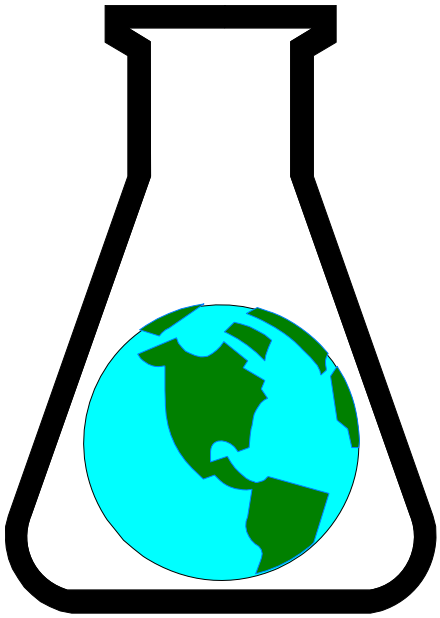
ENVIRONMENTAL ANALYSIS

LABORATORY MANUAL

CAE 345



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1

INTRODUCTION

Role of Laboratory

The role of the analytical laboratory is to provide qualitative and quantitative data to be used in decision making. To be valuable, the data must accurately describe the constituent characteristics and concentrations in the sample submitted to the laboratory. Decisions made using water and wastewater data are far reaching.

In wastewater analysis, the laboratory data define the treatment plant influent, the status of the steps in the treatment process, and the final load imposed upon the water resources. Decisions on process changes, plant modifications or even the construction of a new facility may be based upon the results of laboratory analysis. The financial pressures alone are significant reasons for extreme care in analysis.

Research investigations in environmental pollution control rest upon a firm base of laboratory data. The progress of the research and the alternate pathways available is generally evaluated on the basis of laboratory data. The value of the research effort will depend upon the validity of the laboratory results. Thus, learning to perform laboratory tests on water, wastewater, air, and solid wastes plays an important role in the environmental engineering profession.

Use of Equipment

Laboratory equipment is expensive and care should be taken when using it. This means that the equipment should be properly maintained to provide reliable experimental results. Calibration should be done regularly. It is necessary to see that all equipment being used is clean before and after use. Better results can be obtained when the equipment being used is clean, so always maintain your equipment as if it is your own.

Recording Data

In any experiment, you should make a habit of taking proper notes and recording data in the proper table form immediately after it has been taken**. Please keep a separate notebook to record your data**. Record information using waterproof pen. Surprise notebook checks will be conducted from time to time.

Laboratory Basics

- Check the summary of special sampling and handling requirements (*Standard Methods*, 1995 p. 1-22) before collecting samples. Check the MSDS for any chemical that you are not familiar with.

- Label all beakers before placing solutions in them. Do not pipette directly from the primary solution container. Place an aliquot of the solution into a labeled beaker and pipette from the beaker. This procedure will help prevent contamination.

- Wash all glassware after each experiment. Wash with laboratory grade detergent and rinse with tap water. A final rinse should be done with the class III water available at the microbiology bench in the teaching laboratory.

- To determine the volume of a stock solution needed to prepare a standard, use the following expression:

$$C_{standard} = (C_{stock}\ V_{stock})/\ V_{standard}$$

  where C represents concentration and V represents volume. Note that standards can be prepared in two ways: by sequential dilution or by carefully measuring required volumes from the stock solution. Sequential dilutions are recommended for very dilute standards. An example of each dilution method is given by the following example.

  Example: You are given a 100 mg/L stock solution of sodium chloride. A) Describe how you would prepare 100 ml of a 1 mg/L standard, using 10-fold sequential dilutions. B) Describe how you would prepare 100 ml of a 1 mg/L standard by transferring sodium chloride directly from the stock solution.

  Answer to A: Place a little more than 10 ml of stock solution into a pre-labeled beaker. Carefully transfer 10 ml of the stock solution from the beaker into a pre-labeled 100 ml volumetric flask. Bring the sample up to volume with de-ionized distilled water and mix thoroughly. Then to a second pre-labeled 100 ml volumetric flask, transfer 10 ml of solution from the first volumetric flask to the second one. Bring the sample in the second flask up to volume with de-ionized distilled water and mix thoroughly. Please note that the first volumetric flask has a sodium chloride concentration of 10 mg/L which is a 10 fold dilution of the stock. The second volumetric flask has a concentration of 1 mg/L which is a 10 fold dilution of the solution in the first flask.

  Answer to B: Place a few ml of the stock solution into a pre-labeled beaker. Carefully transfer 1 ml of stock solution from the beaker into a pre-labeled 100 ml volumetric flask. Bring the sample up to volume with de-ionized distilled water and mix thoroughly.

2

LABORATORY SAFETY

The laboratory safety manual for the University of Miami presents a series of recommendations on general safety practices that will help you work safely in your laboratory. You can obtain a copy of this manual from the internet at: http://www.miami.edu/health-safety/Laboratory_Safety_Manual.pdf A supplemental safety manual developed specifically for the Enviromental Engineering Laboratories is available at: http://www.cae.miami.edu/files/docs/Laboratory_Safety_03.pdf .

Please go over the guidelines and procedures given in the current chapter and within the resources listed above. These guidelines are intended to ensure both your safety and compliance with appropriate state, local, and federal safety and environmental guidelines. It is important that you read, understand, and follow these guidelines to ensure your safety and the safety of all who are working with you.

Never attempt to carry out an experiment without knowing the safety rules and procedures. Determine the potential hazards of all chemicals using Material Safety Data Sheets (MSDS) and any other appropriate information on chemicals, equipment, and procedures. A three-ring notebook binder with the MSDSs of the chemicals used in this course is kept in the laboratory for reference. A wall chart for "Laboratory Safety" and "How to Use and Understand Material Safety Data Sheets" are also present in the laboratory for your assistance. Your instructor can answer any questions you have on laboratory safety. Remember laboratory safety is a full time job, all day, every day.

Safety Check List:

1. No food or drinks in the laboratory.
2. No smoking in the laboratory.
3. Never work alone.
4. Use fume hood if noxious gases are involved.
5. Dilute acids in the fume hood. Always add acid to water. DO NOT add water to acid.
6. Properly dispose chemicals.
7. Lab coats, gloves, and safety goggles must be worn at all times. Avoid chemical contact on skin.
8. Use eye glasses rather than contact lenses.
9. Only enclosed shoes are to be worn in the laboratory. No sandals.
10. Keep long hair pulled back.
11. Do not pipette by mouth.
12. Keep work area clean and uncluttered.
13. Read MSDS sheets for chemicals of concern. (Located in shower cabinet in lab)

3

LABORATORY STATISTICS

Introduction

An understanding of statistics is an absolute necessity in the laboratory for determining the accuracy and precision of quantitative analytical data. Many new and specific mathematical terms will be used in the study of statistics. For example, accuracy means the correctness of a given analysis while precision means the "reproducibility" of an analytical procedure. A measure of accuracy can be obtained by analyzing a sample of known concentration and noting the deviation from the known, whereas a measure of precision is obtained my measuring a sample multiple times.

Statistical Terminology

Some additional common terms in laboratory statistics are mean, median, mode, absolute error, relative error, deviation, relative deviation, standard deviation, coefficient of variation, and confidence limits intervals. These terms are defined as follows:

A) *Mean* (y) - The technique of "taking an average" by adding the numerical values (y_1, y_2, y_3 ....etc.) of an analysis and dividing this sum by the number (n) of measurements used.

$$\bar{y} = \frac{\sum y_i}{n}$$

The experimental mean, y, is our best estimate of the true mean, :.

B) *Median* - The same data used to calculate the mean can be displayed in increasing or decreasing series. The median is defined as the "middle" value. If the total number of measurements is an even number, there will not be a single middle value; the median in this case will be the average of the two middle values.

C) *Mode* - The measurement value that appears most frequently in the series.

Statistics Used to Describe Measures of Accuracy (D&E)

D) *Absolute Error* - The difference between the true value and the measured value with the algebraic sign indicating whether the measured value is above (+) or below (-) the true value.

$$Y_i - Y_t$$

Y_i = Measured Value
Y_t = True Value

E) *Relative Error or Percent Error* - Relative error is the absolute error (difference between the true and measured value divided by the true value. It is usually expressed as a percentage (percent error).

$$\frac{(Y_i - Y_t)}{Y_t} * 100\%$$

Y_i = Measured Value
Y_t = True Value

Note the difference between relative error (no absolute value) and the deviation from the mean which contains an absolute value.

Statistics Used to Describe Measures of Precision

F) *Deviation from the mean* (dy) - How much each measured value differs from the mean.

$$dy = |\bar{y} - y_i|$$

where $\bar{y}$ = mean
dy =deviation
y_i = measured value

Deviation from the mean can also be expressed as a percent deviation.

$$\text{Percent Deviation} = \frac{|\bar{y} - y_i|}{\bar{y}} \times 100\%$$

G) *Standard Deviation* (s) - A measure of the distribution of values about its mean. The standard deviation for small data sets (n<20) can be calculated in five steps:

1. Determine the mean ($\bar{y}$)
2. Subtract the mean from each measured value
3. Square each difference
4. Sum the squared terms in step 3 and divide by "n-1"
5. Calculate the square root of the average found in step 4 by dividing by one less than the actual number of measurements

$$s_y = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \bar{y})^2}{n - 1}}$$

The value of "s" is an estimate of the true standard deviation, Φ. The variance is equal to Φ^2.

H) *Coefficient of Variation,* CV - The relative standard deviation which is calculated by dividing the standard deviation by the mean.

$$CV = \frac{\sigma \times 100\%}{\mu} \approx \frac{s \times 100\%}{\bar{x}}$$

I) *Confidence Limits*. The interval around an experimental mean within which the true result can be expected to lie with a stated probability. Confidence limits for small data sets are estimated through the following expression.

$$\text{confidence limit} = \bar{y} \pm \frac{ts}{\sqrt{n}}$$

The value of t is determined from the following table.

Degrees of Freedom	Factor for Confidence Interval, %				
	80	90	95	99	99.9
1	3.08	6.31	12.7	63.7	637
2	1.89	2.92	4.30	9.92	31.6
3	1.64	2.35	3.18	5.84	12.9
4	1.53	2.13	2.78	4.60	8.60
5	1.48	2.02	2.57	4.03	6.86
6	1.44	1.94	2.45	3.71	5.96
7	1.42	1.90	2.36	3.50	5.40
8	1.40	1.86	2.31	3.36	5.04
9	1.38	1.83	2.26	3.25	4.78
10	1.37	1.81	2.23	3.17	4.59
11	1.36	1.80	2.20	3.11	4.44
12	1.36	1.78	2.18	3.06	4.32
13	1.35	1.77	2.16	3.01	4.22
14	1.34	1.76	2.14	2.98	4.14
	1.29	1.64	1.96	2.58	3.29

Table: Values of t for Various Levels of Probability
Note that Degrees of Freedom equal "n-1"
(from Skoog and West, 1986)

EXAMPLE: Calculate the average, standard deviation, and 95% confidence limits for these four weights:

36.78 mg, 36.80 mg, 36.87 mg, and 36.94 mg

=> Mean ($\bar{y}$) = (36.78 + 36.80 + 36.87 + 36.94)/4 = 36.85 mg

=> Measurement	Deviation (y_i - $\bar{y}$)	Deviation Squared (y_i - $\bar{y}$)^2
36.78	0.07	0.0049
36.80	0.05	0.0025
36.87	0.02	0.0004
36.94	0.09	0.0081
	Total =	0.0159 mg

=>Standard Deviation (ds) = $[(0.0159)/(4-1)]^{0.5}$ = 0.07 mg

=>From table, t = 3.18 (degrees of freedom =3 and 95% confidence interval)

=>confidence limit = 36.85 ± (3.18□0.07)/($4^{0.5}$)
= 36.85 ± 0.11 mg

Fitting a Line to a Set of Data Points Using the Method of Least Squares (Establishing Calibration Lines)

Data can be plotted on a graph but not all data fall on a single straight line. One solution to this is to draw a straight line using a ruler through as many data points as possible. A better approach involves using statistics to define the "best possible fit" line fit to the data. A straight line relationship should be equal to y' = mx + b, where y' represents the dependent variable (for example concentration), x represents the independent variable (for example absorbance, NTU, peak area, peak height etc.), m is the slope of the curve, and b represents the ordinate (y-axis) intercept. Mathematically, it has been determined that the best fit line through a series of data points is that line for which the sum of the squares of the deviations of the data points from the line is minimum. This is known as the Method of Least Squares. Computation of the best-fit line by Method of Least Squares is accomplished by solving the following two equations simultaneously.

$$nb + m\,(\sum x_i) = \sum y_i$$
$$b(\sum x_i) + m\,(\sum (x_i^2)) = \sum x_i y_i$$

A measure of how well the data fit to a straight line is given by the "goodness of fit" parameter, R^2. R^2 is computed by subtracting, from unity, the ratio of the variance of the y_i data with respect to the fitted line (y') over the variance of the y_i data with respect to the average of the y's. Y_i's are the values of the standards used to establish the calibration line. **Note that standards used as "controls" are not to be used in a calibration line/curve.**

Standards
(Solutions of Known Concentration)

Controls ⟷ **Calibration Curves**

An R^2 value of 1 implies a perfect fit whereas an R^2 value less than or equal to 0 implies an extremely poor fit. The value of R^2 is computed as follows:

$$R^2 = 1 - \frac{\frac{1}{n}\sum (y_i - y_i')^2}{\frac{1}{n}\sum (y_i - \bar{y})^2}$$

Furthermore, it is important [illegible] *nt shaped curves* to available data. An example of "curve fitting" rather than "line fitting" is given in the BOD laboratory. For more information on "curve fitting" polynomials, students are referred to Chasen, 1978. For more information on "curve fitting" BOD-type curves, students are referred to Droste, 1997.

EXAMPLE: For the following three data points, determine the best fit line and the R^2 value

.

Signal x_i	Known y^a y_i

-1.1	-1.3
+0.1	+0.2
+1.2	+0.9

[a]Values From Standards

=> The best fit line is computed as follows:

$\Gamma x_i = -1.1 + 0.1 + 1.2 = 0.2$

$\Gamma y_i = -0.2$

$\Gamma(x_i^2) = (-1.1)^2 + (0.1)^2 + (1.2)^2 = 2.66$

$E(x_i\ y_i) = (-1.1x-1.3) + (0.1 \times 0.2) + (1.2 \times 0.9) = 2.53$

Substituting into the formulas above:

$$3b + 0.2\ m = -0.2$$
$$0.2\ b + 2.66\ m = 2.53$$

Solving for m and b

$$m = 0.96$$
$$b = -0.13$$

The best fit line is therefore

$$y' = 0.96\ x + -0.13$$

=> The R^2 value is computed as follows:

x_i	y_i	y_i'
-1.1	-1.3	-1.19
+0.1	+0.2	-0.03
+1.2	+0.9	1.02

$\Gamma(y_i - y')^2 = (-1.3 - -1.19)^2 + (0.2 - -.03)^2 + (0.9 - 1.02)^2 = 0.0794$

$= (-1.3 + 0.2 + 0.9)/3 = -0.0667$

$\Gamma(y_i - \ )^2 = 2.527$

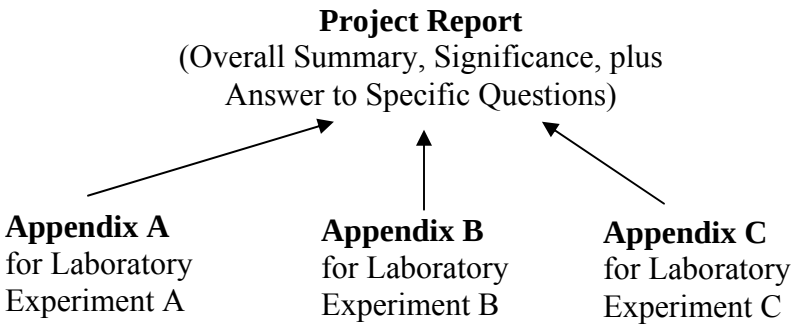
$R^2 = 1 - (0.0794/2.527) = 0.97$

Quality Control Analyses

Control samples are routinely analyzed along with environmental samples. These control samples include blanks, replicates, standards, and spikes. Blanks are used to assure that there was negligible contamination in carrying out the experimental procedure. Replicates are used to obtain a measure of the reproducibility (or precision) of the experiment. Standards are samples of known concentration which can be prepared from raw materials or purchased directly from commercial manufacturers. *Standards are used for one of two purposes: to establish calibration lines/curves OR to determine the accuracy of an experiment. Standards used to determine the accuracy of the experiment are called "controls." Control standards are NOT to be used in establishing calibration lines or curves.* Spike samples involve adding a known amount of material to a sample and measuring the increase in concentration. Spike analyses are useful for determining whether interferences exist in an analytical procedure.

4

PROJECTS AND LABORATORY EXPERIMENTS



Preparation of Project Reports

Project reports are due the week after the completion of a project. A project is a group of experiments which is centered around a particular theme. Each student is expected to write his or her own report. Only raw laboratory data can be shared among group members. Data manipulations, calculations, and laboratory write-ups must be completed independently.

The report should be neat, clearly written, and professional in its presentation. Consider yourself as an engineering consultant who is documenting analytical results for a very important client. You want to make a good impression because you want to continue business with them. So reports should be readily legible and convey the information in a well organized manner. As a minimum, the project report should include the following.

1. Cover Page - This should include the title of the experiment, the name of the individual submitting the report, the course name (CAE 345), and in the lower right-hand corner a list of the laboratory group partners.
2. Main Body of Report - The main body of the report consists of a comprehensive summary of the results of the experiments. Format as a letter to the client (e.g. Dear client, as requested we have evaluated….). The main body of the report should briefly describe the methods used, summarize the data in a form that is quickly assimilated (e.g. in tabular form or as a figure), a thorough discussion and conclusion concerning the significance of the grouped results. Some questions are included within the project list that should be answered as part of the discussion and conclusions portion of the report.
3. Appendices - One appendix corresponds to each weekly experiment. Appendix sheets are provided at the end of the lab manual and electronic versions are available on the class web site (http://cae.miami.edu/~hmsolo/345/cae345.html). Appendices are designed to convey the details from each experimental procedure. Each appendix should have the following components.

   3a. Data and Data Analysis- This section should include data tables, sample calculations, and required graphs. See appendix sheets. Please remember that a description of the samples analyzed should be included within this section. The description should include the date of sample collection and analysis. Report all data in significant figures. Table columns should be clearly identified and units should be included. These should be prepared as neatly as possible. Graphs should be large and properly labeled. An answer must be provided to each question included in the laboratory procedure. This section should be organized and easy to follow. For experiments where the data are obviously incorrect, try to manipulate the data in an attempt to salvage some results. When such manipulations are necessary, the experiment should be repeated; however the exercise is helpful in evaluating where the experimental error occurred and the fuzzy results can help in better planning future experiments.

   3b. Discussion and Conclusion - This section should include a discussion of the results of the project, a discussion concerning data quality, and a list of possible errors that could have occurred during the experiment. An answer must be provided to each question included in the laboratory procedure.

Do not wait until all experiments are completed to begin writing the final report. It is strongly recommended that appendices be prepared immediately after completion of each weekly laboratory experiment.

PROJECT NUMBER 1
Removal of Metals Utilizing the Brita™ Filtration System

Laboratory Procedure: AA Analysis of Metals in Water

Project Report

Your client has hired you to determine whether the Brita™ filtration system is capable of removing copper from water. Your final report should document the performance of the Brita™ filtration system and state whether it perform as well as claimed. When discussing the performance of the filter, the filter media should be described along with a discussion concerning what types of contaminants such media are expected to remove.

EXPERIMENT #1: METALS ANALYSIS USING ATOMIC ABSORPTION

PURPOSE:

This experiment will demonstrate the use of atomic absorption spectrometer to find the concentration of metals in aqueous solutions.

INTRODUCTION:

Atomic absorption spectroscopy is a technique based on the absorption of radiated energy by atoms. The process involves the atomization of a liquid or solid by use of a flame or furnace. Atomization is the process by which the molecules in the liquid or solid are broken to form individual atoms or ions. These atoms (or ions) are capable of absorbing energy when in contact with a beam of electromagnetic energy. The concentration of an element in the original sample is proportional to the amount energy absorbed. The atomic absorption spectrometer measures the amount of radiation absorbed when the atom goes from its ground state to the its excited state. Many different metals can be measured using atomic absorption spectroscopy. Since different metals absorb energy at different wavelengths, analysis for a specific metal will require a source (usually a hollow cathode lamp) which emits radiation at a wavelength characteristic of the metal of interest.

APPARATUS:

- Perkin Elmer Atomic Absorption Spectrometer, AAnalyst 800
- Hollow Cathode Lamp for Cu
- Brita® Filter with Filtration Apparatus
- 100 ml and 500 ml Volumetric Flasks
- Pipettes and pipette bulbs

REAGENTS:

- Copper standard (1000 ppm)
- 1:1 Nitric Acid, HNO_3

GASES:

- Air and acetylene

PROCEDURE:

Sample Preparation

All samples will be prepared in 1% HNO_3 prior to analysis on the AA. Note that acid provided is 1:1 HNO_3. (1:1 HNO_3 is 50% concentrated HNO_3 and 50% deionized distilled water). All samples will be prepared in 100 ml volumetric flasks except the Copper control for the Brita® filter which will be prepared in a 500 ml volumetric flask. Standards should be diluted "sequentially." Samples are as follows.

1) Blank, deionized water in 1% HNO_3.
2) Standards: 1, 4, 5, 10 and 20 mg/L Cu in 1% HNO_3.
3) Tests on Brita® filter. Prepare 500 ml of a 6 mg/L Cu standard *without acid addition*. Open the "new" Brita® filter and pass about 250 ml of deionized water through to wet the filter. Discard the effluent. Pass approximately 200 ml of the Cu standard through the filter. Acidify the Cu influent standard and the effluent from the Brita® filter in a 100 ml volumetric flask to 1% HNO_3.

Distribution of Work for Sample Preparation:

Group 1: Prepare the 20, 5.0, and 1.0 mg/L Cu standards. Prepare Brita® influent control.Complete Brita® filter tests and provide a sample of the influent control and effluent sample for analysis on AA (Replicate 1).

Group 2: Prepare the 10, and 4.0 mg/L standard and the blank. Prepare Brita® influent standard. Complete Brita® filter tests and provide a sample of the influent standard and effluent sample for analysis on AA (Replicate 2).

Start-up of the Atomic Absorption Spectrometer with Flame Atomization

1. **Cleaning**
   - Clean work area by removing unnecessary clutter and wiping off dust with a wet paper towel.
   - Wipe optics (softly) with Kimwipe.
   - Wipe the burner head to remove any loose deposits.
2. **Safety Check**
   - Check that shield is installed properly
   - Check that ventilation is operating
   - Warning: strong magnetic field in instrument once turned on. This may represent a health hazard for those with pace makers or other metallic implants.
3. **Gases**
   - Open air and acetylene tanks (counter clockwise is open). The acetylene tank is the short-wide tank and the air tank is the tall one next to the acetylene tank.
   - Check that the outlet gauge pressures (psig) are within the proper range (See the below table).

Gas	Outlet Gauge Pressure		Tank Pressure
	Pmin	Pmax	Pmin
Acetylene	13.5	14.5	85.0
Air	65.0	72.5	
Nitrous Oxide	65.0	72.5	

3. **Power on AA**
    - Place lamps within AA (maximum 4 lamps at one time). Wipe (softly) the end of the lamp with a Kimwipe to remove dust and fingerprints.
    - Make sure that lamps are pushed fully in.
    - Turn on AA (green switch on bottom left of instrument).
4. **Power on PC, Computer, and Printer**
    - Make sure that there is paper in the printer.
    - Hit "return" on first pop_up screen which requests a password. (The pop-up window does not show if computer is already on).
    - Double-click Winlab 32 for AA and wait for initialization
    - Click "close" on tip of the day window. (This does not always pop-up).
5. **Lamps**
    - Click the lamp button on the main toolbar.
    - Check the wavelength and slit of the lamp you want to use. (for Cu use a wavelength of 324.8 and 0.7H slit)
    - Click the lamp button ON.
    - Click the button for "Lamp 1" assuming that Cu is listed under Lamp 1.
    - Click Set Midscale (wait a few seconds)
    - Background Corrector (Not necessary because Cu does not require this because analysis uses a wavelength greater than 250 nm)
    - Close Lamp Window.
6. **Method**

Decide which method you want to use, new or old.

a. If you want to use *old* method,
   i. Click the Method Button on the main menu. Browse the file you want to use

b. If you want to establish *new* method, go [File _ New _ Method on main menu]
   i. In the New Method Window, Under starting conditions Select element: Cu and hit OK
   ii. Click the Spectrometer tab and specify that you wish to analyze for Cu. Use the default values for Cu (wavelength of 324.8 and a slit width of 0.7H, Signal type AA and measurement of time average)
   iii. Click on the Settings tab on right of window and provide for 3 replicates for each sample
   iv. Click the Calibration tab at the bottom of the window. Select: nonlinear through zero
   v. Click the Standard Concentration tab on right of window. Input calibration ID and concentrations (Provide concentrations values within the sample ID. This will make it easier to follow the standards during calibration). Note our blank is a calibration blank. Shaded cells do not require input.
   vi. Click the Options tab. Make sure that there is a check mark in the summary calibration and calibration curve boxes and in the analysis list box.
   vii. Click File _ Save _ Method on the main menu.
   viii. Type name for file and click OK

7. **Sample Information**
    - Establish sample information, go [File _ New _ Sample Info File]
    - Click OK for default on the pop up window
    - Enter the Batch ID –> CAE345_Fall_2003
    - Input the Sample Ids (include a "known" solution at the beginning and analyze again every 10 samples at most)
    - Click [File _ Save _ Sample Info File]. Type file name and click OK.
    - Check that the Method Name and Sample Info File Name appear on toolbar.
8. **Auto Zero AA**
    - Click Cont button on the main toolbar to open the continuous graphics window. Click autozero graph when signal appears. Close this window.
9. **Analysis**
    - Click the Flame button on the main toolbar
    - Click the Manual button on the main toolbar. Click open to set "Results Date Set Name". Input name. Input description. Click OK.
    - Click the Results button on the main toolbar.
    - Click the Calibration button on the main toolbar.
    - Re_arrange the windows on the screen.
    - Place the nebulizer inlet in deionized water.
    - Turn on flame using the Flame window.
    - Begin Analysis
        i. Carefully wipe capillary tube quickly and put in end of capillary tube in blank standard solution (the one we prepared for this class).
        ii. Analyze blank by clicking the appropriate button in the manual analysis control window. Wipe capillary tube and put tube inlet in deionized water between analyses.
        iii. Analyze standards by clicking the appropriate button
        iv. Analyze samples by clicking the appropriate button
        v. Run with D.I. water for a while to clean up nebulizer
        vi. Run sample blank (1% nitric acid) for a few minutes to clean up nebulizer
        vi. Make sure that your results printed out. Click File _ Print _ New Page to get the last page to print out. The last page should also print out when you close the "Manual" window.
10. **Shutdown**
    a. Turn off flame from the flame control window
    b. Click lamps button from main toolbar and turn off lamps
    c. Close gases (acetylene and air) and record the tank pressure readings (right gauge)
    d. Bleed gas by clicking the Bleed Gas button on the flame control window.
    e. Check that gages are showing zero
    f. Write remaining tank pressure in log book.
    g. Close Winlab 32
    h. Power off AA
    i. Shutdown computer and monitor
    j Turn off fan

NOTES for Instructors. If there are problems you may wish to check the following.

If the AA is set up for Graphite Furnace

- Once you enter Winlab 32, click [File_Change Method_Flame].

Burner Head Alignment

- Click the Flame button on the tool bar to open Flame Control
  - Click the "Align Burner" button on the Flame window
  - Select "Automatically align the burner". Click next.
  - Click "adjustment" to adjust vertical position and then click "OK"
  - Click "next"
  - Put end of inlet capillary tube in DI water.
  - Turn on flame by clicking the on the switch in the Flame window.
  - Aspirate the sensitivity check standard (Use 4 mg/L for Cu. This standard should provide an absorbance of 0.20).
  - Click "adjustment" to set the horizontal adjustment.
  - Click Ok. Click Finish.

Nebulizer Flow Rate

- Open the Continuous Graphics window by clicking Cont button on the tool bar
- Open the Flame Control window
  - Ignite the flame
  - Aspirate the DI water and click "Auto Zero Graph"
  - Aspirate the sensitivity check standard (Should be at 0.20)
- If absorbance of sensitivity check is less than 0.20, unlock the locking ring on the nebulizer.
  - Turn the adjustment knob counterclockwise and clockwise until the maximum absorbance is obtained and record the maximum absorbance
  - Turn off the flame
- Calculate the characteristic concentration
  - Open the Characteristic Concentrations window by going [Analysis _ Characteristic Concentrations]
    - Input the sensitivity check standard as **Sample Concentration** (mg/L)
    - Input the recorded maximum absorbance as **Instrument Reading (A)**
    - Click the sample concentration column
  - Compare the measured characteristic concentration and the comparison characteristic concentration.
    - Those values should be within 20%

Cleaning Burner

- Make sure that the burner is not hot.
- Open the end cap of the burner system
- Wipe the openings and the inside

- Remove the burner head
- Wash the head using soap and rinse it using deionized water
- Pour deionized water into the burner unit from the top opening
- Dry the burner head and re-attach it after it dries.

Suspect Matrix Interference Problems

- Run a Spike sample and check recovery.

Other Information

- Use background correction for elements with: an analytical wavelength below 250 nm, high dissolved solids, low absorbance, or extremely low concentrations.

Other Reference Materials

- Recommended conditions are provided in the "cook book" (Perkin Elmir Analytical Methods for Atomic Absorption Spectrometery)
- Winlab32 Offline: AA should not be running when accessing the offline mode. Click [Tool_Recommended Conditions]. Specify the element you wish to analyze. Record the wavelength, slit width, relative noise, characteristic concentration, and sensitivity check (= characteristic concentration check).

PROJECT NUMBER 2
Analysis of Wastewater

Laboratory Procedure: BOD Analysis
COD Analysis

Project Report

Your client would like to update the wastewater analysis laboratory at the treatment plant and wishes to determine whether the laboratory should be equipped for BOD only, COD only, or both. You are to recommend the analytical capabilities that should be included and explain why. In the report to the client you are expected to compare the results of the BOD and COD analysis for the wastewater sample(s) analyzed. Discuss why the analyses provide similar or different results. Also your report should comment on the efficiency of the wastewater treatment plant at removing BOD/COD from the wastewater. To simplify your comparison use the 5-day BOD data for comparing influent to effluent BOD concentrations.

EXPERIMENT #2: BIOCHEMICAL OXYGEN DEMAND (BOD)

PURPOSE:

To determine the amount of oxygen necessary for biological oxidation of wastewater, effluents, and polluted waters. To determine the amount of oxygen required by bacteria while stabilizing decomposable organic matter.

INTRODUCTION:

Biochemical oxygen demand (BOD) is the amount of oxygen required by microorganisms to biologically degrade organic wastes. Usually the BOD test is used to measure the strength of organic pollution. One of the major factors in determining the performance of wastewater treatment plants is the BOD reduction that they achieve. Complete stabilization of a waste by microorganisms requires too long an incubation period for practical purposes; therefore, the 5-day period has been accepted as a standard. The 5-day BOD (BOD_5) is the total amount of oxygen consumed by microorganisms during the first 5 days of biodegradation. Samples are incubated at 20^oC in darkness. This prevents algae from adding oxygen to the air tight bottle. The typical composition of untreated domestic wastewater has a BOD_5 concentration of 100 - 300 mg/L.

The experimental procedure is based upon diluting the wastewater with a known amount of dilution water and measuring dissolved oxygen concentrations over the course of 5 days. One set of experiments are conducted on the dilution water only and another set is conducted on the mixture of dilution water and wastewater. The BOD of the dilution water at any time t, $BOD_{D,t}$, is given by the following expression.

$$BOD_{D,t} = DO_{D,0} - DO_{D,t}$$

where $DO_{D,0}$ is equal to the dissolved oxygen concentration (mg/L) of the dilution water at time t = 0 and $DO_{D,t}$ is the dissolved oxygen concentration (mg/L) of the dilution water at time t = t. A similar expression can be developed for the BOD of the mixture of dilution water and wastewater at any time t, $BOD_{M,t}$.

$$BOD_{M,t,} = DO_{M,0} - DO_{M,t}$$

Mass balance considerations are then used to determine the BOD of the wastewater from the BOD of the mixture. Within the BOD bottle the "mass of BOD within the bottle" is equal to the sum of the "mass BOD from the wastewater" plus the "mass BOD from the dilution water." In other words, the expression can be written as follows.

$$BOD_{M,t}\, V_M = BOD_{W,t}V_W + BOD_{D,t}V_W$$

where: V_D = Volume of Dilution Water
V_w = Volume of Wastewater
V_M= Vol. of Dilution water plus wastewater, equals the volume of the BOD bottle.
$BOD_{w,t}$ = BOD of the wastewater at time t (mass/volume)

The above equation can be re-arranged and solved for the BOD of the wastewater at time t.

$$BOD_{W,t} = \frac{BOD_{M,t}V_M - BOD_{D,t}V_D}{V_W}$$

In many situations the data are fit to a BOD equation which provides BOD concentration of the wastewater as a function of time. This equation can be derived by assuming that the rate of oxygen utilization is linearly related to the amount of oxygen-consuming organic matter present in a sample or,

$$\frac{dL_t}{dt} = -k_e L_t$$

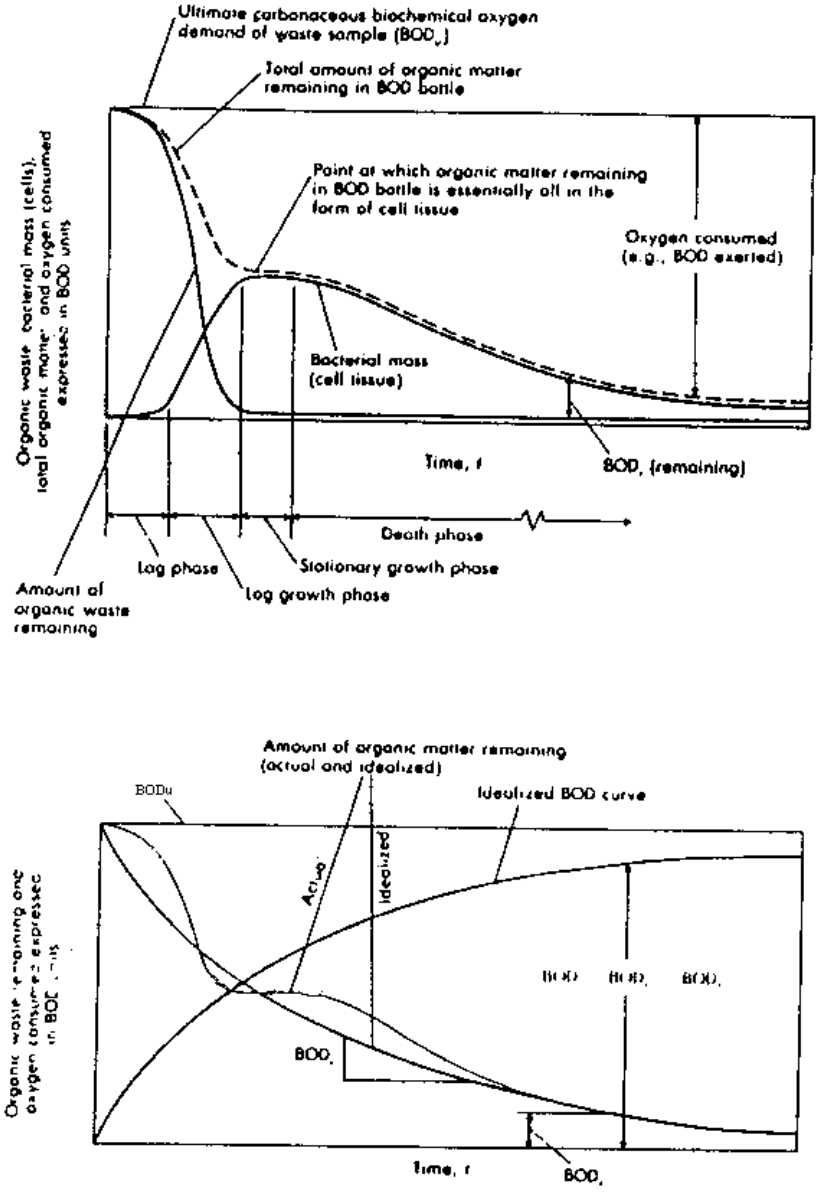
where: L_t = Oxygen equivalent of organic matter present at time t. (mass/volume)
k_e = Reaction constant (inverse time). Please note that this constant is a function of temperature and therefore, constant temperature conditions are needed throughout the experiment.

Integrating the above expression, then

$$BOD_t = BOD_\infty(1 - e^{-k_e t})$$

where: BOD_ = ultimate BOD (mass/volume). The expression above requires the computation of BOD_ and k_e from experimental data. The results of the 5 day BOD test can be used in conjunction with one of two solution methods for computing BOD_ and k_e . These methods include: 1) the method of least squares, and 2) the Thomas method.

Figure A: Functional Analysis of the BOD Test, (a) Interrelationship of Organic Waste, bacterial mass (cell tissue), total organic waste, and oxygen consumed in BOD test; (b) Idealized epresentation of the BOD test.



APPARATUS:

- BOD bottles; 300 ml capacity (20 per student group)
- Air Incubator (20°C ± 1°C)
- Stir plate, stir bar, ring stand, burette, 200 ml beaker, and burette holder
- 250 ml Graduated Cylinder
- Containment vessel, baking soda, wash beaker, solution beaker, and pipette for handling concentrated sulfuric acid.
- Large 40 liter carboy, with diffuser stone attached to a source of pressurized air

REAGENTS:

- Concentrated Sulfuric Acid, H_2SO_4: **Caution, may cause severe burns. Please note safety precautions when handling**. Maintain the concentrated acid within a labeled beaker set inside a secondary containment vessel inside the fume hood. Keep baking soda nearby in case of spill. Also keep a labeled wash beaker and distilled water bottle next to the acid for pipette washing. Rinse the pipette with distilled water prior to setting it down. Keep the pipette inside the hood for use by all student groups.
- Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4.7H_2O$, and 1.7 g NH_4Cl in approximately 500 ml distilled water and dilute to 1 liter. The pH of this buffer should be 7.2.
- Magnesium sulfate solution: Dissolve 22.5 g $MgSO_4 \bullet 7H_2O$ in distilled water and dilute to 1 liter.
- Calcium chloride solution: Dissolve 27.5 g $CaC1_2$ in distilled water and dilute to 1 liter.
- Ferric chloride solution: Dissolve 0.25 g $FeCl_3 \bullet 6H_2O$ or 0.225 g $FeCl_3$ in distilled water and dilute to 1 liter.
- Glucose-glutamic acid standard: Dissolve 150 mg each of reagent grade glucose and reagent grade glutmatic acid (dried at 103 °C for 1 hr) in distilled water and dilute to 1 liter.
- Settled sewage seed, collect from mixed liquor tank: Store settled domestic sewage for 48 hr in a 20 °C incubator. Sewage can be obtained from the Central District Wastewater Treatment Plant, (786)558-4208, the contact person is Evan. If the Central District Wastewater Plant is unable to provide access, then samples can be obtained from the South District Wastewater Plant (8950 SW 232 Street) by contacting Clive Powell (305-989-3399). The number for the chemist at the South Plant is 305-258-8508.
  Also collect environmental wastewater samples while at the wastewater treatment plant.
- Dilution water: Place the desired volume of 20 °C distilled water in a suitable bottle and add 1 ml each of phosphate buffer, magnesium sulfate, calcium chloride, and ferric solution for each liter of water. Aerate or shake to saturate with DO. Just prior to use, add 1ml seed for every L of dilution water. (Approximately10 liters of dilution water needed per student group.) Be sure to utilize <u>settled</u> sewage seed (mixed liquor from aeration tank). Do not shake the sample bottle prior to adding sewage seed to dilution water.
- Manganous sulfate solution: Dissolve 480 g $MnSO_4 \bullet 4H_2O$ or 400 g $MnSO_4 \bullet 2H_2O$, or 364 g $MnSO_4 \bullet H_2O$ in distilled water, filter, and dilute to 1 liter.
- Alkali-iodide-azide reagent: Dissolve 500 g NaOH, and 135 g NaI, in distilled water and

dilute to 1 liter. To this solution add 10 g NaN_3, dissolved in 40 ml distilled water.

- Sulfuric acid, approx 3.6N: Dilute 1 volume of concentrated H_2SO_4 with 9 volumes of distilled water. Remember to add acid to water - not water to acid.
- Starch solution: Heat up water to dissolved soluble starch reagent. Add enough starch until solution appears a clear milky color.
- Distilled water
- Stock sodium thiosulfate solution, 0.10 N: Dissolve 24.82 g $Na_2S_20_3 \bullet 5H_2O$ or 15.82 g $Na_2S_2O_3$ in distilled water and dilute to 1 liter.
- Standard potassium biniodate solution, 0.025 N: Dissolve exactly 0.8124 g $KH((IO_3)_2$ in distilled water and dilute to 1 liter.
- Standard sodium thiosulfate titrant, 0.025 N: Dilute 250 ml sodium thiosulfate stock solution to 1 liter. Standardize with standard $KH(IO_3)_2$ solution by dissolving approximately 2 g KI in 100 to 150 distilled water; add 10 ml of 3.6N H_2SO_4 followed by 20 ml of 0.025 N potassium biniodate ($KH(IO_3)_2$) solution. Dilute to 200 ml and tritrate with thiosulfate, adding starch toward the end of the titration when a pale straw color is reached (To be performed by the T/A).

SAMPLES:

Glucose-Glutamic Acid (control).
Wastewater: Collect one sample from inlet and another from the outlet of the WWTP.

PROCEDURE:

1. a) Using your best engineering judgment, determine the dilution for the glucose-glutamic acid control (BOD_5= 198 mg/L) and for the raw and treated wastewater samples. Suggestions: read section 23-3 in Sawyer, et al., note table 23-1.
   b) Check proposed dilutions with instructor before proceeding.
2. a) Prepare four dilution water blanks by filling each bottle with the dilution water/seed mixture.
   b) Prepare four raw wastewater samples. Add the appropriate volumes of raw effluent, X1 ml, as determined in step 1. Add (300-X1) ml of dilution water. Make sure you shake the sample bottle thoroughly prior to pipetting sample. Samples for this lab can be pipetted directly from the sample bottle to assure that uniform samples are pipetted. Shake bottle between additions of raw wastewater to different BOD bottles.
   c) Prepare four treated wastewater samples as in part b with appropriate volumes of treated wastewater, X2, and dilution water (300-X2) as determined in step 1. Make sure you shake the sample bottle thoroughly prior to pipetting sample as in part b above.
   d) Prepare eight glucose-glutamic acid samples as in part b, with appropriate volumes of glucose-glutamic acid,X3, and dilution water (300-X3) as determined in step 1.
3. Add water to the reservoir at top of BOD bottle. Refill during incubation period to avoid total evaporation of water.
4. Determine the DO (day = 0) on one bottle of raw wastewater, treated wastewater, and dilution water blank, and two bottles of the glucose-glutamic acid control. (See DO determination below)
5. Put remaining samples in incubator (20^oC).
6. Repeat step three for days 3, 4, and 5.

DO ANALYSIS - Winker Titration, Azide Modification (Modified from Jenkins et al. 1980)

To a 300 ml BOD bottle full of sample, add 2 ml $MnSO_4$ solution followed by 2 ml alkali-iodide-azide reagent; stopper and exlude air bubbles; mix by inverting the bottle several times. When the floc has settled to approximately 1/2 the height of the BOD bottle, remove the stopper and add 2 ml of concentrated H_2SO_4 (located in the hood), restopper, and mix until dissolution is complete. Pour 203 ml sample measured with a graduated cylinder into a beaker and titrate with thiosulfate solution to a pale straw color. (The 203 ml volume is equivalent to 200 ml of original sample because dilution by reagents.) Add a few drops of starch solution and continue the titration to the first disappearance of the blue color. Record the volume of thiosulfate solution used.

EXPERIMENT #3: CHEMICAL OXYGEN DEMAND (COD)

PURPOSE:

To estimate the oxygen demand of organic matter when it is subjected to oxidation by a strong chemical oxidant in water.

INTRODUCTION:

The Chemical Oxygen Demand (COD) is the amount of oxygen needed to chemically oxidize wastes. In the COD test, a strong chemical oxidizing agent is used to oxidized the organics. The primary advantage if COD over BOD is that it is relatively fast, taking 2 to 3 hours, whereas BOD requires 5 day to complete. Another difference in the test methods is that BOD is a biochemical process as measured by the ability of mircrobes to degrade the organics, whereas COD is purely a chemical process.

APPARATUS:

- COD reactor with cover and test tube rack
- Spectrophotometer (Milton Roy, Spec 20) with red plastic filter and phototube for reading within the 600 to 620nm range (CE-A30)
- Heat Resistant Gloves
- 1, 25 or 50 ml graduated cylinder
- Pipettes (5 & 10 ml) and pipette bulbs
- 100 ml volumetric flasks (4 per student group)
- 50, 100, or 150 ml beakers (3 per student group)

REAGENTS:

- HACH COD Ampules, Range 0 to 1500 ppm
- Stock Solution of Potassium Hydrogen Phthalate, $C_8H_5O_4K$ (2500mg/L as KHP): Dry approximately 3.5g $C_8H_5O_4K$ at 120° C overnight. Dissolve 2.500g in water and dilute to 1 liter.

PROCEDURE:

Samples include: one blank, three standards for calibration curve (500mg/L, 250mg/L, 62.5 mg/L potassium hydrogen phthalate, KHP), control (125 mg/l KHP, do not use for calibration curve), and two environmental samples (wastewater before and after treat treatment). Replicate the environmental sample to obtain a measure of precision. Prepare the standards (500, 250; 62.5mg/l) using sequential dilutions. Prepare the 125 mg/L control directly from the 2500 mg/L stock solution.

DIGESTION STEP (Follow Safety Instructions Carefully)

1. Turn on the COD reactor to preheat to 150°C. Safety precaution should be taken for the next process. Wear goggles, gloves, and apron. Wrap the COD Digestion Reagent Vial in a towel in case of breakage. Cautiously remove the cap.
2. Carefully pipette 2.5 ml of sample into the vial. Spilled reagent will affect test accuracy and is hazardous to skin and other materials.
3. Make sure that caps are well-tightened. Over the sink, thoroughly mix the contents in the vial that is still wrap in a towel. WARNING: Vial will become very hot during the mixing and could break easily. The plastic shield should be in place on the heater block before vials are placed in the reactor.
4. Prepare a reagent blank by repeating steps 1-3, replacing the sample with distilled (demineralized) water. For two hours, heat the vials at 150°C and then cool for 20 minutes.

COLORIMETRIC MEASUREMENT

1. With the spectrophotometer disconnected form the power source, insert red plastic filter into spectrophotometer and check that the proper lamp is placed inside (CE-A20). Turn on the spectrophotometer and allow it to warm-up for 20 minutes. Adjust wavelength (600 to 620 nm). Adjust 0% T with left knob while cuvette chamber is empty. Adjust 100% T with right knob with the blank sample from the digestion step. **(Handle ampules carefully. Do not shake)**
2. Record Absorbance and Transmittance of samples.

PROJECT NUMBER 3
Volatile Organic Compounds

Laboratory Procedure:

- Analysis of Volatile Organic Compounds in Liquid Samples Using Gas Chromatography.
- Analysis of Volatile Organic Compounds in Gaseous Samples Using Gas Chromatography

This project consists of two parts: one focuses on the analysis of VOCs in water samples and the other focuses on a demonstration lab that illustrates the analysis of VOCs from air. The analytical laboratory method for VOCs is very similar for both liquid and gaseous samples. The primary difference is in how the samples are processed. Project #3 is to include a project summary report and two appendices. One appendix corresponds to the VOC – liquid lab and another appendix corresponds to the VOC - gases lab. Below is a summary of the minimum information that should be contained in the project summary report.

Project Summary Report

Your client owns a laboratory that has the capability to analyze for VOCs in water. Your client wishes to expand his/her analytical capabilities to measure VOCs in gaseous samples. The project summary for project #3 is to provide a set of recommendations to the client by which he/she can upgrade his/her laboratory facilities. Specifically, you are to mention the potential differences in instrumentation and sample processing between liquid and gaseous samples.

EXPERIMENT # 4: VOLATILE ORGANICS IN *LIQUID* SAMPLES USING GAS CHROMATOGRAPHY (GC)

PURPOSE:

In this experiment, the student will use a gas chromatograph (GC) to analyze volatile compounds in water.

INTRODUCTION:

Gas Chromatographic (GC) methods are highly microanalytical procedures. In gas chromatography, a mobile phase (a carrier gas) and a stationary phase (column packing or capillary column coating) are used to separate individual compounds. The carrier gas is nitrogen or helium. In a packed column, the stationary phase is a liquid coating on an inert granular solid called the column packing which is placed in a borosilicate glass tube. In a capillary column, the stationary phase is a polymer coating on to the inner wall of the fused silica. The column is installed in an oven with the inlet attached to a heated injector block and outlet attached to a detector.

When the sample solution is introduced into the column, the organic compounds are vaporized and carried through the column by the carrier gas. These are then analyzed by various detectors that are specific to the particular analyte. The commonly used detectors are flame ionization detector (FID), electron capture detector (ECD), photoionization detector (PID) and the electrolytic conductivity detector (ELCD) or Hall detector.

Using these detectors, compounds can be analyzed both qualitatively and quantitatively. For quantitative analysis, a calibration curve is constructed which is linear when the concentration of standard is plotted versus the area counts under the curve or peak height. From this calibration curve, then the unknown concentration can be determined.

APPARATUS:

- A Hewlett-Packard 5890 Series II Gas Chromatograph equipped with an electron capture dectector (ECD). Fitted with: 1) a Megabore capillary column DB-608 (30 m length, 0.53 mm internal diameter, 0.83 m film thickness) from J&W Scientific Co., and 2) an IBM compatible computer with ELAB chromatography software to process the data.
- Syringes, 1 cc and 5 cc
- 40 ml I-Chem bottles
- Shaker
- Beakers
- 2 ml amber bottles with teflon septa

REAGENTS:

- Methanol
- Pentane
- Stock Solution for Standards Preparation (See Instructor)

GASES:

- Nitrogen
- Helium

PROCEDURE:

Sample Preparation

Stock solutions (100 ppm) are provided for 1,2 dichloroethane (DCE); 1,1,2 trichloroethene (TCE), and chloroform ($CHCl_3$). Each of these solutions were prepared in methanol (MeOH).

Samples:

Combined Standards: Prepare a combined standard of 1000 ppb and another combined standard of 250 ppb. The 1000 ppb combined standard has 1000 ppb of DCE, 1000 ppb of TCE, and 1000 ppb of $CHCl_3$. The 250 ppb combined standard has 250 ppb of DCE, 250 ppb of TCE, and 250 ppb of $CHCl_3$. These standards will be used for your calibration curve.

Control Samples: Prepare one sample with 500 ppb chloroform and 500 ppb DCE; Prepare another sample with 500 ppb of DCE and 500 ppb of TCE. These samples will be used to determine which peak corresponds to chloroform versus DCE versus TCE and will be used to determine the accuracy of the calibration curve. Since 500 ppb DCE standard will be run twice, then these analyses can also be used to determine the reproducibility of the results.

Tap Water and Raw Untreated Water: Process the laboratory tap water and raw untreated water for GC analysis. Use the results from the calibration curve to determine the concentrations of $CHCl_3$, TCE, and DCE in these samples.

Blank Sample: Teaching Assistant will prepare the blank sample during demonstration. This sample was prepared in the same way as the others except, deionized distilled water was used rather than the solutions of chloroform, DCE, or TCE.

Separation of work for sample preparation:

T/A:	Will prepare the blank as part of a class demonstration of the procedure
Group 1 prepare:	the 2 control samples, and 1000 ppb combined standard
Group 2 prepare:	UM tap water, untreated groundwater and 250 ppb combined standard.

Sample Preparation: Skip step "a" for the blank, tap water, and untreated water samples.

a. Place 20 mL of distilled water into an I-Chem bottle and keep uncovered. Remove volume of water that will be added to prepare the standards. Remove calculated amounts of stock solution with a 1 cc syringe by inserting the syringe tip into the stock solution and extracting the required volume of stock solution. Transfer the stock solution to the I-chem bottle and cover bottle. Mix the sample by inverting the bottle a few times.
b. This step is performed in the hood. Take top off I-Chem bottle.Using a 5 cc syringe, add 4 ml of clean pentane to each sample. Close I-Chem bottle.
c. Shake the samples by inverting a few times and by placing them on the Gyratory shaker (300 rpm). Shake for about 1 minute. Remove the sample from the shaker, and place in the hood. Let the samples sit for a few minutes to allow the pentane and aqueous fractions to separate.
d. This step is performed in the hood. Using a 1 cc syringe, remove the pentane layer (approx. 1

ml) and place in small 2 ml sample vials for analysis. The covers to the I-Chem bottle and 2 ml bottle can be removed during the transfer. Use a new 1 cc syringe for each sample.

e. Analyze samples using the gas chromatograph.

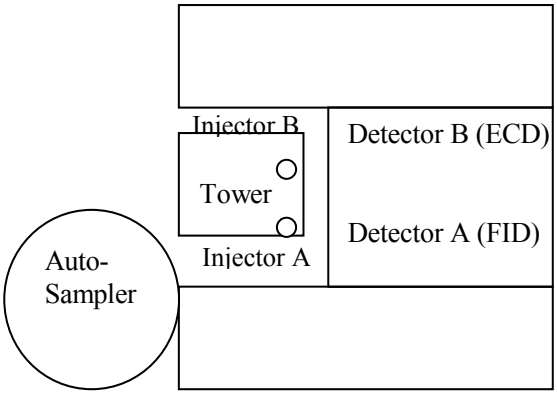
Maintenance and Operation of Gas Chromatograph

1. Check Gases

N_2 gas, 25 psi outlet pressure
He gas, 40psi outlet pressure
H_2 gas, 20 psi outlet pressure*****
Air, 40 psi outlet pressure*
*Needed to run FID detector. Not needed for ECD. We are using an ECD.

N_2 and He gases needed for stand by mode. Tanks must be changed once depleted (approximently every 3 months). Use teflon tape and snoop to assure that there are no leaks after tanks are changed.

2. Location of Detectors

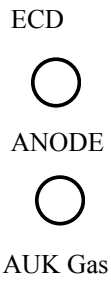


3. Turn on controller to HP7673 (on shelf behind computer) and check that green light on autosampler tower turns on.

4. Check Program on GC (Top Row)
   a. init. temp –> 35
   b. init. time –> 1.00
   c. rate –> 5 deg/min
   d. final value –> 80
   e. final time –> 1.00
   f. inj B temp –> 250
   g. Det. B temp (ECD) –> 325
   h. equib time –> 1

5. Hit Sig1, on, enter and Sigl again and it should indicate that the reading corresponds to signal "B". Hit signal again so that a number is observed on the right of the digital display. The display usually equilibrates at about 40 (may be higher right after changing tank).

6. Check ECD knobs on upper left - should be fully counter clockwise



7. Start-up computer
    a. Turn on computer next to GC., turn on monitor, turn on printer. Make sure there is paper in the printer.
    b. Go to C:/ directory and double click on DOS prompt that says "Go."
    c. Use arrow keys and go to methods, retrieve, and then CAE345 and hit return.
    e. Hit escape to go to main menu.
    f. Use arrow keys to put cursor on "Go." Hit return.
    g. Enter 1 and return when asked for the number of samples to process.
    h. Enter a file name for that sample and hit return (record filename on appendix sheet).

8. Inject 8 ul of sample into the GC (Inject into Injector B). Wash syringe 5 times with pentane prior to and after injection.

9. Perform the following steps one quickly after the other: a) Hit "start" on the GC and b) press any key on the computer keyboard to initiate data collection by the computer.
10. After the sample completes the run (approximately 20 minutes) hit escape on the computer to go to the main menu. Go to "Reprocess" and save results as a PRN file. Record file names for reference later. Also save the chromatogram and peak files.
11. Repeat steps 8 and 9 for the remaining samples.
12. Copy the PRN files to disk.
13. Shut down-turnoff computer, monitor, printer, and controller.
14. Import PRN files into Excel sheets and manually integrate the peaks in Excel.

EXPERIMENT # 5: ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN GASEOUS SAMPLES USING GAS CHROMATOGRAPHY

PURPOSE:

In this experiment the students will learn how to process gaseous phase samples for analysis of VOCs on a gas chromatograph.

APPARATUS:

- Thermometer
- Gasoline VOC collection device (side-arm flask fitted with a vacuum pump, SKC Catalog number 210-1000 MHK, 20 to 250 ml/min)
- 2, Stainless Steel Gas Collection Tubes fitted with carbon sorption media. Media includes a Carbopack C (n-C8 to n-C30) and Carbopack B (n-C4 to n-C14)
- Perkin Elmer Auto System XL Gas Chromatograph.
  Temp Program: 50°C for 5 min, 250°C for 2 min, at 12.5°C/min
  Carrier gas: helium
  Detector: FID
  Column pressure: 11.5 psi.
  Elite-5 column: 30 meter, 0.32mm ID, 0.25mm df
- Thermal desorber: Turbomatrix ATD400
  Purge: 1.0 min
  Desorb: 2.0 min, sampling tube 120 °C, cold trap –30 °C
  Trap hold: 1.0 min at 150°C (40 °C/min)
  Inlet split 40.0 ml/min
  Transfer line: 200°C, valve 150°C

REAGENTS:

- Gasoline
- o-xylene (0.004 mg/ml, 0.16 mg/ml, and 0.4 mg/ml)

PROCEDURE:

Students will work in one large group and as a group they will be asked to participate in the following activities:

Set up for the gasoline vapor collection device
(Completed before the laboratory meeting day).

i. Pour 500 ml of gasoline into one side arm flask. Estimate the surface area that is exposed to the air and record this surface area.
ii. Insert gas sorption tubes (orient spring on the inlet side; the rings on the metal housing should go on the outlet side) into the vapor collection device.
iii. Set vacuum pump to 150 ml/min and hook-up pump to vapor collection device. Turn on the pump and record the time the pump was turned on.

iv. Measure the ambient air temperature in the room.
v. One student will be assigned to shut off the pump about 18 to 24 hours later. This student needs to record the time that the pump was shut off and the volume of gas that was pumped through the sorption device. The gas sorption tubes are to be removed and the end caps are then to be placed on the sorption tubes in preparation for analysis.

Preparation of the Stainless Steel Gas Collection Tube

1. Background: There are two ways to pack the gas sorption tubes. One packing method (figure 1) is for the sorption of gas samples (which was already done for us prior to the experiment) and the other packing method is for standards. The primary difference is the omission of a spring for the "standard" tubes. Standards are prepared by adding a known quantity of chemical (o-xylene) to a sorption tube that is packed as shown in figure 2. The spring is not used in the "standard" tubes because it would block the syringe used to transfer the o-xylene to the tube.

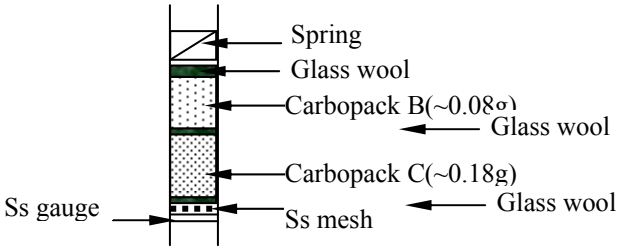


Fig.1. Sampling tube

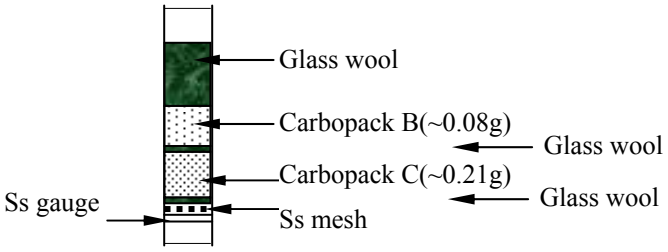


Fig. 2. "Standard" tube

2. Prepare 3 standard tubes (figure 2) by first inserting a stainless steel mesh at the bottom of the tube. The rings on the stainless steel housing should be oriented towards the bottom of the tube. Add a small amount of glass wool. Be careful. If you add too much wool the carbon will not fit and you will need to start over. About 0.21 g of Carbopack C are to be added on top of the first layer of glass wool. Add another layer of glass wool to separate Carbopack C from Carbopack B. About 0.08 g of Carbopack

B is added on top of the second layer of glass wool. The top of the tube is then filled with a third layer of glass wool. Label the tubes #1, #2, and #3.

3. Add measured amounts of o-xylene to each calibration tube. Add 5 ul of 0.004 mg/ml to one tube (tube #1), add 5 ul of 0.16 mg/ml to the second tube (tube #2), and 5 ul of 0.4 mg/ml to the third tube (tube #3). Remember to rinse the syringe with hexane 5 times. The best way to measure the 5 ul when adding the o-xylene to each calibration tube is to extract 6 ul of o-xylene and then push out 1 ul so the plunger is now on 5 ul. Wipe the tip with a Kimwipe and then add the 5 ul to the tube. Then insert the syringe to the top of the Carbonpak (figure 3) and then push the plunger slowly.

   Please note that tubes #1 and #3 will be used for establishing the calibration line for the experiment and tube #2 will be used as a control.

Fig.3. Injection of calibration standard into calibration tube

4. Provide these tubes to Dr. James' laboratory supervisor for analysis. Dr. James' laboratory supervisor will describe the operation of the equipment that will be used to analyze the samples.

PROJECT NUMBER 4
Analysis of Potable Water

For this set of experiments students are welcome to bring-in their own water samples for analysis. Please note the ultimate source of water and treatment method for the water supply.

Laboratory Procedure:

Chlorine Residual
Alkalinity
Hardness

Project Report

Your report should include a discussion concerning the treatment methods associated with each water supply and whether the results of the analyses reflect the treatment methods utilized. Note whether samples are surface water or groundwater and the treatment process that each water supply had undergone prior to analysis.

EXPERIMENT #6: CHLORINE RESIDUAL

PURPOSE:

To determine amount of the various forms of residual chlorine by using the DPD Method.

INTRODUCTION:

Chlorination is the most commonly used method of disinfection. Although there are alternatives like ozone, chlorine is an inexpensive and a powerful oxidizing agent. The key reactions are:

$$Cl_2 + H_2O \quad <=> \quad HOCl + H^+ + Cl-$$
$$HOCl \quad <=> \quad H^+ + OCl-$$

The prime disinfecting agent is the hypochlorous acid (HOCl) in which dissociation is pH dependent. At a higher pH values, the hypochlorous acid yields less effective hypochlorite (OCl-) ions. Together, HOCl and OCl- are called *free available chlorine.* The relative proportion of these free chlorine forms is pH and temperature-dependent. The free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride. The chloramines formed depend on the pH of the water, the amount of ammonia available, and the temperature.

$HOCl + NH_3$	=>	$H_2O + NH_2Cl$	(Monochloramine)
$HOCl + NH_2Cl$	=>	$H_2O + NHCl_2$	(Dichloramine)
$HOCl + NHCl_2$	=>	$H_2O + NCl_3$	(Trichloramine)

One disadvantage of chlorination is the potential formation of trihalomethanes (THMs) in the presence of organic matter.

The two most common methods for measuring the chlorine residual in water are the iodometric method and the DPD method. The iodometric method is suitable for measuring total chlorine concentrations greater than 1 mg/L. The DPD method can measure lower concentrations of total chlorine residual (down to 0.1 mg/L) and can measure individual chlorine species.

APPARATUS:

- Pipettes (5 ml, 3 per group) and pipette bulb.
- Magnetic stirrer, stir bar, ring stand, burette, funnel, and burette holder
- Four, 250ml Erlenmeyer flasks
- Balance Scale, spatulas, and weighing boats
- 100 ml graduated cylinder
- 500 ml volumetric flask

REAGENTS:

- Chlorox solution (approximately 50,000 mg/L as Cl_2): Purchase a fresh bottle of Chlorox bleach from the supermarket.
- Prepare a 100mg/L Chlorine standard from the Chlorox solution.
- Phosphate buffer solution: Dissolve 24 g anhydrous Na_2HPO_4, and 46g anhydrous KH_2PO_4 in distilled water. Combine this solution with 100 ml distilled water in which 800 mg disodium ethylenediamine tetraacetate dihydrate (a.k.a. Titra Ver Hardness Reagent or Ethylene diaminetetraacetic Acid, Disodium Salt, Dihydrate) have been dissolved. Dilute to 1 liter with distilled water, and to prevent interference in the free available chlorine test caused by any trace amounts of iodide in the reagents.
- N,N- diethyl - p- phenylenediamene (DPD) solution: Dissolve 1g DPD oxalate in chlorine - free distilled water containing 8 ml of approximately 9 N sulfuric acid and 200 mg disodium ethylenediamine tetraacetate dihydrate (a.k.a. Titra Ver Hardness Reagent or Ethylene diaminetetraacetic Acid, Disodium Salt, Dihydrate). Make up to 1 liter, store in a brown glass - stoppered bottle. Discard when discolored. Caution: DPD oxalate is toxic-take care to avoid ingestion.
- Standard ferrous ammonium sulfate (FAS) titrant: Dissolve 1.106 g $Fe(NH_4)_2(S0_4)_2 \cdot 6H_2O$ in distilled water containing 1 ml of approximately 9 N sulfuric acid and make up to 1 liter with distilled water. 1 ml FAS titrant = 0.1 mg available residual chlorine as Cl_2. For a 100 ml sample 1ml FAS = 1 mg/L of available residual chlorine as Cl_2.
- Potassium iodide,crystals
- Sulfric acid, approximately 9 N: Slowly add 250 ml concentrated H_2SO_4 to approximately 750 ml distilled water.

Samples: 2 mg/L control (analyze two times)
Blank (de-ionzied water)
Environmental Samples (treated potable water and untreated groundwater)

PROCEDURE:

1. Using the standard provided, prepare 500 ml of a 2 mg/L solution of Cl_2.
2. **In a separate titration flask**, mix 5 ml of buffer reagent and 5 ml of DPD indicator solution.
3. Add 100 ml of the 2 mg/L chlorine solution to the titration flask. Mix the solution.
4. Free Chlorine (Reading A): Fill burette to zero reading at top with ferrous ammonium sulfate titrant (FAS). Titrate rapidly until the red color is discharged. Observe the volume at which this occurs. If the color reappears, do not titrate further.
5. Monochloramine (Reading B): Add one small KI crystal to the solution from part 4 and mix, continue titration and observe the volume at which the red color disappears. Do not titrate further if the red color reappears.
6. Dichloramine (Reading C): Add several KI crystals to the solution from part 5 and mix for approximately 2 minutes, continue titration and observe the volume at which the red color disappears. Allow the sample to stand for an additional 2 minutes if color reappears. Titrate again and use new reading as reading "C".
7. Nitrogen Trichloride (Reading D): Fill burette to zero reading. Obtain a clean titration

flask. Place a small KI crystal in the titration flask. Add 100 ml of sample and mix. Add the contents to a second flask containing 5 ml of buffer solution and 5 ml of DPD indicator solution. Titrate rapidly with FAS until the red color disappears. Observe the volume at which it occurs.

8. Repeat procedure for U.M. tap water, untreated groundwater, and de-ionzed water. Perform a replicate on the 2 mg/L control.

COMPUTATIONS:

Reading	NCl_3 Absent	NCl_3 Present
A	Free Chlorine	Free Chlorine
B-A	NH_2Cl	NH_2Cl
C-B	$NH\ Cl_2$	$NHCl_2 + ½\ NCl_3$
D	------	Free chlorine + ½ NCl_3
2(D-A)	------	NCl_3
C-D	------	$NHCl_2$

EXPERIMENT #7: ALKALINITY AND THE CARBONATE SYSTEM

PURPOSE:

To determine the concentration of different carbonate species from alkalinity measurements.

INTRODUCTION:

The alkalinity of water is defined as its capacity to neutralize acid or the amount of acid required per liter to lower the pH to about 4.3. The alkalinity of many surface waters is a function of carbonate, bicarbonate, hydroxyl content, hydrogen ion concentration and thus dependent on the concentration of these constituents. For these waters, the alkalinity is defined mathematically by the following equation.

$$\text{Total alkalinity} = [HCO_3^-] + 2[CO_3^{-2}] + [OH^-] - [H^+]$$

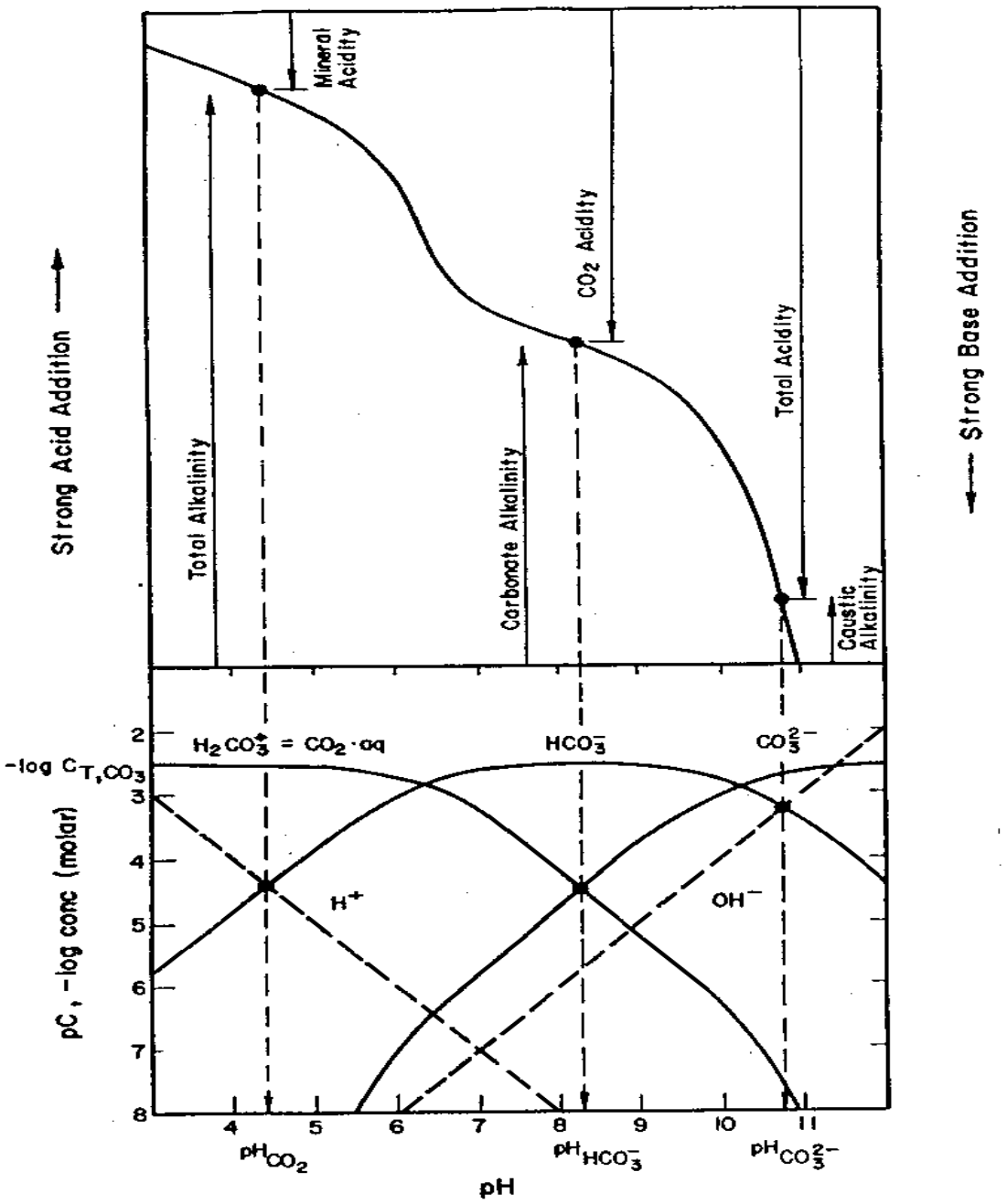
Where total alkalinity has the units of eq/l or moles H^+/l and
[ ] refers to the molar concentration.

The pH of natural fresh waters is between 6 and 9 indicating that the major carbonic species in this range is bicarbonate, thus the alkalinity under these circumstances is approximately equal to the bicarbonate concentration.

APPARATUS:

- pH meter with Calomel reference electrode and a glass electrode. Stand for pH meter.
- pH buffer solutions, (pH 4,7, and 10)
- Magnetic stirrer with stirring bars and a 100 ml beaker
- Burette, ring stand, funnel, and burette holder
- 50 ml graduated cylinder

Figure B: Titration and Species Relationships in the Carbonate System



Titration and Species Relationships in the Carbonate System. ($C_{T,CO_3} = 10^{-2.5}M$)

REAGENTS:

- Sodium Carbonate Primary Standard Solution, 0.02N: Dry Na_2CO_3 at 140 ^oC. Weigh 1.060g of Na_2CO_3 and dissolve in 500 ml of boiled and cooled distilled water. Make up volume to 1 liter of boiled and cooled distilled water
- Sulfuric Acid Solution, approximately 0.02N. To 500 ml distilled water, carefully add 2.8 ml of concentrated H_2SO_4 and dilute to 1 liter (approx. 0.1 N). Transfer 200 ml of this solution to 1 liter volumetric flask and dilute to 1 Liter (approx. 0.02 N). 500 ml needed per student group. Normality of concentrated H_2SO_4 is approx. 36 N.
- Phenolphthalein Indicator Solution
- Bromecresol green - methyl red indicator solution: Dissolve 100 mg of bromecresol green sodium salt and 20 mg of methyl red sodium salt in 100 ml of distilled water. pH should be at about 4.5.
- Control Solution, 0.005 M $NaHCO_3$. Dilute 0.42 g of oven-dried $NaHCO_3$ in 500 ml of distilled water and dilute to 1 liter.

SAMPLES:

Environmental Samples
Control (0.005 M $NaHCO_3$, [C_T = 0.005 M)]). Note initial pH of this solution

PROCEDURE

1. Calibrate pH meter. Use the 4 and 10 buffer solutions to calibrate meter and check accuracy with the pH 7 buffer solution.
2. Use the 0.02N sodium carbonate solution supplied to standardize the sulfuric acid solution. Place 20 ml of the 0.02 N sodium carbonate solution in a 100 ml beaker. Add 30 ml of distilled water. Titrate with the acid until the endpoint of the titration is reached at a pH of approximately 4.3. Repeat.
3. With the standardized acid, titrate 50 ml volumes (50 ml of sample, no dilution with distilled water) for each sample. To each sample add 5 drops of phenolphthalein. (If no color develops proceed to the next step.) Titrate each sample **and** record ml of acid added versus pH. It is recommended that the pH be recorded after every mL of additional acid. When color begins to fade, record the pH change after every 0.25 mL. When the solution turns colorless (pH approx. 8.3) the phenolphthalein endpoint has been reached - Record the mL of acid needed to reach this point.
4. Add five drops of bromecresol green-methyl red indicator and continue the titration as above. When the solution turns from blue-green to another color (pH approx. 4.5) stop the titration. Record the mL of acid needed to reach this point.

EXPERIMENT #8: HARDNESS

PURPOSE:

The purpose of the experiment is to introduce the concept of complex formation and stability and to use this concept to measure the calcium and magnesium concentration in water.

INTRODUCTION:

Hardness of water is a property caused by calcium and magnesium ions. The total hardness is defined as the sum of the multivalent cations in water. Calcium and magnesium concentrations usually dominate and both expressed as calcium carbonate in milligrams per liter or eq/ L. Hardness is measured by forming chelates with metal ions using the commonly used chelating agent ethylene diamine (N, N, N', N'), tetraacetic acid usually called EDTA.

In the determination of hardness using EDTA, the sample is buffered at pH 10.0 to prevent the precipitation of metal ions. Since EDTA and hardness complexes are not colored, an additional chelating agent Eriochrome black T (EBT) is used for the endpoint determination. A small amount of EBT is added to the test solution before the titration with EDTA.

APPARATUS:

- Burettes, ring stand, funnel, and burette holder
- Volumetric flasks
- 150 ml beaker
- Stirrer and magnetic stir bar
- 50 ml graduated cylinder

REAGENTS:

- Standard $CaCO_3$ Solution: Weigh 1.000 g anhydrous $CaCO_3$ into 500 mL Erlenmeyer flask. Place a funnel in the flask neck and add a little at a time 1 + 1 HCl until all the $CaCO_3$ has dissolved. Add 200 ml distilled water and boil for a few minutes to expel CO_2. Cool and adjust pH to a value of 5.0 using either 3N NH_4OH or 1 + 1 HCl as required. Transfer to a 1 liter volumetric flask and bring-up to volume.
- Buffer Solution: Dissolve 16.9 g ammonium chloride (NH_4Cl) in 143 ml concentrated ammonium hydroxide; add 1.170 g of disodium ethylene diamine tetracetate dihydrate and 780 mg of $MgSO_4 \cdot 7H_2O$ and dilute to 250 ml with distilled water. Keep in the hood in a tightly stoppered plastic or resistant glass container.
- EDTA Titrant, approx. 0.01 M (1 ml = 1 mg hardness as $CaCO_3$): Dissolve 3.723 g disodium ethylene diamine tetraacetate dihydrate (a.k.a. Titra Ver Hardness Reagent or Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate) in distilled water and dilute to 1 liter.
- EBT indicator: Dissolve 0.5 g of Eriochrome Black T and 4.5 g hydroxylamine hydrochloride in 100 mL of 95% ethyl alcohol.

SAMPLES:

Blank, de-ionized distilled water
Control, 200 mg/L as $CaCO_3$, prepare from 1000 mg/L as $CaCO_3$ standard
Environmental Samples

PROCEDURE:

1. Use the 1000 mg/L $CaCO_3$ standard solution supplied to standardize the EDTA titrant. Fill burette with EDTA titrant. Measure 20 ml of standard 1000 mg/L $CaCO_3$ solution and place in beaker. Add approximately 30 ml of distilled water and 2 ml of buffer solution (in hood). pH should be at approximately 10.0. Add EBT indicator drop-by-drop until a wine red color develops. Titrate the standard solution to a blue color. The titration should not exceed 5 minutes. Repeat.
2. With the standardized EDTA, titrate the a distilled water blank by placing 50 ml of distilled water to a beaker. Add 2 ml if buffer solution (in hood). Add EBT indicator drop-by-drop until a wine red color develops. Titrate the solution to a blue color. The titration should not exceed 5 minutes. Repeat for the environmental samples and for the controls. Titrate controls 2 times.

PROJECT NUMBER 5
Analysis of a Surface Water

This project involves field and laboratory work centered around Lake Osceola. The class will be separated into the three following teams for the field work portion of this project.

Team A: Field Sampling Preparation Crew (clean bottles, gather equipment for turbidity sampling, analyze turbidity samples, take equipment to field, etc...) Bring separate cooler for "people" drinks. Put ice packs in cooler just before field sampling. Need two carboys to collect samples for gravimetric analysis – one for inlet sample and one for outlet sample.

Team B: Field Measurements and Sample Collection Crew (prepare list of equipment needed, gather this equipment, learn how to use YSI probe, calibrate YSI probe, learn how to use the D.O. kit, responsible for coordinating depth measurements, turbidity sample collection, YSI measurements [D.O., salinity, temperature, pH], D.O. kit measurements, etc...)

Team C: Map Preparation (obtain map of lake, identify sampling locations, record data in field (include the preparation of the data sheets) and put into Excel spreadsheets, provide class with antecedent rainfall(from South Florida Water Management District web page, etc..)

Each team will summarize their work in written form for distribution to the rest of the class. Each team will also briefly describe their work to the class (10 minutes) and will be available for questions.

Laboratory Procedures: Solids in Water Using Gravimetric Analysis
Phosphate

Project Report

You have been hired by the University President's Office to document the aesthetic quality of Lake Osceola and to evaluate measures by which the quality can be further improved. Your work includes a field study of the lake documenting spatial variations in lake dissolved oxygen, salinity, temperature, pH, turbidity, and depth. You will also document the solids content, and phosphate levels of the lake.

The report should include a figure of Lake Osceola with sampling points. Use contour maps to present the depth, dissolved oxygen, and salinity data. All field data collected should be summarized in tabular form and plotted. Compare the values from the turbidimetric analysis with the concentrations of suspended solids obtained during the gravimetric solids experiment and discuss. Is the concentration of phosphate considered elevated for the environmental samples?

EXPERIMENT #9: SOLIDS IN WATER USING GRAVIMETRIC ANALYSIS

PURPOSE:

To illustrate the various operations involved in gravimetric analysis. To determine the various categories of solids that are commonly present in water and wastewater, and to investigate the types of materials that these solid categories define.

INTRODUCTION:

Solids may affect water or effluent quality in a number of ways. Waters with high dissolved solids (> 500 mg/L) generally are not palatable. Waters high in suspended solids are aesthetically unsatisfactory for bathing and are generally difficult to disinfect properly.

Solids are determined by gravimetric analysis based upon weight measurements. "Total Solids" is the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids include "total suspended solids," the portion of total solid retained by a filter, and "total dissolved solids," the portion that passes through the filter. In our analytical procedure, dissolved and suspended solids are operationally defined by filtration through a 1.5 □m pore size filter. It is important to note that this operational definition does not always coincide with the true chemical definition of dissolved versus suspended materials.

This experiment illustrates the principles of weighing and demonstrates separation and categorization techniques used to define the various types of solids in waters and wastewater. These techniques involve weighing, filtration, evaporation, and combustion. Filtration is used to separate "suspended" or particulate (nonfilterable) fractions from "dissolved" or "soluble" (filterable) fractions. Evaporation separates water from material dissolved or suspended in it. Evaporation of water and wastewater samples is normally conducted at two temperatures, 103^{o} - 105^{o} C and 179^{o} - 181^{o} C. The lower temperature is usually used with samples containing high concentrations of organic matter that may suffer significant weight loss due to volatilization and decomposition at the high evaporation temperature.

The analytical objective of the combustion in the solids determinations is to differentiate between volatile and non-volatile matter. Organic matter will be destroyed completely by burning at 550^{o} C for 30 minutes.

APPARATUS:

Balance, analytical - sensitive to 0.1 mg
Oven, 103^{o} - 105^{o} C and 179^{o} - 181^{o} C
Muffle furnace capable of 550 ^{o}C
Glass fiber filters, 90 mm diameter (Whatman 984H or equivalent), 6 needed per student group
Büchner filtration funnel with rubber stopper, sidearm flask, aspirator, vacuum tubing, and vacuum source
Forceps
Aluminum evaporating dishes (to contain > 25 ml), 6 needed per student group
Desiccator and dessicant
Graduated Cylinders, 1 liter and 50 ml capacity
Aluminum foil
Blotting container and Kimwipes

SAMPLES:

Lake Osceola Water, Inlet and Outlet

Control, DS = 3500 mg/L, SS = 20 mg/L (Use 2L for the control filtration)

Each student group will need approximately 4 liters per sample. A total of 8 to 12 Liters of Lake Osceola water will be needed per student group. Each sample requires 2 weighing dishes (one for dissolved solids and one for total solids) and 1 filter (for suspended solids). Three filter blanks should be carried through the experiment to check for filter weight change.

PROCEDURE:

==> **Record all necessary weights of dishes and filters before TS, DS, and SS determinations. Use the same scale for all measurements.**

Label all evaporating dishes by etching a number and a symbol unique to your group on the aluminum tab. Place filters on a piece of aluminum foil. Label the filters by etching a number and symbol onto the foil.

TOTAL SOLIDS

1. Weigh the evaporating dish.
2. Add 15 or 25 ml of the sample. Use 15 ml for small evaporating dish and 25 ml for large dish.
3. Evaporate to dryness in an oven set at 103^{o} Celsius.
4. Cool in a desiccator.
5. Reweigh.

SUSPENDED SOLIDS

1. Pre-ignite filters in muffle furnace (place in muffle furnace at 550 ^{o}C) Ignite for 1 hr. Wrap filters in aluminum while in furnace.
2. Weigh the filter to the nearest 0.1 mg.
3. Place filter, wrinkled side up, in the Büchner funnel. Apply vacuum and set filter by adding a minimal amount of distilled water to the edges of the filter.
4. Filter a known volume of the sample solution through the filtration apparatus. Volume used is dependent upon the turbidity of the water. Filter until suspended material is visible on the filter. Use the first 500 ml of filtrate to rinse the sidearm flask and discard. Continue filtration but do not discard all of the remaining filtrate. A portion of the filtrate is used for the dissolved solids determination.
5. Repeat the analysis for three filter blanks as in the step above. Filter 300 ml of distilled water through the filters. The results of this step will be used to estimate whether the filter weight changed significantly before and after drying/ignition. If a difference is noticeable, this difference must be used as a correction in your computations.
6. Remove the filter with the forceps. Drain as much water from the filter as possible. Blot the filter onto absorbent paper to further drain water. Draining is necessary to prevent the filter from "sticking" to the filter support during the drying process.
7. Dry the filter for one hour in the oven at 103^{o} C.
8. Cool in a desiccator.
9. Reweigh to nearest 0.1 mg.

TOTAL DISSOLVED SOLIDS

1. Weigh an ignited cooled aluminum dish. Prepare a filtrate from the suspended solid sample and transfer 25 ml to an ignited cooled aluminum dish.
2. Evaporate to dryness in an oven set at 180^{o}C.
3. Cool in a desiccator
4. Reweigh.

VOLATILE SOLIDS

1. Take samples from the total, suspended, and dissolved solids test and place them inside a muffle furnace set at 550 ^{o}C for one hour. Cool samples in a desiccator and reweigh.

EXPERIMENT #10: PHOSPHATE, OPTICAL METHOD WITH A SPECTROPHOTOMETER

PURPOSE:

To demonstrate the operation of a spectrophotometer. Learn one experimental technique for determining the amount of phosphate in water.

INTRODUCTION:

Optical methods of analysis can be used to determine satisfactorily the concentration of many dissolved substances. The law relating the amount of light-transmitted by a solution to the concentration of light absorbing constituent is the Beer-Lambert law known as "Beer's Law".

$$\text{Log } (I_o / I) = A = k_{10}\, l\, c$$

where

- I = Intensity of monochromatic light transmitted through the test solution
- I_o = Intensity of light transmitted through the reference solution, "the blank"
- A = Absorbance (dimensionless)
  - k_{10} = Absorptivity, a constant for a given solute/system at a given wavelength. (Subscript corresponds to the “base 10" logarithm)
- l = Light path length (usually in cm)
- c = Concentration of solute g/l.

Thus Beer's law states that for a given solution, Absorbance is directly proportional to the light path and concentration of absorbing substance. In the instrumental measurement of color intensity, the light transmitted through the solution is measured. The transmittance of a solution (T) is defined as I/I_o and % T as I/I_o x 100.

A spectrophotometer is an instrument by which you can measure light passing through a clear solution in the visible and ultraviolet range. The light source for an instrument such as the Spectronic 20 used in our experiment is a tungsten lamp. Light from this is dispersed by a diffraction grating and the desired wavelength region is regulated by passing the desired range of light through a slit. This selected wavelength band is then passed through the sample solution. The light that is not absorbed by the solution is received by the phototube, the signal from which is displayed on the instrument scale.

The sensitivity of analysis depends on the choice of wavelength. This can be determined by experimentally evaluating a solution at different wavelengths and measuring the absorbance or transmittance as a function of wavelength. Maximum sensitivity or the largest absorptivity (a) is found at wavelengths where maximum light absorption occurs. Similarly minimum absorbance is found where minimum light absorption occurs. Thus, if possible a wavelength corresponding to a relatively flat portion of the absorption spectrum is used.

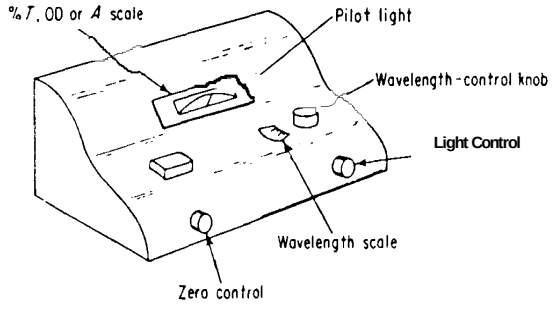
Phosphate exists in several forms in natural water. Two common analytical methods include the vanodomolybdophosphoric acid method and the ascorbic acid method. The vanadomolybodophosphoric acid method is based upon the reaction of ammonium molybdate with orthophosphate in acidic solution. The molybdophosphoric acid formed gives a yellow color

in the presence of vanadium, the intensity of which is proportional to the phosphate concentration. The ascorbic acid method is based on the reaction of ammonium molybdate with orthophosphate in acidic solution (equation 1). The molybdenum in the molybdophosphate complex formed is readily reduced to molybdenum blue in the presence of ascorbic acid to produce a blue colored solution (equation 2).

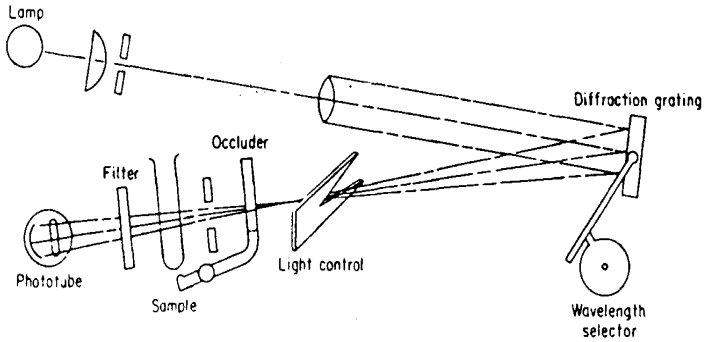
$$PO_4^{-3} + 12(NH_4)_2MoO_4 + 24H^+ \rightarrow (NH_4)_3PO_4 \bullet 12MoO_3 + 21NH_4^+ + 12\ H_2O \qquad (1)$$

$$(NH_4)_3PO_4 \bullet 12MoO_3 + C_6H_8O_6 \rightarrow$$
$$\text{(molybdenum blue)} + \text{(oxidized form of ascorbic acid)} \qquad (2)$$

Figure C: a) Bausch and Lomb Spectronic 20
b) Schematic Optical Diagram of the Spectronic 20



Bausch and Lomb Spectronic 20



Schematic optical diagram of the Spectronic 20

APPARATUS:

- Bausch and Lomb or Milton Roy Spectronic 20 Spectrophotometer, equipped with red filter and CE-A30 phototube.
- Cuvettes, (1 cm light path) and test tube holder
- 50 and 100 ml graduated cylinders, 100 ml volumetric flasks, and funnels
- 2, 5 ml pipette and pipette bulb (Use one pipette for phosphate standards and other for the remaining reagents to prevent cross-contamination)
- 200 ml Erlenmeyer flask
- Beakers

REAGENTS: Note: Glassware should be washed with phosphate free soap and 1 N HNO_3.

- 5N Sulfuric Acid, H_2SO_4: Dilute 70 ml of concentrated H_2SO_4 to 500 ml with de-ionized distilled water.
- Potassium antimonyl tartrate (a.k.a. antimony potassium tartrate): Dissolve 1.3715 g $K(SbO)C_4H_4O_6$ ½ H_2O in 500 ml distilled water. Store in glass stoppered bottle.
- Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6Mo_7O_{24}$ 4H_2O in 500 ml distilled water. Store in a glass-stoppered bottle.
- Ascorbic acid, 0.1 M: Dissolve 8.80 g ascorbic acid in 500 ml distilled water. The solution is stable for about 1 week at 4 ^oC.
- Concentrated Stock Phosphate Solution, 50 mg/L: Dissolve 219.5 mg of KH_2PO_4 in 1 L of distilled water. (50 mg/L as P) (T/A please remove this sample from lab so that students do not use this by mistake.)
- Stock Phosphate Solution, 1 mg/L: Dilute 20 ml stock phosphate solution to 1000 ml with distilled water. (50 mg/L as P)
- T/A should test this experiment prior to student exercise to check for potential contamination.

SAMPLES:

Lake Oceola Inlet and Outlet (Replicate 1)
0.1 mg/L control

PROCEDURE:

1. With the power turned off check that the red filter and correct phototube (CE-A30) are installed in the spectrophotometer. Turn on the spectrophotometer and allow it to warm-up for at least 20 minutes.
2. Prepare the combined reagent by mixing in a 250 ml Erlenmeyer flask, 100 ml of 5N H_2SO_4, 10 ml potassium antimonyl tartrate solution, 30 ml ammonium molybdate solution, and 60 ml ascorbic acid solution. Mix after the addition of each reagent. Let all reagents reach room temperature before they are mixed and mix in the order given. The reagent is stable for 4 hours.
3. Determine the absorption spectrum. Add 10 ml of stock phosphate solution (1 mg/L) and 40 ml of distilled water into a 125 ml Erlenmeyer flask. Add 8.0 ml of combined reagent and mix thoroughly. After at least 10 minutes but no longer than 30 minutes, measure the absorption spectrum of the solution by making measurements at 775, 800, 825, 850, 875, 900, 925 nm in the following manner for each wavelength.

_ Set 0% T with the zero control knob (left) when no cuvette is in the cell holder.
_ Set 100% T with light control knob (right) when the blank is in the cell holder.
_ Insert the curvette containing the sample.
_ Read and record %T and A.

4. Prepare standard curve at a wavelength of 880 nm. The standard curve is prepared using the following method.
   a) Prepare phosphate standards containing 0, 0.01, 0.025, 0.1, 0.25, 0.50 mg/L PO_4 as P by pipetting appropriate amounts of the stock (1 mg/L) phosphate solution into 50 ml volumetric flasks. Also prepare a reagent blank (0 mg/L). Place each standard and blank solution into an separate 125 ml Erlenmeyer flask and add 8 ml of combined reagent to each solution. Wait for color development.
   b) Prepare environmental samples (replicates). Add 50 ml of sample to a 125 ml Erlenmeyer flask. Add 8 ml of combined reagent. Wait for color development.
   c) Set 100% T for distilled water blank. Measure the absorbance of each sample at 880 nm. Samples include the environmental sample(s) as well as the standards. Use five standards (0, 0.01, 0.025, 0.25, and 0.50 mg/L PO_4 as P) for the calibration curve. The sixth standard (0.1 mg/L) is used for quality control.

PROJECT NUMBER 6

Analysis of CCA-Treated Wood Using X-Ray Fluorescence Spectroscopy

Your client has asked you to analyze a set of 3 wooden boards for their metals content. Your client provided you with the following information.

Sample ID	Retention Level at Store (pcf)[b]	Measured Metals Concentration, (mg/kg)			Measured Retention Level (pcf, CCA oxide basis)
		As	Cr	Cu	
U (for untreated)	0				
TL (for treated low retention)	0.25				
TH (for treated at high retention)	2.5				

[a]pcf = pounds of chemical per cubic foot of wood. Retention is a term used by the wood treatment industry to describe the amount of CCA chemical (oxide basis) contained within wood. Expressing concentration in units of retention will require a conversion of the arsenic concentration as As to As_2O_5, Cu to CuO, and Cr to CrO_3. These three components (As_2O_5, CuO, and CrO_3) are then added together to get the pcf of CCA on an oxide basis. Typically CCA is composed of the following 47.5% CrO_3, 18.5% CuO, and 34.0% As_2O_5.

In particular your client wants you to fill-in the missing columns of data in the table above. You should assume a wood density of 32 pounds of wood per cubic foot of wood. In your "Dear Client letter" please provide a completed summary table (above), explain the method utilized in collecting the data (e.g. instrument used [Innov-X, Model alpha 2000s] and experimental design), and briefly describe how the computations were performed. You should also provide the computations and the organized data within an appendix of your report. The appendix should include a description of relevant information including qualitative descriptions observed during experimentation. The appendix should also include attempts to quantify accuracy and precision. Please put the data in a table that includes the proper column headings and units. Additional tables would also be likely helpful. Two wood boards will be analyzed per type of wood (2 U, 2 TL, and 2 TH). Each board to be analyzed in triplicate.

NOTE: No appendix sheet provided for this report. Students are to organize the data into tables.

EXPERIMENT #11: ANALYSIS OF SOLID SAMPLES USING AN XRF METHOD

PURPOSE:

In this experiment, x-ray fluorescence spectroscopy (XRF) will be used to analyze metals within solid media (wood).

INTRODUCTION:

An atom consists of a nucleus and orbiting electrons. Respective electron orbits have their corresponding energies; orbits of the same energies are grouped into shells. A given electron will occupy the lowest energy shell available. When the incident x-ray, from the XRF spectrometer hits the atom, the innermost electron (the one with the lowest energy) absorbs it (Williams 1987). This ejects that electron creating a vacancy in the shell. Next an electron from a higher energy shell falls to the created vacancy, and in the process excees x-ray energy is emitted that is equal to the difference of energy between those shells. An XRF analyzer registers the emitted energy and compares it to the known energies of a given element (Figure D).

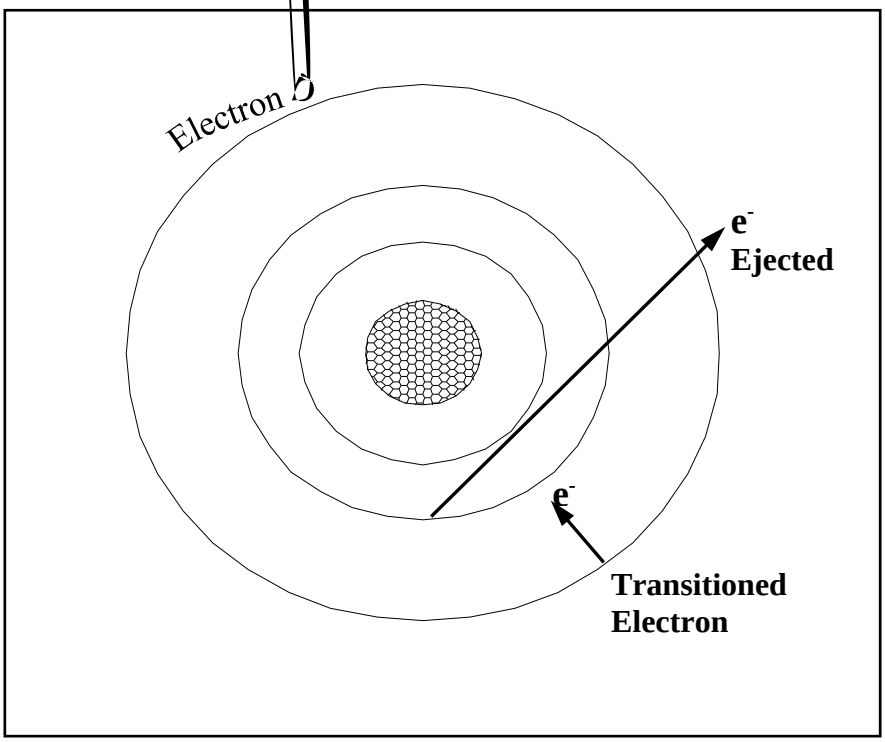


Figure D: Emission of Characteristic X-Ray

Elements can be distinguished from one another by the wavelength or energy level of the x-ray emitted. The concentration of a particular element is determined from the frequency or intensity of the emitted x-ray which is usually compared with calibration standards (Williams 1987).

APPARATUS:

XRF Unit (Innov-X, Model XXX)

REAGENTS:

None.

SAMPLES:

- Untreated wood sample.
- CCA-treated wood at a low retention level.
- CCA-treated wood at a high retention level.

PROCEDURE:

To Be Written by Students.

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APPENDIX SHEETS

Goto: http://www.cae.miami.edu/~hmsolo/345/cae345.html