEMPºC - % SLOPE

Temperature compensator: (black knob in the center of clear plastic dial on instrument panel) is used to adjust electrode slope when calibrating or changing temperature.

CALIB

Calibration control: (lower left of instrument panel) is used to calibrate the instrument and electrodes on a standard of known activity, concentration, or pH. On some models the calibration control has a two-mode, vernier drive which provides a fine adjustment over one turn and coarse adjustment over the remaining nine turns.

INPUT

Two input jacks: (lower right of instrument panel). **Large input jack** accommodates specific ion, pH, or redox electrodes. **Small red input jack** accommodates reference electrode.

Zero adjust screw: (center of instrument panel) is used to position needle exactly on **center scale.** Function switch must be in the **OFF** position before making any adjustments.

TWO-BUFFER STANDARDIZATION

1. Set up instrument and electrodes. Turn function switch to pH position.
2. Select two buffers, e.g. pH = 4 and pH = 10.
3. Place pH and reference electrodes or combination electrode in one buffer solution.
4. Turn the calibration control until the needle (on the black scale) points to the pH value of the buffer.
5. Remove electrodes from this buffer solution. Rinse with distilled water and place in the second buffer solution.
6. Turn the temperature compensator knob until the meter needle points to the pH value of the second buffer solution.
7. Turn the slope indicator until the arrow of the temperature compensator points to the temperature of the solution. The percent of theoretical slope can be read on the slope scale. A slope of less than 90% may be caused by a defective pH electrode or contaminated buffer solution.
8. Remove electrodes from the second buffer solution, rinse with distilled water, and place in unknown solution. Read the pH value of the unknown on the black pH scale.

GUIDE FOR USING THE 420A pH METER [DIGITAL]

METER OVERVIEW

1. Results are displayed in the large field of the display

2. Mode is indicated by SET UP, CALIBRATE or MEASURE along the top of the display.

3. The parameters in the setup menu are preset to typical values and usually do not require changing.

4. If you want to escape the sequence you are in, or you are not sure of which function you are in, pressing **mode** will take you to MEASURE mode where you can start again.

METER CONNECTIONS

Power

1. Plug line adapter into an appropriate wall outlet.
2. Plug line adapter firmly into power input on rear panel of meter.

ELECTRODE CONNECTIONS

1. Remove BNC shorting cap.
2. Attach electrode with BNC connector by sliding BNC connector onto electrode input, then push down and turn clockwise to lock into position.
3. Attach ATC probe with DIN connector by sliding the DIN connector into the ATC probe input until firmly in place.

pH MEASUREMENT OVERVIEW

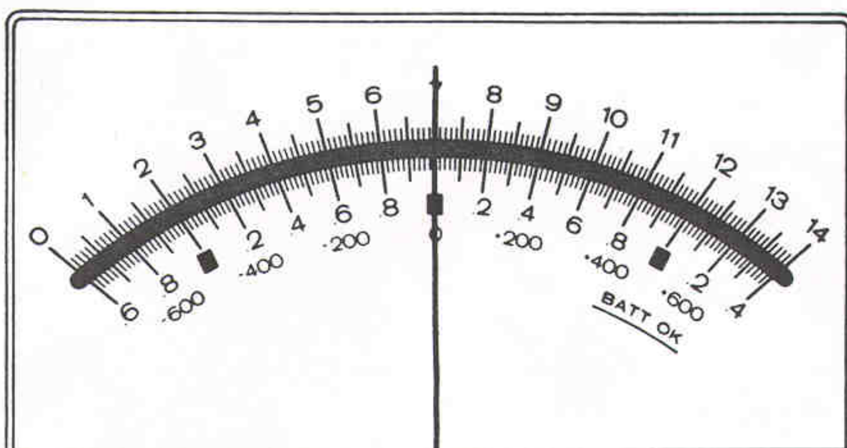
The meter/electrode system is calibrated with one to three buffers that bracket your sample pH. For maximum accuracy a three buffer calibration is recommended, but for this experiment the use of two buffers is adequate.

The autocalibration feature automatically recognizes 4.01, 7.00, and 10.01 buffers.

pH AUTOCALIBRATION WITH TWO BUFFERS

1. With power on, select the CALIBRATE mode, by pressing **mode** until CALIBRATE indicator, is aligned with the pH legend along top of display.
2. Place electrode(s) into pH 7 buffer and stir moderately.
3. Press **yes** to select the two buffer calibration which brackets your sample range.
4. When **"READY"** is displayed beside the reading, indicating electrode stability, press **no** to change buffer value or press **yes** to accept buffer value. The buffer value is stored and meter display freezes for three seconds. The meter automatically switches to buffer two, indicated by the "P2" on the display.
5. Remove electrode(s) from first buffer. Rinse with deionized water.
6. Place electrode(s) into second buffer and stir moderately.
7. When "READY" is displayed beside the reading, indicating electrode stability, press **no** to change buffer value or press **yes** to accept buffer value. The buffer value is stored and meter display freezes for three seconds.
8. After the second buffer value has been entered, press **yes**. The electrode slope will be displayed. SLP appears in the lower field while the actual electrode slope, in percent, appears in the main field. Press **yes** to accept. The meter will then automatically go into measure.
9. Remove electrode(s) from buffer. Rinse with deionized water. Place electrode(s) into sample. When "READY" is displayed beside the reading, record the sample results.

<u>METER SCALES</u> [ANALOG]



pH scale (black – Numbered from pH 0 to pH 14 in divisions of 0.1 pH.

pH EXP (red) – Full-scale 2.8 pH units in divisions of 0.02 pH. Red squares mark integral pH values.

MV scale (blue) – Numbered from –700 mV to +700 mV in divisions of 10 mV.

BATT OK (green) – Indicates condition of batteries.

EXPERIMENT 6

DETERMINATION OF TRACE IRON USING A UV-VISIBLE SPECTROPHOTOMETER

INFORMATION AND TECHNIQUES

1. Understanding the fundamentals of spectroscopy.
2. Learning to use a spectrophotometer
3. Determination of trace iron using a UV-Vis spectrophotometer.

Background

Fundamentals of Spectroscopy: Light (infrared, visible, ultraviolet) is electromagnetic radiation. The interaction of electromagnetic radiation with matter is useful in many ways to determine both the identity of compounds and their concentration in mixtures. The electromagnetic spectrum ranges from high energy γ-rays to very low energy radio waves. Many regions of the spectrum are used for obtaining information about material samples. Because of the wide range of energies involved, the methods used in the various spectral areas seem quite different, but they all are based on similar principles.

Electromagnetic radiation has both a wave and a particle character. It is often envisioned as a wave being propagated through space, which can be characterized by its frequency. At the same time, it can also be envisioned as a flux of particles (photons) which are characterized by their energy. *Frequency, υ,* is defined as the number of oscillations passing a point in a specified time interval. The units of frequency are given in Hertz (Hz). 1 Hz = 1 oscillation/sec. The *wavelength*, the distance measured from the maximum of one wave to the next, is related to the frequency by the speed of the wave. Both wavelength and frequency are related to the photon energy. The relationships are expressed as:

$$E = h\upsilon = \frac{hc}{\lambda} \quad \text{and} \quad \upsilon = \frac{c}{\lambda}$$

where E is the energy of the photon, h is Planck's constant, 6.626×10^{-34} J sec., λ is the wavelength (in meters), υ is the frequency, and c is the speed of light in a vacuum, 3.00×10^{8} m/sec. Since the speed of the wave propagation depends on the matter through which the wave passes, the frequency and the energy are the only truly inherent characteristics of a wave. The wavelength will change when the medium changes. The relationship between the speed of light in any material and that in a vacuum is described by the *refractive index*, η, of the material.

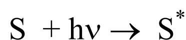
$$\eta = \frac{speed\ of\ light\ in\ vacuum}{speed\ of\ light\ in\ material}$$

It should be noted that the speed of light in a material, and therefore the refractive index of the material changes with wavelength.

While the entire spectrum of radiation obeys the same laws, travels at the same speed, and has the same basic nature, different spectral regions are used in different ways. Short wavelength, high energy radiation in the x-ray region has sufficient energy to cause changes in the inner electron structures of atoms. The chemical state of an atom has little effect on the inner shell electrons. Therefore, x-rays can be used to probe the elemental composition of a sample, without regard to the chemical state of the atoms present. Ultraviolet radiation interacts with the outer electronic levels, promoting electrons to higher energy levels, so this region of the spectrum will yield information on the bonding of the atoms in molecules, and their oxidation state. At even lower energy, infrared radiation affects the rotational and vibration energy levels in molecules, giving spectra that indicate the identity of functional groups and are rich in molecular structure information. In the radio frequency area the energies are so low that only reorientation of nuclear spins occurs in the molecules. The absorption of energy in this area is informative about structure and conformation of molecules.

Spectroscopy: The interaction of radiation and matter can be put to use in either the emission or absorption modes. In emission spectroscopy, molecules or atoms are stimulated in some way to raise them to a higher energy level. When they return to the ground state, energy is emitted as radiation. The radiation is detected and its frequency and intensity give information about the identity as well as the amount of the radiating species. In absorption, radiation is passed through the sample. The reduction in the intensity of the radiation emerging from the sample indicates the concentration of absorbing species, while the wavelengths absorbed provide information about the identity of the absorbing species.

According to quantum mechanics, atoms and molecules may only exist in certain energy states. Their lowest energy level is called the ground state. They can be promoted to a higher energy level or an excited state by irradiation with an electromagnetic wave of suitable wavelength:



where S is a low energy species, and S* is its excited state. The amount of energy absorbed is exactly equal to the energy difference between the lower energy state and the excited state. This energy can only be supplied from radiation of the specific wavelength which has the same energy as that needed for the transition. The excited species can then lose its energy by a process in which no radiation is emitted, or through a radiative process that involves emission of radiation.

Both atoms and molecules exist in discrete energy states. These energy levels can be attributed to the electronic states, as well rotational and vibrational levels. In an electronic transition, an electron is promoted to a higher energy level. In rotational and vibrational transitions, the molecule absorbs or emits energy to undergo rotational or vibrational changes. The total energy is expressed as E_t:

$$E_t = E_e + E_r + E_v$$

where E_e, E_r and E_v denote the energy associated with electronic, rotational, and vibrational states. Single atoms have no rotational or vibrational levels. In absorption or emission spectroscopy with atomic vapors only electronic transitions are possible. Therefore, the spectra of atoms consist of a series of narrow lines (0.2 - 0.4 nm wide), each corresponding to a discrete electronic transition. This is called a *line spectrum*. Since each element produces a unique set of spectral lines, they can be used to identify the element. Atomic spectroscopy is, therefore, an excellent tool for qualitative analysis, even in a complex sample.

In a molecule, each electron state contains rotational and vibrational sublevels, in addition to the electronic levels. Consequently, for a molecule, there are numerous possible transitions which are quite close in energy. As a result, a *continuous spectrum* is produced, which contains broad absorbance or emission bands, rather than discrete lines

Absorption Spectroscopy and Beer's Law: In absorption spectroscopy, a sample is irradiated with electromagnetic radiation, and the amount which passes through is monitored. An absorption spectrum is a plot of amount of radiation absorbed as a function of wavelength. Based on the wavelengths which are absorbed, the absorbing atoms and molecules may be identified. Similarly, from the amount of energy absorbed, the amount or concentration of the analyte can be

determined. Consequently spectroscopy provides both qualitative and quantitative information. Monochromatic light is passed through the solution, and the power of the incident beam, P^o, as well as the power of the emerging beam, P, are measured.

Transmittance (T) is defined as:

$T = P/P^o$

A parameter called *absorbance* (A) is defined as -log T or log P^o/P, and Beer's law states that:

$A = \varepsilon bC$

The constant ε, the molar absorptivity, ($Lmol^{-1}cm^{-1}$) is a function of the compound that is absorbing the radiation and the wavelength of the radiation. The units for concentration (C) are moles per liter, while the path length (b) is given in centimeters.

Beer's law predicts a linear relationship between absorbance and concentration. However, in practice, nonlinearity at higher concentrations is often found when absorbance is plotted versus concentration. There are several reasons why nonlinearity may occur.

Spectroscopic Apparatus: A spectrophotometer comprises of a light source, a monochromator, and a radiation detector. After light is transmitted through the sample, it is necessary to measure its intensity at one or more wavelengths. A detector is a device to convert the energy of the radiation into a current or voltage in the measuring circuitry. Often the electrical signal is very small and requires amplification before it can be analyzed. The type of detector needed to determine the intensity of emitted or transmitted light depends on the wavelength. Finally, it is often necessary to select a band of wavelengths for use in the measurement. This is done by filtering out unwanted wavelengths or by dispersing the radiation from the sample or from the source into its component wavelengths, thus separating them in space

A schematic diagram of a UV-Vis spectrophotometer is shown in Figure 1. Quantitative analysis is performed by determining the molar absorptivity coefficient, ε, using solutions of known concentrations. A wavelength best suited for analysis is chosen. This is usually selected at a place in the spectrum where the absorbance is not changing rapidly, and where interfering substances have low absorbance. While it is not necessary to select a point at which the absorbance is at a maximum, selecting the highest absorbance point will give the highest sensitivity. If the samples to be analyzed are not at trace levels, a wavelength at which lower absorbance takes place may be preferable. The best accuracy in absorbance spectroscopy is achieved at fairly low absorbances. The point of best accuracy is at an absorbance of 0.38. At higher concentrations, because of the log term in Beer's law, a large change in concentration

causes only a small change in the transmitted light. Therefore it is better to use a wavelength at which absorbance is less, if it avoids measuring solutions with absorbances above 1, or diluting the sample.

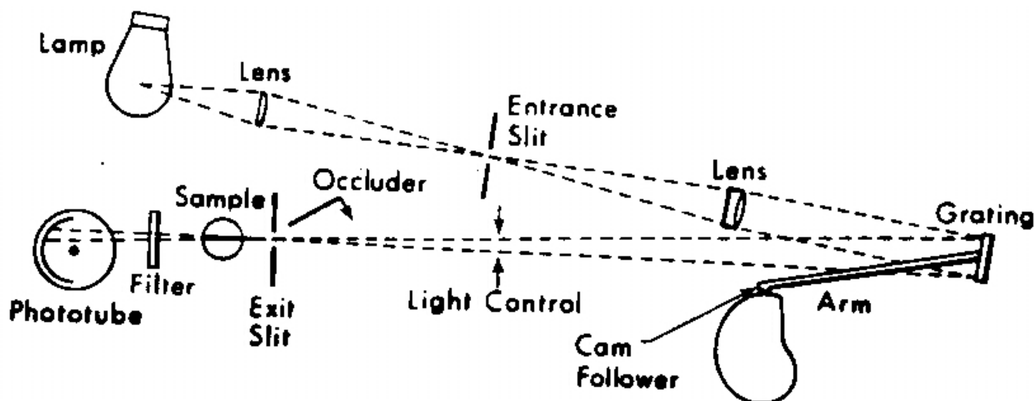


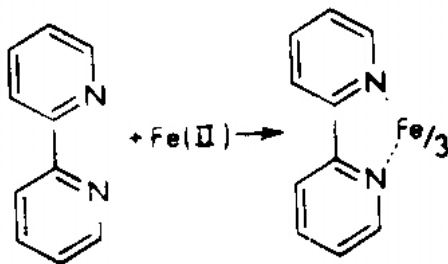
Figure 1: Schematic diagram of the Spectronic 20 spectrophotometer

Colorimetry: To determine individual compounds in samples, a reagent which reacts specifically with the component of interest is added to the sample, forming a colored species. The intensity of color produced is proportional to the concentration of analyte in the sample and can be measured using the UV-visible spectrophotometer.

Analyte + Colorimetric reagent $\rightarrow$ Colored complex

The selectivity is provided by the colorimetric reaction and the absorbance in the visible or UV region is used only for quantitation.

Colorimetric Determination of Iron: Bipyridine is a suitable colorimetric reagent for iron as it forms an intensely red complex with Fe(II), which absorbs in the visible region and quantitation at ppm level is possible using a UV-Vis spectrophotometer. Each Fe^{2+} ion is coordinated with three bipyridine molecules to form the colored complex.



The stable complex forms rapidly in the pH range of 3-9. The absorption coefficient is 8650 liter/mole-cm at 522 nm. If Fe(III) is present, it must be reduced to Fe(II) using hydroxylamine hydrochloride ($NH_2OH.HCl$) before the analysis is carried out.

It should be noted that a comparison method may be used only if a system follows Beer's law. For the iron (11) – bipyridine system the law is followed over the concentration range of 0.5 to 8 ppm. In order to fulfill this condition several dilutions of solutions are necessary.

PROCEDURE

In this experiment ferrous ammonium sulfate, $FeSO_4. (NH_4)_2SO_4.6H_2O$, is used as a standard. Calculate and weigh out the amount of this compound needed to make a 0.002 M iron solution (you may check your calculations with the instructor before proceeding). Transfer the salt to a 250 ml volumetric flask, dissolve it in water, add 8 ml of 3M H_2SO_4 and dilute to volume with distilled water and mix. Pipet 10 ml of this solution into a 100 ml volumetric flask, add 4 ml of 3 M H_2SO_4 and dilute to volume. This is your working standard solution. Calculate and record its concentration in mg Fe/mL sample. Note: After the dilution, the final molarity of the standard solution will be close to 0.0002M (but known exactly). The iron concentration of this standard solution should be known to within about 0.5%.

QUANTITATIVELY transfer the entire unknown solution to a 100 ml volumetric flask, rinsing the bottle several times to be sure all the unknown is transferred. Add 4 ml of 3M H_2SO_4, mix thoroughly, dilute to volume with distilled water and thoroughly mix again. This is your sample solution. Obtain 5 volumetric flasks (100 mL) and label two of these "sample", two of them

“standard” and the last one “blank”. Pipet 10.00 mL of sample solution into/each of the “sample flasks”. Into the “standard flasks”, pipet 10.00 mL of the working standard solution. Put about 10 mL of distilled water into the “blank” flask and add 0.4 mL (about 10 drops of 3 M. H_2SO_4).

To each of these five flasks add 1 ml of 10% hydroxylamine hydrochloride solution, 10 ml of 0.1% bipyridine solution and 4 ml of 10% sodium acetate solution. The sodium acetate plus sulfuric acid gives an acetic acid-sodium acetate buffer in the pH region of about 4.5 to 5. Mix after each reagent is added. Dilute each to volume. On the spectrometer, set the absorbance to zero using the blank solution. Set the wavelength at 522 nm, and measure the absorbance of each of the five solutions. Rinse the cuvet with the solution to be measured before filling, and wipe the outside before inserting into the spectrometer, to avoid smudges and fingerprints. Place the cuvet in the same position each time, with the mark to the front.

Calculate the concentration as:

A_{std}/A_{smp}

$$\frac{A_{std}}{A_{smp}} = \frac{[Fe]_{std}}{[Fe]_{smp}}$$

Report: The total weight in mg of iron in the entire sample issued to you.

OPERATION OF THE VISIBLE SPECTROPHOTOMETERS

STANDARD MODEL SPECTRONIC 20

THE SPECTRONIC 20 HAS THREE MAIN CONTROLS FOR ROUTINE OPERATION:

Wavelength Control
Power Switch/Zero Control
100%T Control

WAVELENGTH CONTROL

The Wavelength Control selects the desired analytical wavelength of the instrument. The selected wavelength is indicated on the Wavelength Scale in the window next to the knob. The Scale is color-coded to correspond to the operating range of the instrument's phototube: Black gradations for the basic 340nm – 600nm and red gradations for the 600nm – 950nm range of the optional red phototube/filter combination. All gradations are in 5nm intervals.

POWER SWITCH/ZERO CONTROL

The ON-OFF main power switch is operated by the Zero Control Knob. Allow about 10 minutes for warm up. The Zero Control is used to set the meter to a 0%T readout WHEN THE TEST TUBE ADAPTER IS EMPTY AND THE ADAPTER COVER IS CLOSED.

100% T CONTROL

This control is used to set the meter to 100%T (0.0A) with a blank reference solution. It must be reset whenever the analytical wavelength has been changed. When operating at a fixed wavelength for an extended period of time, periodically check the 100%T (and 0%T) readout and readjust if necessary. The 0% Transmittance is obtained when there is no test tube in the sample holder. These controls are needed because the source (tungsten lamp) does not emit light of equal intensities at all wavelengths and also the sensitivity of the phototube varies with wavelength. Further, the test tube or the "blank" solution (the solvent) may absorb light of certain wavelengths. To measure only the light absorbed by the sample these side effects which influence % T must be compensated for.

OPERATION

GENERAL

The Spectronic 20 Spectrophotometer features uncomplicated adjustment and operation. However, successful use of the instrument requires close attention to correct laboratory procedures and analytical techniques, i.e., clean glassware, correct dilutions, etc.

1. All solutions must be free of bubbles.
2. All sample holders must be at least ½ full.
3. For greater accuracy, use square cuvettes.
4. Always rise the test tube with several portions of the sample before making a measurement.

FOR OPTIMUM PERFORMANCE WITH TEST TUBE SAMPLE HOLDERS, INSURE THAT THE INDEX MARK ON THE TEST TUBE IS ALIGNED IN THE SAME DIRECTION EACH TIME

FOR SAMPLE MEASUREMENT

1. Select Wavelength	WAVELENGTH CONTROL
2. Set Meter Zero	
Sample compartment empty, cover closed	ZERO CONTROL
3. Insert reference blank	
Set 100%T (O.OA)	100%T CONTROL
4. Insert unknown sample	

(READOUT IN % T OR ABS)

DIGITAL SPECTRONIC 20D

The Spectronic 20D Spectrophotometer is a single beam spectrophotometer with an overall wavelength range of 340 nm to 950 nm. The wavelength accuracy is 2.5 nm and the readibility is 1.0 nm.

OPERATING FEATURES

The main controls for routine operation are Wavelength Control, Power Switch/Zero Control, the MODE selector, and the Factor Adjust controls.

DIGITAL READOUT

The Digital Readout displays wavelength and data readings. The four LED status indicators, next to the labels TRANSMITTANCE, ABSORBANCE, CONCENTRATION, and FACTOR, indicate the MODE currently active.

WAVELENGTH CONTROL

The wavelength control selects the desired analytical wavelength of the instrument. The selected wavelength is indicated on the left side of the LED display.

POWER SWITCH/ZERO CONTROL

The ON-OFF main power switch is operated by the Zero Control knob.
Zero Control sets the display to a 0%T readout WHEN THE TEST TUBE ADAPTER IS EMPTY AND THE ADAPTER COVER IS CLOSED.

TRANSMITTANCE/ABSORBANCE CONTROL

This control sets the display to 100%T (0.0A) with a blank reference solution. IT MUST BE RESET WHENEVER THE ANALYTICAL WAVELENGTH HAS BEEN CHANGED. When operating at a fixed wavelength for an extended period of time, check the 100%T (and 0%) readout and readjust if necessary.

MODE SELECT

This control selects the TRANSMITTANCE, ABSORBANCE, CONCENTRATION, or FACTOR mode.

FACTOR ADJUST CONTROLS

The pushbuttons labeled INCREASE and DECREASE are used in the CONCENTRATION and FACTOR modes. To set a lower CONCENTRATION or FACTOR value, press and hold down the DECREASE button until the desired value is displayed. To set a higher value, press and hold down the INCREASE button until the desired value is displayed.

SAMPLE MEASUREMENT: SPECTRONIC 20D

Transmittance and Absorbance

The sequence for sample measurement is:

a. Select wavelength using Wavelength Control.
b. Set the mode to TRANSMITTANCE (press the MODE select control until the "transmittance" LED.on the right of the display is lit).
c. With sample compartment empty and cover closed, adjust Zero Control so that the meter reads zero.
d. Choose the mode that you require by pressing the MODE selector control until the appropriate LED (on the right of the display) is lit.
e. Insert reference blank into the sample compartment and set 100%T or 0.0A.
f. Insert unknown sample into the sample compartment and read measurement from display in percent transmittance or absorbance.

CARY 50 UV/VIS SPECTROPHOTOMETER

This spectrometer uses a dual beam, Czerny-Turner 0.28 m monochromator, has a 190-1100 nm wavelength range, approximately 1.5 nm fixed spectral bandwidth, full spectrum Xe pulse lamp single source with exceptionally long life, dual Si diode detectors, quartz overcoated optics, scan rates up to 24 000 nm/min, 80 data points per second maximum measurement rate, non measurement phase stepping wavelength drive, room light immunity, centrally controlled by PC with Windows interface. The wavelength accuracy is 0.07 at 541.92 nm and 0.24 at 260.54 nm.

The Cary 50 is controlled by the new Cary WinUV software. This Windows based software features a modular design which makes it easy to use.

With the Cary 50, you can benefit from the following features:

- The maxiumum scan rate is 24 000 nm per minute. That means you can scan the whole wavelength range of 190-1100 nm in less than 3 seconds.
- With a data collection rate of an impressive 80 points per second you'll have all the information you need about your kinetics assay.
- The Cary 50 can measure samples up to 3 Abs so you won't have to dilute as often.
- The Xenon lamp flashes only when acquiring a data point, unlike a diode array which exposes the sample to the whole wavelength range with each reading, causing degradation of photosensitive samples.
- As the Xenon lamp is very intense, the Cary 50 can use a beam splitter without the loss in energy causing excessive photometric noise. The beam splitter allows simultaneous reference beam correction, so peaks will not shift as the scan speed changes.
- The Cary 50's super-concentrated beam makes it ideal for fibre optic work.
- The light beam is narrow and very intense, so even if you are using microcells you will still get excellent noise performance.
- The Cary 50 is unaffected by room light. You can operatte with the sample compartment open or closed, you won't notice the difference.

OPERATION

The operation of the instrument is given below as a set of computer screen attachments.

EXPERIMENT 7

SPECTROPHOTOMETRY OF A TWO COMPONENT MIXTURE: DETERMINATION OF COBALT AND NICKEL AS EDTA COMPLEXES

INFORMATION AND TECHNIQUES

1. Learn how to analyze mixtures using a UV/VIS spectrometer.
2. Learn about complex formation using EDTA.
3. Learn how to make a solution from materials that are difficult to dissolve.

Background:

It is possible to analyze a complex mixture containing several species simultaneously without prior separation. In this experiment the concentration of Co and Ni in a mixture will be determined. Both these metals react with EDTA at a pH of 4 or more. Although the complexes (and therefore the color) are stable, the rate of reaction with EDTA especially for Co is relatively slow and the solutions have to be warmed for a long time to ensure complete reaction. This method is applicable only at high concentrations.

Beer's Law: According to Beer's law, the absorbance (A) of a solution is equal to the absorbtivity (ε) times the cell length (b) times the concentration (C) at a given wavelength or:

$$A = \varepsilon\, b\, C$$

Since b is usually one cm, the working equation reduces to $A = \varepsilon c$. If a solution contains two (or more) absorbing species (chromophores), then the measured absorbance at a given wavelength will be the sum of all absorbances at that wavelength. By determining the absorptivities (ε) of each species at each wavelength, we can theoretically determine the concentration of each component in a mixture by measuring the total absorbance at each wavelength and solving the resulting equations simultaneously. For the binary mixture of this experiment, we have:

at wavelength λ_1 $\quad A_1 = \varepsilon_1\,[Co] + \varepsilon_2\,[Ni]$ and

at wavelength λ_2 $A_2 = \varepsilon_3$ [Co]+ ε_4 [Ni] where

A_1 & A_2 = absorbance of the mixture at λ_1 and λ_2 respectively

$\varepsilon_1 - \varepsilon_4$ = the four absorptivities

[Co] = the cobalt concentration (M)

[Ni] = the nickel concentration (M)

If we measure A_1 & A_2 for our unknown and obtain ε_1, ε_2, ε_3 and ε_4 from the calibration standards, we can solve the two equations for the concentrations of Co and Ni. The highest sensitivity and precision are attained when the measurements are made at wavelengths at which the absorbance difference is a maximum and the spectral overlap is minimum.

Procedures:

Preparation of Co and Ni standard solutions: Accurately weigh enough Co metal to prepare 100 ml of approximately 0.05 M solution. Place it in a 100 ml volumetric flask and add 15 ml of dilute HNO_3.(about 2M) Heat gently on a hot plate until dissolution is complete. Do not stopper the flasks. Use the Fume Hood for this procedure. Neutralize with NaOH (about 2M) until the first permanent precipitate of cobalt hydroxide is visible. Then add a few drops of acetic acid to clear the solution and dilute to volume. Similarly, prepare 100 ml of a 0.05 M Ni solution.

Analysis procedure: If the sample is solid, weigh accurately an amount corresponding to 0.1 to 0.2 g each of Co and Ni and transfer to a 100 mL volumetric flask. If it is a mixture of oxides, add 8 ml of conc. HCl, dissolve on a hot plate, neutralize the solution, add acetic acid to clear the solution, dilute to volume and mix. If the original sample is a liquid the dissolution step is not necessary. If a liquid unknown is used, it should be transferred quantitatively to a 100 mL volumetric flask and diluted to volume.

Pipet duplicate 40 ml aliquots of Ni and Co standard samples into four 100 ml volumetric flasks. Prepare 200 ml of a buffer solution which is 1M in NH_4Cl and 1M in NH_3. (This solution may be available from the stockroom). Add 10 ml of this buffer to each of the volumetric flasks. Add1.6g of the disodium salt of EDTA to each of the Ni and Co standards. Use a powder funnel to add the EDTA. Remove the stopper and warm the flasks on a hot plate for 20 min. to complete formation of the complex. Cool and dilute to volume. Prepare a blank

containing 12 ml buffer solution, and 1.6 g. of EDTA in 100 ml of solution. Determine the absorbance of one Co and one Ni standard at 20 nm intervals between 350 and 650 nm. (Use one standard for Co and one for Ni) **NOTE:** ***Each time the wavelength is changed, the zero and the*** *100% adjustments must be made.* Plot the absorbance spectra of Co and Ni. From the curves choose the suitable wavelenghts for analysis of the unknown sample solutions. Pipet 40 ml aliquots of the unknown into two 100 ml volumetric flasks. Add 3.0g of the EDTA and 10ml of the buffer to each of the unknown solutions. Dilute to volume. Measure the absorbance of each of the standards and the unknown solution at the two selected wavelengths. Make at least five sets of readings for the solutions at each of the two wavelengths.

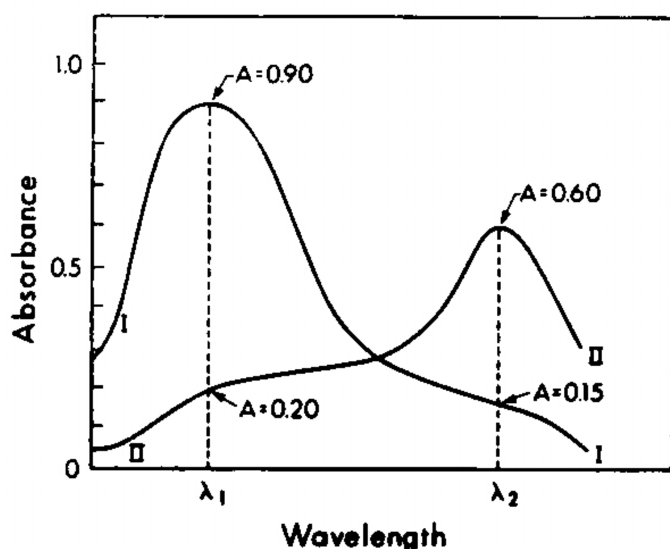


Figure 1: Spectra of two hypothetical compounds. The best wavelengths are λ_1 and λ_2, where the difference in absorbance of the compounds are greatest.

Notes:

1. You will need to solve two equations simultaneously since you have two unknowns - both metals will absorb to some extent at both wavelengths, so the absorbance you measure will be a sum at that wavelength.
2. After you finish, you may want to try using a couple of other wavelengths. You should get the same results.
3. If the absorbance is to too high, dilute the samples quantitatively until an optimum absorbance reading can be obtained.

CALCULATIONS

Wavelength (nm)	A (Co)	A (Ni)
350	__	__
370	__	__
390	__	__
410	__	__
430	__	__
450	__	__
470	__	__
490	__	__
510	__	__
530	__	__
550	__	__
570	__	__
590	__	__
610	__	__
630	__	__
650	__	__

Report:

Report the amount of Co and Ni in mg.

ABSORBANCE OF COBALT STANDARD, NICKEL STANDARD AND UNKNOWN AT λ_1

Cobalt St	(1)
Cobalt St.	(2)
Nickel St	(1)
Nickel St	(2)
Unknown	(1)
Unknown	(2)

ABSORBANCE OF COBALT STANDARD, NICKEL STANDARD AND UNKNOWN AT λ_2

Cobalt St	(1)
Cobalt St	(2)
Nickel St	(1)
Nickel St	(2)
Unknown	(1)
Unknown	(2)

EXPERIMENT 8A

DETERMINATION OF SODIUM IN AN UNKNOWN SOLUTION AND IN SNACKS BY FLAME ATOMIC ABSORPTION SPECTROMETRY (AAS).

INFORMATION AND TECHNIQUES:

1. Learn how to extract metals from complex samples.
3. Learn about instrumentation involved in atomic spectroscopy.
2. Learn how to use flame AA.

Background:

The concentration of sodium in food materials is important because of its serious effects on health conditions such as high blood pressure. Sodium can be analyzed by colorimetry, flame emission, as well as flame AAS. Flame emission has a detection limit of approximately 0.1 ppm and the detection limit for AA is around 2.0 ppm. Thus flame emission is actually more popular for measuring Na. In this experiment you will determine Na concentrations in an unknown solution and in snack foods (potato chips, pretzels, peanuts etc.) using acid digestion followed by flame AA analysis.

Flame AAS is widely used for analysis of most metals because of its simplicity, effectiveness, and relatively low cost. In flame AAS, a liquid sample is aspirated into a flame and atomized. A light beam is directed through the flame into a monochromator and detector which measures the amount of light absorbed by the atomized element in the flame. For dilute solutions (usually in the ppm level), Beer's Law is followed and the absorbance is proportional to the concentration of the element in the sample. Each element has its own characteristic absorption (line) spectra in the UV-VIS region. A hollow cathode lamp is used as a light source and is specific for the element of interest. For example, in this experiment, a Na hollow cathode lamp will be used as a source of radiation.

The characteristic wavelength for AA analysis of sodium is 589 nm. In AA, interference can come from molecular species present in the flame background and also from the lack of absorption by atoms bound to molecular species. This occurs when the flame is not hot enough to provide dissociation of molecules in the flame, or when dissociated atoms are oxidized immediately to a compound that is refractory (i.e., stable at high temperatures). An acetylene/air flame is generally used in flame AA and the typical flame temperatures are 1800^oC to 2500^oC.

Sample Preparation: The first step in the analysis of real-world samples is usually sample preparation. Unfortunately many of our analytical instruments do not allow the whole sample to be introduced for direct measurement of the analytes of interest. Instead the analyte is extracted from the sample matrix (usually in a solution form) and then introduced into the analytical instrument. Sometimes, the extraction can be quite rigorous because the analyte is bound strongly to the sample matrix. For very dilute extracts, a concentration step may also be necessary. Extraction and other intermediate steps used to transfer the analyte from a real-world sample into a form suitable for analysis are referred to as sample preparation.

The most common sample preparation procedure for metals is acid digestion. Most metals are soluble in acids and by boiling the sample in acids such as HNO_3, or HCl, the metals can be brought into solution. Nitric acid is preferred in most cases because nitrates of most metals are water-soluble. Sometimes, $HClO_4$ and H_2O_2 are added to oxidize the organics.

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Experimental Procedure

Note: Eye protection and gloves should be worn during the acid digestion procedure, and at all times in the lab.

Prepare 200 ml of dilute HNO_3 solution by a 1:1 dilution of concentrated HNO_3. Prepare 5, 10, 15 and 20 ppm Na standard solutions by diluting a 1000 ppm stock solution with deionized water. A blank sample containing the dilute HNO_3 is also prepared.

Sample Preparation: Grind and mix some of the snack food given to you. Weigh out three 0.50 g. samples into 150 or 200 ml Erlenmeyer flasks. Add 50 ml of HNO_3 solution prepared in the previous step. Heat each sample on a hot plate inside a fume hood. Bring to a boil and then let it simmer for 30 min. Cool the flasks, and transfer the solution into 100 ml volumetric flasks. Dilute to the mark with distilled water and mix thoroughly. Filter each solution through a filter paper into a clean dry 100-ml flask. If the measurements are outside the range of the standard calibration curve, then further dilution is needed.

UNKNOWN

If the unknown is a solid, follow the same procedure with triplicate samples of 0.1 g. each. If the unknown is a liquid, the absorbance can be measured directly.

AAS analysis: The operation manual for the 2380 AA Spectrometer is available in the laboratory. Follow the procedures carefully. Operations include adjustment of the radiation source (Na hollow cathode lamp), set up parameters for analysis; igniting the flame; and analytical measurements. Before every measurement adjust the 'zero' of the spectrometer with DI water. Measure the absorption of every standard and sample three times. Digits on display will fluctuate during a measurement. Choose the maximum value.

First measure the absorbance of the blank, then the standards starting from the lowest to the highest concentration. Run the blank once again before running the samples.

Treatment of Experimental Data and Calculations:

Calculate the average absorbance (A) of the standards, samples and a blank. Use your judgement or the Q-test to eliminate any gross error. If the absorbance of the blank is more than 0.02, correct your measurements by subtracting the blank. Derive the calibration curve as A=f(C). The plot should be a straight line according to Beer's law. Use regression analysis to determine the best fit. From the calibration curve (or the equation) determine the concentration of sodium in the snack food and the unknown sample.

Report:

For the unknown sample report as ppm. For the potato chips report as a percentage.

Quick Operations Guide for the 2380 AA

1. Make sure that the LAMP control is turned counterclockwise (current is OFF) and install the lamp.
2. Switch POWER on.
3. Make sure that ventilation is on.
4. Make the following control settings:

   SIGNAL ⇒ LAMP

   BG CORRECT ⇒ AA

   GAIN ⇒ fully counterclockwise.
5. Turn the LAMP control unit until the LAMP-ENERGY display shows the lamp current at 8 mA.
6. Set the SIGNAL control to SET UP.

   Set the SLIT control to 0.7.

   Adjust the COARSE ADJUST wavelength control to 589.0 nm (for sodium) (217 nm for Lead).
7. Use the gain control to adjust the maximum reading to 75. Turn the FINE ADJUST wavelength control slowly to obtain a maximum reading on the LAMP-ENERGY display.
8. Set the SIGNAL control to ABSorbance.
9. Make sure that air is opened. Open AIR on the instrument and adjust air flow at about 40-50 ml/min.
10. Open the cylinder with acetylene. Open FUEL (acetylene) and adjust fuel flow at about 20 ml/min.
11. Press FLAME to light the flame.

COLLECTION OF DATA

There are two ways to collect data.

1. The MODE switch should be set on continuous (you will observe continuous fluctuations in the absorbance readings). Press AZ (Auto Zero) to Zero the instrument while aspirating the blank. Press READ while aspirating the standard/sample and collect 3-5 readings by switching MODE to HOLD.
2. The MODE switch can be set on HOLD. Press AZ (Auto Zero) to Zero the instrument while aspirating the blank. Then, collect 3-5 absorbance readings from Readout Display by pressing READ, while aspirating standard/sample.

SHUT OFF PROCEDURE: After finishing your measurements, close acetylene first and then the air supply. Turn off current of the lamp and switch POWER off.

EXPERIMENT 8B

DETERMINATION OF LEAD IN SEDIMENTS BY FLAME ATOMIC ABSORPTION SPECTROMETRY (AAS).

INFORMATION AND TECHNIQUES:

1. Learn how to extract a metal from a strongly bound matrix.
3. Trace analysis using atomic spectroscopy.
2. Environmental application of flame AA.

Introduction

Lead ions and organolead compounds are toxic and particularly harmful to the developing nervous system of young children. Lead is widely used in automobile batteries, paints, solders, gasoline, for radiation protection and in other products. Recently, efforts have been effective in removing lead from paints, piping solders and gasoline. However, many sites in New Jersey have been impacted by lead at concentrations that may pose a risk to humans and/or the environment. The NJ DEP cleanup criteria for lead in soils are 100 ppm for residential and 600 ppm for non-residential areas.

Flame AAS is currently widely used for analysis of most metals because of its simplicity, effectiveness, and relatively low cost. In flame AAS, the liquid sample is aspirated into a flame and atomized. A light beam is directed through the flame into a monochromator and detector that measures the amount of light absorbed by the atomized element in the flame. According to Beer's Law the amount of energy at the wavelength absorbed in the flame is proportional to the concentration of the element in the sample. Each element has its own characteristic absorption wavelength in the UV-VIS spectrum and a source lamp is specific for the element of interest. For example, in this experiment, lead hollow cathode lamp is used as a source of radiation. For more details about the AAS method, before starting the lab, you should consult your textbook.

Lead has two characteristic absorption wavelengths at 217.0 and 282.3 nm. Measurement at 217.0 nm provides better sensitivity, while determination at 282.3 nm is usually less susceptible to interference. The most common interference results from the lack of absorption by

atoms bound in molecular combination in the flame. This occurs when the flame is not hot enough to provide dissociation of molecules in the flame or when dissociated atoms are oxidized immediately to a compound that would not dissociate further at the flame temperature. For atomization of lead, temperatures of 1700-1800°C are sufficient and an acetylene/air flame is generally used for analysis. Lead can usually be determined in aqueous solutions by the flame AAS method in concentrations above 0.5 ppm with the absorbance linearly related to concentration up to 20 ppm.

For determination of metals in solid samples by flame AAS, the analytes must be quantitatively transferred into solution. For this purpose, the most common procedure is an acid digestion. Depending on their nature, solid samples are treated by acids or their mixtures; frequently oxidizing agents like hydrogen peroxide are added to destroy stable organic complexes. After filtration and dilution the liquid extract is used for AAS analysis.

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Experimental Procedure

Note: Eye protection should be worn during the acid digestion procedure, and at all times in lab.

a). <u>Acid digestion.</u>

Weigh two portions of approximately 0.5 g each of a contaminated sample using an analytical balance. Weigh approximately the same quantity of a blank, which contains no detectable amount of lead (pure quartz sand). Transfer samples and the blank into three 200-400 ml beakers for acid digestion.

Add to each beaker about 10 ml of 1:1 nitric acid aqueous solution and heat on a hotplate under the hood for about 15 min at a temperature near the boiling point. Do not allow vigorous boiling, as sample can be lost by splashing. During heating, the beakers should be covered with watch glasses.

Add 5 ml of concentrated nitric acid and reflux for another 10 min near the boiling temperature. Allow the solutions to cool.

Add 2 ml of DW and three portions of 1 ml each of 30% hydrogen peroxide into each beaker. Heat the samples for about 5 min. Cool the solutions to room temperature.

Add about 20 ml of DW to each beaker and filter the samples through a funnel with a Whatman #41filter paper into three 100 ml volumetric flasks. Rinse the residue in the filter with

distilled water, adding the rinsings to the flasks. Dilute to the 100 ml volume with water. The three samples and blank are then ready for analysis.

b). Preparation of standards.

Pipet 5.00 ml of a stock solution, containing 1000 ppm of lead into a 100 ml volumetric flask, dilute with water to 100 ml and mix. An intermediate solution containing 50 ppm of lead is obtained.

Using pipets and 50 or 100 ml volumetric flasks prepare five solutions containing 1.0; 2.5; 5.0; 10.0 and 20.0 ppm of lead. Use these solution standards for AAS analysis. After preparation, these standard solutions may be transferred from volumetric flasks to any clean and dry beakers or bottles for storage.

c). AAS analysis.

A brief manual for operation of the 2380 AA Spectrometer is available in the laboratory. Follow the step by step instructions in the manual. Operations include adjustment of the source of radiation (lead hollow cathode lamp), set up parameters for analysis; igniting the flame; analytical measurements and turning off the equipment.

Before every measurement adjust the "zero" of the spectrometer with distilled water. Measure the absorption of every standard and sample three times. Digits on display will fluctuate during a measurement. Choose the maximum value. First measure absorbance of standards starting from the lowest concentration. Then measure absorbance of samples and a blank.

Treatment of Experimental Data and Calculations.

Calculate average absorbance (A) of standards, samples and blank. Use a common sense and/or a Q-test to eliminate "suspicious" data. If absorbance of a blank is more than 0.02, subtract it from each absorbance measurement of the samples.

Using data for the standards, derive a calibration plot in coordinates A=f(C). It is expected that the plot approximates a straight line. The "best" straight line from the points may be found using an appropriate computer program or calculations based on the method of least squares.

From the calibration plot or equation of a straight line determine the concentrations of lead in the two sample extracts in ppm. For diluted aqueous solutions concentration in ppm is equal to the concentration in μg/L.

For determination of lead in the sample, 100 ml of solution were used. So, mass of lead in the first volumetric flask in μg is **M=C x 100/1000**;

For determination of lead concentration in the sample C_{sam}, divide M by the weight of the sample in grams. $\mathbf{C_{sam}=M/G}$ **[μg/g] or [mg/kg]**.

Report: Carry out these calculations for each sample and find the average. Report concentrations and standard deviation. Discuss possible sources of errors and compare your results with cleanup criteria for soils in New Jersey.

EXPERIMENT 9

PART A - GAS CHROMATOGRAPHY: SEPARATION AND ANALYSIS OF A MIXTURE OF CYCLOHEXANE AND TOLUENE

INFORMATION AND TECHNIQUES:

1. Learn how to use a gas chromatograph (GC)
2. Learn about some of the practical issues involving separations
3. Qualitative and quantitative determination using a GC

GAS CHROMATOGRAPHY:

In chromatography a sample is separated into its component parts by putting the mixture into a mobile phase, which flows over or through a stationary phase. The sample components are distributed between these two phases, depending on their relative solubility or affinity for each of the phases. If the components differ in this affinity, they will be separated as they pass through the system. Materials which have a greater affinity for the stationary phase will take a longer time to pass through the system, and those which remain more in the mobile phase will exit the system more rapidly. In a gas chromatograph, the mobile phase is an inert gas, the stationary phase is a high molecular weight non-volatile liquid or a polymeric solid packed into a column. The separation depends on the vapor pressure of each component, with those of highest vapor pressure traveling through the column more rapidly.

At the end of the column, a detector is needed to determine when the components are exiting the column (eluting) and to record the quantity of each component. In this case a flame ionization detector (FID) detects organic compounds by passing them into a small hydrogen-air flame. As they burn, ions are formed in the flame, collected on an electrode, amplified and recorded. The thermal conductivity detector (TCD) measures the transfer of heat from a warm filament to the walls of the detector, and indicates the elution of compounds as the thermal conductivity of the gas stream changes with concentration. This detector can be used for inorganic gases as well as organics.

RETENTION TIME

The retention time t is the time taken for the component peak to appear after the time of injection of the sample.

OPERATING THE GAS CHROMATOGRAPH

A guide to the operating procedure for the Varian CP-3800 Gas Chromatograph is given at the end of this experiment. Normally, the teaching assistant will have set up the equipment in advance.

PROCEDURE

Study the GC instrumentation your group is going to use. Draw a neat sketch of the GC. Specify the type of detector, column etc. that are being used. Also report all the operating conditions such as flow rates, temperature etc.

The given sample contains cyclohexane and toluene. Using a microsyringe, first inject 1 μl each of the pure substances provided to you and report the retention times. This procedure will be done once and copies of the chromatograms will be given to each student. Make sure that there are no air bubbles in the syringe. Also inject the syringe into the rubber septum rapidly and release it rapidly. Prepare accurately a known mixture of the compounds by weighing one gram of each into a weighing bottle; then inject this sample. Then inject your sample of unknown composition into the GC. Based on retention time identify the unknown components. Carry out these procedures quickly, making sure to cover the weighing bottles because these compounds are volatile and vaporize rapidly and any evaporation would change the composition of the mixture, due to different volatilities. Use the peak areas to estimate the concentration of each component in the given mixture as:

Area of Std./Area of Unknown = mg of standard/mg of Unknown.

For your information a typical chromatogram obtained for the separation of diethyl ether, hexane, toluene and octane is attached.

Report: Weight percent of the components in the unknown mixture. Also label the **chromatogram** to show the individual peaks for cyclohexane and toluene in addition to any peaks due to air bubbles or impurities in the solvents.

PART B – DETERMINATION OF CAFFEINE IN A BEVERAGE USING HPLC

High pressure liquid chromatography (HPLC) uses a column filled with a fine packing, which is coated with an organic **stationary phase.** As the sample is moved through this column by a liquid **eluent**, the **mobile phase,** the various compounds move at differing rates, depending on their affinity for the stationary phase. Compounds which have a stronger attraction to the stationary phase will be retained longer and **elute** (come out of the column) later. The identities of the sample components are found by matching their **retention times** (how long it took to pass through the column) with retention times of standards under the same conditions. The sample is detected when it emerges from the sample by an ultraviolet spectrometric detector.

Your sample is a beverage which contains caffeine. Cola, tea, coffee, decaffeinated coffee are all possible samples. The sample should be prepared as it would be used. If tea, brew it in boiling water. Instant coffee can be dissolved in hot water as you would do to prepare it. Sodas should be degassed by stirring and shaking until all bubbles are gone. Gentle heating of some of the samples will also help degas it. If there is any cloudiness in the sample, it should be filtered through a syringe-tip filter. Draw several ml of sample into a 5 ml syringe, attach the filter, and inject it into the sampling valve on the HPLC.

The eluent used in this experiment is a 30/70 mixture of methanol and water. (It may be necessary to use a 40/60 mixture to alter the polarity.) The eluent will have already been filtered under vacuum to remove particles and dissolved gases before

use. Set the flow at 1.5 ml/min, and the detector wavelength at 254 nm. Note the column specifications for your report.

Using the supplied stock caffeine solution of 1000 mg/1, prepare three standard solutions containing caffeine at 100, 50 and 10 mg/l. The dilutions may be done in small test tubes, using a graduated pipet. Keep them stoppered, to avoid evaporation. Use the 30/70 methanol-water solution supplied to make all

standards and to dilute samples. For caffeinated beverages, dilute 1 ml of the sample with 4 ml of eluent, (a 5 fold dilution). For decaffeinated coffee or tea, use it directly, without dilution.

Inject 10 microliters of diluted standard or sample into the column. As you turn the injection valve, press the start button on the integrator. Run the standard first, so you know when to expect the caffeine peak. Press the stop button after the caffeine peak elutes. Identify the caffeine peak in the samples by its retention time, and read the integrated area from the integrator printout. Do some replicates as time permits, to obtain a measure of the reproducibility of the method.

Make a plot of peak area vs. concentration for the standards and find the concentration of caffeine in your sample. Do not forget to take the dilution into account. If the sample does not fall within the range of standards, either make another standard or dilute the sample more or less to bring it into the proper range.

APPENDIX I

pH Amplifier

How the pH Amplifier Works

The pH Amplifier is a circuit which allows a standard combination pH electrode (such as the Vernier Software 7120B) to be monitored by a lab interface. The pH electrode is connected to the BNC connector on one end of the box. The cable from the pH Amplifier ends in a 5-pin DIN plug for connection to a variety of lab interfaces.

The pH Amplifier does three primary tasks:

- It steps up the voltage produced by the pH electrode to a range where it can be monitored by the lab interface. A fairly common op-amp circuit is used.
- It offsets the voltage so it is always in the range of 0 to 3.5 volts. There is a potentiometer inside the pH Amplifier which allows this offset to be adjusted
- It provides the negative voltage required for the amplifier circuit. One of the Ics on the circuit board converts the +5 volt power supplied by the lab interface to-5 volts.

When a combination pH electrode is connected, the pH Amplifier will produce a voltage of 1.75 volts in a pH buffer. The voltage will increase by about 0.25 volts for every pH number decrease. The voltage will decrease by about 0.25 volts/pH number as the pH increases.

CALIBRATION

In most cases you can simply load an experiment file which is designed for pH measurement, and calibration of the pH system will be handled at the same time. For the best accuracy, you may want to calibrate your pH system. This allows for minor differences in pH electrodes and pH Amplifier circuits. To calibrate the system, you will need distilled water and two pH buffers of different pH. Calibration is done by first removing the electrodes from its storage container and rinsing it in distilled water. Place it in one of the buffer solutions (e.g., 4.0 buffer). Follow the instructions on the screen and use this pH as one calibration point. Now rinse the electrode in distilled water again and move it to the second buffer solution (e.g., 7.0 buffer). Use this pH as the second calibration point. Save this calibration for later use.

Using the pH Amplifier with your computer interface.

1. Connect the lab interface (Serial Box Interface, ULI, MPLI, or VIU) to the computer.
2. Connect the pH Amplifier to the lab interface.
3. Connect a combination pH electrode with a BNC connector to the pH Amplifier.
4. Start up your laboratory interface program. Make sure you select the correct probe with the program.
5. Load a pH calibration file using your data collection program. Calibration files for pH measurement are provided on all Vernier Software lab interface program disks.

6. Try out the system measuring the pH of some known solutions or pH buffers.

7. For the best accuracy, you may want to calibrate your pH system. Follow the calibration instructions on the screen and in the lab interfacing program manual. Additional calibration tips are included on the pH electrode information sheets. Save the new calibration as a file on the disk so that it can be used at a later time.

ELECTRODE PREPARATION

Remove the Protective soaker bottle or cap from the electrode and thoroughly rinse the electrode with distilled water. Wipe carefully with a clean lab wipe.

During shipment, air bubbles may have entered the electrode. Hold the electrode up to the light and inspect the internal chambers for air bubbles. If air is seen, carefully shake the electrode downward (like a thermometer) to dispel the air from the sensing elements inside the electrode.

Position the cover to expose the electrode reference chamber fill hole (for sealed, gel filled electrodes, disregard this operation).

For refillable designs, fill the reference chamber with the appropriate pH reference filling solution. Use the appropriate reference filling solution. Use of the incorrect reference fining solution will damage the electrode and render it inoperable.

ELECTRODE STANDARDIZATION

1. Carefully connect the pH electrode to the input connector on the pH meter. Ensure that the electrode connection is secure.
2. Place the electrode into a beaker containing pH 7.00 buffer and a stir bar. Stir at a moderate speed.
3. When the reading is stable, adjust the meter to read the value of the pH 7.00 buffer at the temperature in the laboratory.
4. Remove the electrode from the buffer. Rinse with distilled water and blot with a lab wipe.
5. Place the electrode in a beaker containing pH 4.01 buffer. Stir as before.
6. When the reading is stable, adjust the meter to read the value of the 4.01 buffer at the temperature in the laboratory.
7. Rinse the electrode with distilled water and blot with a lab wipe. Place the electrode in a beaker containing the sample. Stir. Record the pH when the reading is stable.

ELECTRODE STORAGE

Between measurements, store the pH electrode in a beaker containing pH electrode storage solution.

When storing for longer periods, store the pH electrode in the storage bottle or refit the cap which came with the electrode. Ensure that there is adequate pH electrode storage solution in the storage bottle or cap.

When taking the electrode out of service for extended periods, rinse the electrode thoroughly, wipe with a lab wipe and refit the storage bottle or cap. Maintain the level of pH electrode storage solution in the storage bottle during storage.

If the pH electrode is equipped with a fill hole cover, slip the cover over the fill hole.

. Soak the electrode in pH electrode storage solution for 30 minutes after this procedure.