Instrumental Methods in Chemistry

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Written by

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Instrumental Methods in Chemistry

Course Description:

Quantitative analysis is a one-year course in chemistry offered to junior and senior students that emphasizes quantification of substances based on wet-chemical methods and modern instrumentation techniques. Topics will include statistics, sampling strategy, gravimetry, multiple chemical equilibria, titrimetry, potentiometry, voltammetry, absorbency and fluorescence spectroscopies, gas and liquid chromatographies, and capillary electrophoresis. This course will be correlated to various scientific areas including, but not limited to, medical sciences, chemical engineering and the environment. Students should have no less than two class lectures and two periods to conduct laboratory exercises per week. ***All topics and laboratory exercises meet and /or exceed the New Jersey Core Curriculum Content Standards for Science (NJCCCSS).***

Prerequisites:

- Biology
- Chemistry
- Algebra I and II

Credits: 5 (Honors level)

Rationale and Objectives:
The primary rationale and objectives of this course are to introduce students to current analytical methods and to cultivate sound experimental technique through a hands-on, inquiry-based, problem-solving approach. This course provides numerous opportunities to advance students beyond the realm of scientific literacy and into the realm of scientific inquiry and discovery by emphasizing competency in skills such as experimental design, collection and analysis of empirically-derived data, and review of findings by the scientific community.

While it is clearly impossible to cover the entire spectrum of modern analytical instrumentation in a single course, several broad categories, incorporating the principles of most common types of analyses, will be examined.

The major goals of this course are as follows:

- To provide students with scientific understanding in the following areas:
  - ✓ spectroscopic instrumentation and separation techniques used to identify and quantify different chemical species.
  - ✓ principles, design(s) and function(s) of the most commonly used instruments
  - ✓ the information that can be gained from specific instruments
  - ✓ proper use of instrumentation and/or techniques.
  - ✓ effective interpretation of results.
- To provide students with an understanding of the importance of chemistry in society (i.e. health fields, environment, government industry, and research).

- To develop correct analytical laboratory techniques, including:
  - ✓ proper sample preparation specific to the instrument
  - ✓ the use of standards, calibrations and blanks to insure the accuracy of analytical results
  - ✓ ability to perform calculations related to solution chemistry and analysis, including: concentrations, dilutions, and manipulation of data
  - ✓ ability to explain the significance of each step in an analytical procedure

- To develop good scientific data manipulation and communicative skills, specifically related to:
  - ✓ effective laboratory notebook record keeping
  - ✓ accurate statistical analysis of results
  - ✓ effective utilization of computer software for the manipulation and presentation of experimental information, including graphical analysis
  - ✓ effective presentation of scientific findings in written and oral formats

- To provide students with an academic experience that expands, promotes, and supports learning and high achievement in Chemistry, Mathematics, Technology, Language Arts and other key disciplines through an integrated curriculum that is challenging, viable and relevant.

Approach

The curriculum will focus primarily on *Spectroscopic, Electrochemical (a.k.a. Electroanalytical) and Methods of Separation.*
Methodologies will be presented with a special emphasis on each of the following:

- Principles and theories of operation
- Instrumental design (focusing on peculiar or unique feature(s))
- Figures of merit (relative to other methods with similar capability)
- Application(s) of technique(s) to a variety of research areas (i.e. medical science, biology, biochemistry and all areas of chemistry)

Recommendations for teachers:

Remember that this course is designed to expose students to a number of analytical skills in chemistry that can be used to solve problems in other scientific areas. It is important that the laboratory exercises explore the various applications of these technologies to biological, biochemical, chemical, medical and environmental sciences. You should encourage students to discuss their methodologies and findings (i.e. discussions of topic(s) being researched and its practical application to the real world). Without that interaction, the course can quickly become grade-oriented, intimidating and tedious instead of a fascinating learning, empowering, and fun experience. Therefore, do not allow students to simply get an 'A' in the class and flunk in learning! They must learn these techniques such that they will use them in a research setting. Have students do all the problems sets associated with each activity.

Remind students that this course will require diligent and focused attention to detail. The homework, quizzes and examinations should assess their understanding of concepts and **not** the ability to memorize facts.

Grading Quizzes and Exams
There should be eight (8) one-hour examinations that will be announced at the beginning of the course. Three practical assessments will also be scheduled. In those, students will have to determine identifications and quantification of unknown compounds. Quizzes will be given as the need arises. **Students must come to class prepared for work.**

Students' overall grade will be weighted as follows:

Eight one-hour exams	30% (10% each)
Quizzes & Homework	20%
Lab Reports	20%
Finals	30%
Total	100%

Grade Scale:

- A's = 90% or greater
- B's = 80 - 89 %
- C's = 65 - 79 %
- D's = 50 - 64 %
- F's = <50

***Attendance*: Students must attend all classes.**

Unit 1: Introduction to the tools of Analytical Chemistry/ Statistics

Introduction to Analytical Methods, Acid-Base and Oxidative Titration, Separation and Purification

Objectives:

- Description of analytical methods, theory of processes
- Acid-Base titration theory and application
- Oxidative titration theory and application
- Purpose and theory of separation and purification of chemical products

Content:

- Overview of analytical chemistry
- Acids and bases in water
- Oxidation as an analytical tool
- Separation methods
- Purification methods

Skills:

- TSWBAT organize analytical methods into logical sequences
- TSWBAT relate appropriate analytical methods depending on product type

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

- Acid-Base titration of known chemicals
- Acid-Base titration of unknowns
- Oxidative titrations

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments and Techniques In Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

- Science: 5.1, 5.2, 5.4, 5.5, 5.8
- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 10 Hours [4 class days]

Unit 2: Purification of solids, Determination of Purity of Solids, Melting Point Depression, Molality, Molecular Weight Determination

Objectives:

- Description of analytical methods, theory of processes
- Acid-Base titration theory and application
- Oxidative titration theory and application
- Purpose and theory of separation and purification of chemical products

Content:

- Purification of solids by Recrystallization
- Melting point determination
- Freezing point depression
- Molecular weight from freezing point depression
- Molality

Skills:

- TSWBAT do recrystallizations
- TSWBAT perform melting point determinations
- TSWBAT determine molecular weight from freezing point depression data

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

- Recrystallization of an impure compound
- Melting point determination of an unknown
- Molecular weight determination of an unknown

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments and Techniques in Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

- Science: 5.1, 5.2, 5.4, 5.5, 5.8
- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 10 Hours [4 class days]

Unit 3: Distillation; Boiling Points; Vapor Pressure Determinations; Theoretical Plates; Product Isolation and Purity

Objectives:

- Distillation Theory and purification
- Vapor pressure and distillation efficiency
- Purification via distillation

Content:

- Purification of liquids by distillation
- Boiling point determination
- Vapor pressure determination
- Theoretical plate determination

Skills:

- TSWBAT do a distillation
- TSWBAT perform boiling point determinations
- TSWBAT achieve proficiency in product isolation with distillation
- TSWBAT calculate theoretical plates

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

- Distillation of a wide boiling mixture
- Distillation of an impure compound
- Boiling point determination of an unknown

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments and Techniques In Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

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- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 10 Hours [4 class days]

Chemistry Laboratory: Instrumental Methods

Unit 4: Chromatography [Gas and Liquid]; Solid and Liquid Supports; Solvent Effects; Band Movement; Elution Techniques; Product Isolation

Objectives:

- Chromatography theory
- Mole fractions
- Component Calculations
- Isolation of eluted products

Content:

- Purification of liquids by gas liquid phase chromatography, glpc
- Purification of solids by liquid-solid phase partition chromatography
- High performance liquid chromatography, hplc
- Band movement observation

Skills:

- TSWBAT perform a simple chromatographic separations
- TSWBAT watch band movement with visible and ultraviolet light
- TSWBAT use printers to see band movement in glpc applications
- TSWBAT practice elution techniques

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

- Liquid phase chromatographic separation of two solids
- Gas phase chromatographic separation and isolation of two liquids

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments And Techniques In Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

- Science: 5.1, 5.2, 5.4, 5.5, 5.8
- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 10 Hours [4 class days]

Chemistry Laboratory: Instrumental Methods

Unit 5 Product Identification; Elemental Analysis; Index of Refraction; Infra Red Absorption; Ultraviolet and Visible Absorption; Nuclear Magnetic Resonance

Objectives:

- Methods of identification of unknowns
- Elemental analysis and empirical formulas
- Theory of Infra Red Spectroscopy
- Theory of Ultraviolet and Visible spectroscopy
- Identification of structure with NMR

Content:

- Calculation of empirical and molecular formulas from combustion analysis
- Infra Red absorption phenomena and structure
- Electron excitation and UV/Visible absorption as related to structure
- Nuclear magnetic resonance theory and applications

Skills:

- TSWBAT learn the theoretical determination of structure and identity using:
  - Elemental Analysis
  - Molecular Weight
  - Infra Red Spectroscopy
  - UV/Visible Spectroscopy
  - Nuclear Magnetic Resonance Spectroscopy

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

- Paper identification of unknown chemicals

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments and Techniques In Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

- Science: 5.1, 5.2, 5.4, 5.5, 5.8
- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 10 Hours [4 class days]

Chemistry Laboratory: Instrumental Methods

Unit 6 Infra Red Absorption Spectroscopy

Objectives:

- To gain proficiency in scanning with an Infra Red Spectrometer
- To use Infra Red data for structure elucidation

Content:

- Practice scanning and interpretation in a real time environment

Skills:

- TSWBAT perform and evaluate known structures utilizing Infra Red Absorption spectroscopy

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

- Infrared Absorption Spectroscopic evaluation of known chemical structures

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments and Techniques In Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

- Science: 5.1, 5.2, 5.4, 5.5, 5.8
- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 10 Hours [4 class days]

Chemistry Laboratory: Instrumental Methods

Unit 7 Ultraviolet and Visible Absorption Spectroscopy

Objectives:

- To gain proficiency in scanning with a UV/Visible Spectrometer
- To use UV/Visible data for structure elucidation

Content:

- Practice scanning and interpretation in a real time environment

Skills:

- TSWBAT perform and evaluate known structures utilizing Ultraviolet and Visible spectroscopy

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

- Ultraviolet and Visible Spectroscopic evaluation of known chemical structures

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments and Techniques In Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

- Science: 5.1, 5.2, 5.4, 5.5, 5.8
- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 10 Hours [4 class days]

Chemistry Laboratory: Instrumental Methods

Unit 8 Nuclear Magnetic Resonance Spectroscopy

Objectives:

- To gain proficiency in scanning with a NMR Spectrometer
- To use NMR data for structure elucidation

Content:

- Practice scanning and interpretation in a real time environment

Skills:

- TSWBAT perform and evaluate known structures utilizing Nuclear Magnetic Resonance spectroscopy

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

- Nuclear Magnetic Resonance Spectroscopic evaluation of known chemical structures

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments and Techniques In Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

- Science: 5.1, 5.2, 5.4, 5.5, 5.8
- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 10 Hours [4 class days]

Chemistry Laboratory: Instrumental Methods

Unit 9 Structure Determination of Unknown Chemical Compounds

Objectives:

- To use various instrumental techniques for the elucidation of the structures of unknown chemicals

Content:

- Analysis and prediction of structures of unknown chemicals in a Real Lab environment

Skills:

- TSWBAT perform and evaluate unknown structures utilizing Combustion Analysis, Molecular Weight Determination, Chromatography, Infra Red, UV/Visible and NMR spectroscopy

Assessment:

- Class Participation
- Objective Evaluation [Quizzes, Tests, Worksheets]
- Laboratory Results
- Attitude

Projects and Products:

Determination of chemical structures and purity using Combustion Analysis, Molecular Weight Determinations, Chromatography, Infra Red, UV/Visible and NMR spectroscopy

Resources:

- Text: Principles of Instrumental Analysis, Fifth Ed., D. A.Skoog, F. J. Holler, T. A. Nieman, Saunders, 1998.
- Experiments And Techniques In Organic Chemistry, D.A. Pasto, C. R. Johnson, M. J. Miller, Prentice Hall, 1991.
- Handouts
- Internet Articles

NJ Core Curriculum Content Standards:

- Science: 5.1, 5.2, 5.4, 5.5, 5.8
- Cross Content Workplace Readiness – CCWR standards 1-5

Duration: 17.5 Hours [7 class days]

Curriculum Map: **Chemistry Laboratory, Instrumental Methods**

	September	October	November	December	January
Content	Analytical Methods: Description and Theory; Acid-Base, Oxidative Titrations; Separation and Purifications	Recrystallization; Melting Points; Molality; Molecular Weight by Freezing Point Depression	Distillation; Boiling Points; Vapor Pressure Determinations; Theoretical Plates; Product Isolation	Chromatography; Solid Supports; Solvent Effects; Band Movement; Elution Techniques; Product Isolation	Product Identification: Elemental Analysis; Index of Refraction; Infra Red Absorption; Ultraviolet and Visible Absorption; Nuclear Magnetic Resonance
Skills	TSWBAT: Organize analytical methods into logical sequences Relate appropriate analytical methods depending on product type	TSWBAT: Do recrystallizations; Perform melting point determinations; Determine molecular weight	TSWBAT: Do distillations; Determine boiling points; Achieve proficiency in product isolation; Calculate theoretical plates	TSWBAT: Perform simple liquid solid phase chromatography; Watch band movement via visible and UV light; Practice elution techniques	TSWBAT: Learn the theoretical determination of structure and identity using elemental analysis, molecular weight, infra red, UV/V is, and nuclear magnetic resonance spectroscopy
Assessment	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]
NJ Standards	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5

Curriculum Map: **Chemistry Laboratory, Instrumental Methods**

This course is an opportunity for intensive hands-on operation of the most common instrumentation used in modern commercial and research

	February	March	April	May	June
Content	Infra Red Spectroscopy: Practice Scanning and Evaluation	Ultra Violet/Visible Spectroscopy: Practice Scanning and Evaluation	Nuclear Magnetic Resonance Spectroscopy Practice Evaluation	Unknowns	Unknowns
Skills	TSBAT: Perform and evaluate chemical structures via infra red spectroscopy	TSBAT: Perform and evaluate chemical structures via ultra violet and visible spectroscopy	TSBAT: Evaluate chemical structures via nuclear magnetic resonance spectroscopy	TSBAT: Analyze and predict the chemical structures of unknown chemicals in a Real Lab environment	TSBAT: Analyze and predict the chemical structures of unknown chemicals in a Real Lab environment
Assessment	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]	Class Participation; Objective Evaluation [Quizzes, Tests, Worksheets, Laboratory Results and Attitude]
NJ Standards	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5	Science S 5.1: S 5.2: S 5.4: S 5.5: S 5.8: CCRWR S 1-5

Inquiry-based Activities for Quantitative Analysis in Chemistry

I. Chromatography

Any of various techniques for the separation of complex mixtures that rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass, such as paper, gelatin, or magnesia.

Types of Chromatography:

- Paper
- Thin Layer
- Column
- Electrophoresis
- Gas Chromatography (GC)

II. Rate of Reaction

- Organic Acid ---> ester
- Calculate Rate Constant (K)

III. Titrations

- pH
- Strong acid
- Weak acid
- Buffer
- Conductivity indicator
- Redox

IV. Freezing point depression to calculate GMM

V. Spectrophotometry and Beer's Law

- Calculation equilibrium constant
- Infra-Red Spectrophotometry

VII. Calorimetry

VIII. Lead busters

IX. Independent Study

Analytical Process Category: Chromatography

Background Information:
The general theory underlying all Chromatography is that a mixture is made of components which retain their individual physical properties. Among those physical properties are attractions to different substances. The mixture can be separated by having it move through or across the surface of a material to which some components are more attracted than others, being "dragged" by a solvent, or elutant, to which the components are also differently attracted.
The following information and terms need to be noted:
1. Stationary phase - the material the mixture is moving through (or across).
2. Mobile phase - the solvent, or elutant, which is flowing across the stationary phase and dragging components of the mixture with it.
3. The distance the solvent moves.
4. The distance each component moves.

There are several types of chromatography, determined by the nature of the mobile and stationary phases.
Paper chromatography: The stationary phase is a strip of paper, and the solvent or mobile phase, moves by capillary action, taking the dissolved components with it. The solvent is a liquid, usually water.

Thin Layer Chromatography: The solvent moves across a plate that is coated with a thin layer of inert material such as silica. The plate is usually horizontal and the solvent can be any liquid. Electrophoresis is a special type of thin layer chromatography, where an electric current is used to pull the material across the plate. Electrophoresis is used to separate the components of DNA.

Gas Chromatography: the solvent is an inert gas like Helium and the stationary phase is a long tube. The material being separated is a mixture of volatile substances which travel at different rates.

The ratio of the distance a particular component moves to distance the solvent moves is called the Rate of flow - R_f - and is a property of the component (and the solvent). The R_f can be used to identify the material since it doesn't vary for the material, if the stationary and mobile phases don't vary. Each Chromatography investigation has to consist of two parts. The first part is making or finding, the standard, using known materials, so you can find the R_f. The second part is running the unknown material and comparing its R_f to the standard. The analyst standards might be able to obtain standards from other researchers, providing others have used the same stationary and mobile phases for the material.

INVESTIGATION: Thin Layer Chromatography

PART I. PLANT PIGMENTATION

Background Information: Plant pigments are an essential part of the process of photosynthesis, as well as other plant processes. Pigments are light absorbing compounds whose color depends upon the wavelengths of light that are absorbed. Light energy of different necessary wavelengths is absorbed by the plant during photosynthesis. Chlorophyll absorbs red and blue light, and reflects green; hence chlorophyll containing plants are green. The most common types of chlorophyll are called chlorophyll a and b. Other pigments also occur in chloroplasts. They absorb light at wavelengths other than the ones absorbed by chlorophyll. Most plants contain carotenoids as accessory pigments - of which carotenes and xanthophylls are the most common. These are yellow or orange. Anthocyanins are red or blue pigments. (Anthocyanins are the only pigments which are slightly water soluble.)

There are two parts to this process:

1. **Extraction of pigments**
   Plant pigments are not soluble in water, as they are large organic molecules. They are soluble in organic solvents such as ethanol, octane, and oil. The pigments will be separated using two solvents. The first is ethanol and water mixed in a ratio 90:10. The second is iso-octane and ethanol mixed in a ratio 90:10

2. **Chromatographic separation of the pigments**
   The pigments are light sensitive and need to be handled with care. The fat soluble plant pigments are readily separated on a plastic or glass plate coated with silica gel (the stationary phase) and using a mixture of iso-octane: ethanol:: 90:10 as the mobile phase.
   The quality of the separation depends on the quality of the plate and the saturation of the plate environment with the elutant before the separation takes place.

PURPOSE(S):

1. To identify pigments in leaves
2. To learn to use thin layer chromatography

PROCEDURE:

I. Extraction of pigments from a leaf

A. Select a single type of leaf, chop it and place in a test tube.
B. Add 1 mL of the first extracting solvent, an ethanol and water 90:10 mixture.
1. Stir and "mush" until the liquid is nice and green.
2. Pour the liquid into another tube and add 1 mL of a saturated NaCl solution to the liquid.
3. Discard the solid.
4. Add 1 mL of iso -octane and ethanol 90:10 mixture, shake. Two layers will form.
5. Remove the gasoline (top) layer of solution, let evaporate to reduce liquid.
6. Do not allow it to dry completely. Keep it away from light.

(Additional TINY amounts may be used to make the extraction more manageable)

II. Separation of Components of pigment

C. Obtain a plate with the stationary phase of silica gel. Use the template to spot pigments on the plate and be very careful to keep the spots small. Do several applications and allow them to dry in between. Keep a record (perhaps a little map) of the migration and location of each spot. If you have only one type of leaf, make a few spots and take an average of your results.

D. Place the mesh on the glass plate and add a few drops of mobile phase mixture. This will saturate the system so that the plate does not dry out.

E. Insert the wick in the slot and fill all compartments with mobile phase mixture.

F. Using the octane / ethanol mixture as a mobile phase, allow the solvent to flow for 4 - 5 cm.

G. Measure Distance solvent moved, and the distance each spot moved.

Record all data on a table.

DATA ANALYSIS:

1. Calculate R_f for each spot on the plate. Add to table.
2. Look up the expected R_f for possible components, or compare to the standards you ran and identify the pigments.

STANDARDS

Expected R_f (When iso octane is used)
CAROTENE 0.75
CHLOROPHYLL A 0.30
CHLOROPHYLL B 0.23
LUTEIN 0.19
VIOLAXANTHIN* 0.13
NEOXANTHIN* 0.09

CONCLUSION:
Write a brief summary about the process of thin layer chromatography. Explain how it can be used to analyze materials, as well as separate them. What pigments were in your leaf? How does it compare to other leaves analyzed by other groups? (Be specific.)
Discuss possible reasons for your results not comparing exactly to the standard reference values.

Investigation: Column Separation

COMMENTS:
When a mixture passes through a column packed with gel beads, the molecules will separate according to size. The column is filled with the beads as well as with 0.9% NaCl solution. The beads cannot be allowed to dry out. The NaCl is also the elutant which pushes the mixture through the column. The test for starch is the blue color that forms in the presence of iodine.
The test for glucose is the deep red color that forms in Benedict's solution. This masks the test for starch and both cannot be done on the same sample. Therefore, a few drops of each sample must be removed for performing the iodine test for starch.

MATERIALS/ EQUIPMENT:

10 test tubes labeled 1A - 10 A 10 test tubes labeled 1B - 10 B
Test tube rack
Ring stand with 2 clamps
Water bath
Gel Column
1% glucose/0.5% starch mixture
Hot plate with water bath
Benedict's solution in dropping bottle Iodine solution in dropping bottle
0.9% NaCl solution in large dropping bottle.

PURPOSE(S):

1. To purify substances in a Starch-Glucose mixture using gel columns.

Before you start: predict which material you think will separate out first.

PRELIMINARY ACTIVITIES:

Prepare mixture that is 0.5% starch and 1 % glucose.
Weigh 0.5 grams of starch and 1 gram of glucose and dissolve in 100 mL of warm water. Heat gently and swish until the mixture is hazy, but no sediment is present. The entire class can share this mixture.

Prepare 0.9% salt solution.
Weigh 9 grams of NaCl and add to 991 grams of deionized water.

Prepare 20 test tubes numbered 1 A - 10 A and 1B - 10 B. Use the very small tubes with etched circles and number them in pencil. Place them in order in a test tube rack.
Add 2 drops of iodine to each tube 1B - 10 B.

Put 1 mL of water in a tube of the same size and use it as a guide to filling each tube to that height during the time you are running the separation. About 23 drops from the column is 1 mL.

Set up a hot plate with water baths for each group.

PROCEDURE:

1. Clamp column to a ring stand. Place a clamp below it so that the small test tube is supported and can stand under the tip.

2. Cut the tip off the bottom of the column. Place a beaker under it. Remove the top cap to allow the liquid to flow out into the beaker. Add two or three mL of NaCl solution to the top of the column and let it all flow into the beaker. When the flow is done, cap the outlet spout (at the bottom).

3. Use a plastic pipette to add 2 mL of the starch/glucose mixture to the top of the column. Remove outlet cap and allow the column to drain until the flow stops. (The mixture is held in the column.) Replace the outlet cap.

4. Add more 0.9% NaCl solution to fill the top of the column.

5. Remove the cap on the outlet tip and collect 1 mL of fluid into each test tube 1A - 10A. You will need to add small additional amounts of NaCl solution to keep the mixture flowing out of the column.

6. After all 10 samples have been collected, cap the outlet. Make sure there is NaCl solution filling at least half the space ABOVE the gel in the column.

7. Pour a few drops of solution from test tube 1A into 1B, from 2A into 2B etc. Check the B tubes for the presence of starch by holding each against white paper and looking for the distinctive blue color that is the test for starch.

8. Add 1 mL of Benedict's solution to each tube 1A - 10 A. Place all of the A tubes into the warm water bath and wait until the reddish color that indicates the presence of glucose develops. (You can determine the relative amount of glucose after the precipitate settles by comparing the amount of blue color left in the solution - the more blue, the less glucose.)

9. Create a table and record which tubes test positive for each of the components.

SAMPLE
Test tube Benedict's test Iodine test
1A
1B
2A

2B

CONCLUSION

Was your prediction correct?

What happened in the column that caused the separation?

Suggest two other materials that might be separated in this manner.

Investigation: Paper Chromatography

Materials:
Samples of $AgNO_3$, $Pb(NO_3)_2$ and $Hg_2(NO_3)_2$ salt
Mixture of salts
Glass capillary tubes
Paper strips
Plastic tube with cap
Glacial Acetic Acid Solution.
Color developer
Part I: Make the standards.

1. Each standard is a known solution of $AgNO_3$, $Pb(NO_3)_2$ and $Hg_2(NO_3)_2$
2. Write the name of the salt at the top of the strip. Use pencil.
3. Apply a tiny drop of salt solution to a strip of paper one inch from the bottom. Let it dry and apply a second and then a third drop.
4. Make sure it dries between applications or it will protrude out too far.
5. Attach the strip to the red plastic cap by weaving it between the little prongs.
6. Pour Glacial Acetic in the tube to a level of about 1 cm.
7. Place the strip into the solution and fit the cap to the top of the tube.
8. When the solvent has ascended about 12 cm, remove it from the tube and mark the solvent front with a pencil.
9. Allow it to dry.
10. Spray with the potassium chromate solution to turn the salt spot to its characteristic color.
    You now have a set of three standards.

Identification of Unknown:
Obtain a sample of a unknown mixture of two of the salts. Spot the paper strip and run the chromatogram.
Compare the results to the standards and identify the salts in the unknown mixture.

Investigation: The Equilibrium Constant

Purpose:

- To measure the concentrations of substances in equilibrium with each other.
- To calculate the equilibrium constant for this reaction at the temperature of the room.

Overview: This is the equilibrium reaction:

$Fe^{+3} + SCN^{-1} <==> FeSCN^{+2}$

Both of the reactants are practically colorless, while the product is red.

Two facts help us track the progress of the reaction and analyze the equilibrium.
First, we can always measure the amount of stuff we put into the reaction.
Second, the product can be measured by using a spectrophotometer to calculate its concentration according to the intensity of its color.

$$Ke = \frac{[FeSCN^{+2}]}{[Fe^{+3}][SCN^{-1}]}$$

In order to measure the $[FeSCN^{+2}]$ the spectrometer attachment to the Logger Pro will be used. A standard solution must be made to use for comparison. The red $[FeSCN^{+2}]$ solution absorbs blue light, so the spectrometer will be set to send blue light at a wave length of 448 nm through the solutions and the absorbance will be measured. Absorbance is proportional to the concentration, so a standard solution is needed for comparison.

Once you know the concentration of $FeSCN^{+2}$, you can calculate the remaining concentrations of Fe^{+3} and SCN^{-1}, and then calculate the Keq.

Preliminary Activity: Prepare solutions.
GOGGLES AND APRONS are a necessity.
Handle with caution.

1. **Prepare a 1000 mL of a solution that is 0.2M $Fe(NO_3)_3$**
Use 1.0 M HNO_3 instead of water to prepare this solution.

The formula is Fe $(NO_3)_3$. 9 H_2O, which means that you are going to weigh molecules that have water of hydration in them. The mass of the water has to be included in the GMM.
Calculations:
GMM =
0.2 M = _____ moles / Liter = _____grams/ Liter
100 mL will require _____ grams.

Weigh the correct amount, place in a 100 mL volumetric flask and fill to the line with 1 M HNO_3 solution.

2. Prepare approximately 0.002 M KSCN solution.
Calculations:
GMM =
0.002 M = _____ moles / Liter = _____grams/ Liter
The amount of KSCN is so small that it will be easier if you do not try to measure out exactly 0.002 moles. Weigh as close to the correct amount as you can. It is not as important that you weigh the exact amount calculated above as it is that you know the exact amount you weigh. Make sure that you know the exact mass and calculate the correct molarity.

Mass of KSCN weighed _____ g which is _____ moles

Actual M of the KSCN Solution _____ M

Wherever 0.002M ** KSCN is written, you need to substitute the actual M.

Place KSCN in a 1000 mL volumetric flask and fill to the line with water.

3. Prepare the 0.002 M solution of $Fe(SCN)^{+2}$
You need to dilute the 0.2M Solution.
Assume that you are starting with 10 mL of the 0.2M.
How many mL of solution will you need to make to dilute it to 0.002M?

4. Prepare the standard solution of $Fe(SCN)^{+2}$ to use in the spectrometer.
This is made by the reaction between Fe^{+3} and SCN^{-1} that you made in preliminary steps #1 and #2 above.

Add 2. 0 mL of the KSCN solution to a 25 mL graduated cylinder. Add 0.2 M $Fe(NO_3)_3$ solution to the 25mL mark.
Calculate the concentration of KSCN after it has been diluted from 2 mL to 25 mL.

The standard solution contains so much more Fe^{+3} ion then SCN^{-1} that It can be safely assumed that all of the KSCN has been converted to $Fe(SCN)^{+2}$.
The standard equilibrium concentration of $Fe(SCN)^{+2}$ is _____M.

Procedure:
1. Label five 10-mL graduated cylinders 1-5
2. Mix solutions in each according to the chart provided by your teacher.
3. Set aside until the spectrometer is ready.
4. Connect Spectrometer to the computer via the USB port.

   a. Turn on Computer, Select Logger Pro
   b. Pull down the Experiment Menu
   c. Select Connect Interface → Spectrometer → Scan for Spectrometer
   d. If the computer tells you that you have to calibrate the Spectrometer, prepare a blank by filling a cuvette 3/4 with **distilled water**.
   e. Calibrate (if necessary).
   f. Choose Calibrate → Spectrometer from the Experiment Menu
   g. Place cuvette with distilled water in slot.
   h. Click OK

5. Measure the Absorbance of the standard solution.
   a. Place the cuvette with the standard solution in the Spectrometer.
   b. Click on the Configure Spectrometer Data Collection icon, located fourth from the right end of the toolbar.
   c. When the display opens click Absorbance vs. Concentration, under Set Collection Mode.

The wavelength of 448 nm must be used. Scroll down to 448 in the list of wavelengths. Click OK to close the display.

d. Place the standard solution in the Spectrometer. When you insert the cuvette, **MAKE SURE THE PLAIN FACES ARE IN THE PROPER ORIENTATION.** Click Collect, and then click Keep. The Absorbance will appear in red in the lower left hand corner of the screen. Record it in the table.
e. Fill 5 cuvettes 3/4 full with each of the solutions from Procedure 1, place each cuvette in the spectrometer. The absorbance will automatically be measured. Read the values in the lower left hand corner of the screen and record in the table.

Data Analysis:
1. When the solutions were mixed, and water was added, the original concentration of each solution was diluted. Ex., the first test tube originally had 1 mL of .002 M KSCN. After mixing, the same number of moles was in 10 mL of solution. Calculate the concentration (Molarity) of the Fe^{+3} and the SCN^{-1} ions in each test tube after mixing. ($M_aV_a=M_bV_b$). Record data in the table below.
2. Absorbance is proportional to the [$FeSCN^{+2}$]. The standard gives you one relationship.

$$\frac{\text{Standard } [FeSCN^{+2}]}{\text{Absorbance of standard}} = \frac{\text{sample } [FeSCN^{+2}]}{\text{Absorbance of sample}}$$

Calculate the [FeSCN+2] for each sample and record in the table below.

*
After the equilibrium is established, the original [Fe+3] and the original
[SCN-1] have each been reduced by the amount of Fe (SCN)+2 that has formed, as calculated from the absorbance in table above. Record these final molarities in the table below. Calculate Keq.
*
Calculate Keq for each trial and record in table.
Calculate the average K_{eq} for the five trials. If you were allowed to discard one trial, which would you select? Why? What happens to your average if you discard one trial?
Results analysis

1. The value of Ke should not change from one trial to the next. Using the average value for your trials, calculate the deviation of each trial from the average. The smaller the deviation the more precise your experiment was.

The average deviation is the Precision.

2. The true value for this Ke is _____ (to be given by the teacher)
The difference between the true value and your value is the accuracy of the experiment.
Calculate the % error.
Conclusion and Questions
1. Discuss the errors that might be caused by each of the following procedural mistakes.
   a. Weighing
   b. Measuring Volume
   c. Using improper dilutions
   d. What other procedural errors might be inherent in this protocol?
   (Protocol is another term for procedural steps and methods.)

2. What human errors might you have made during the course of this investigation?

Investigation: Freezing Point Depression to Measure Molar Mass

Purpose(s):

- To use Temperature Probes
- To measure the freezing point of lauric acid
- To measure the freezing point depression of Lauric acid when a known mass of a solute is added
- To calculate the molecular mass of the solute

Background Information:

The presence of a substance dissolved in a solvent will cause the freezing point of the solvent to be lowered. The amount of F. Pt Depression is a constant property of the solvent. The amount the temperature falls is proportional to the number of solute particles in the solvent. This is purely a physical event and is independent of the nature of the substance that s dissolved in the solvent. (It is called a colligative property.)

The ratio of solute to solvent is calculated differently from Molarity. In Molarity, you do not know the exact amount of solvent. You know the amount of solute and the total volume of solution. This is true because the solution is used to deliver measured amounts of solute into a reaction. Nobody cares about the solvent.
For this investigation you are interested in the amount of solute and its effect on the solvent. The total amount of the solution is irrelevant. The ratio of solute to solvent is called molality. The other difference between molality and Molarity (besides the fact that one is M and the other is m) is that the solvent is weighed.

The molality is # moles of solute / 1000 grams of solvent.

When water is the solvent, the difference is minor, since water is 1 g / 1 mL, so 1000 mL is about 1000 g. Often, the solvent is not water.

In Part I of this investigation, lauric acid, an organic acid, is the solvent. It is normally a solid at room temperature. In order to find its freezing point, you must first melt it to cause it to be in a liquid phase. This happens at around 44 -45 ˚C. Then you will allow it to cool in a water bath, using a temperature probe to record the temperature at which it turns back into a solid (i.e., freezes).

The second part of this investigation will be to find out how much a weighed sample of another substance, the solute, will lower the freezing point of lauric acid measured in part 1.
The Molar mass of the solute will be calculated using the relationship
$\Delta T = = K_f m$

ΔT = Lowering of the freezing point
K_f is a constant for the solvent, (3.9˚C kg/mole)
m is the molality of the solution (in moles of solute/kg lauric acid).

Moles is the unknown value in this investigation. You will solve for it and use it to calculate the GMM of the solute.

Materials:
Logger Pro, Computer, Temperature Probe
400 mL beaker
Ring stand
Clamp to hold test tube
18x150 test tube
Lauric acid
Solute
Thermometers
Hot water bath - over 80˚C to melt solvent and solution
Cool water bath - approximately 25˚ C to freeze solvent and solution

Procedure:

Part I: Measure Freezing point of Lauric acid.
1. You must wear goggles!
2. Open computer file icon, select Chemistry with Computers, select Experiment 15, and freezing point depression.
   x axis time scaled from 0 - 10 minutes, y axis temp from 0 to 100 ˚C
3. Add about 300 mL tap of water between 20 and 25 °C to a beaker.
4. Clamp test tube containing melted Lauric acid to the stand. (Careful, it's HOT!!!)
5. Insert Temperature Probe into Lauric acid. Wait about 30 seconds for the probe to achieve the same temperature as the acid.
   Click Collect to begin data collection.
6. Immediately lower the test tube into the beaker. The water level has to be higher than the level of acid inside the tube.
7. Gently use the probe to stir the acid. (DO NOT HOLD THE PROBE BY ITS WIRE!!!)
8. Continue collecting data for 10 minutes. When data collection stops, place the test tube in a hot water bath to melt the Lauric acid and remove the probe. (Do not try to pull the probe out of the solid acid.)
9. Drag the cursor to the start of the flat part of the graph and drag it across the entire plateau. Click on the statistics button. This will list the mean temperature value, which will appear in the statistics box on the graph. Record this value as the freezing point of lauric acid.

Part II. Determination of freezing point of a solution of solute and lauric acid

Procedure:

From Data Menu, choose Store Latest Run. Click Temperature axis label and uncheck Run 1 Box. Click OK. This enables you to collect a second set of data.

1. Weigh approximately 1 gram of solute. Record exact amount.
2. Weigh approximately 8 g of Lauric acid. Record exact amount.
3. Place both samples in the same test tube. Melt the solution in the hot water bath.
4. Repeat the steps from Part I to find the freezing point of the solution.
5. In this case the graph does not turn into a nice horizontal plateau at the freezing point. The graph decreases until the freezing point is reached and then decreases more slowly. To find the exact freezing point, click on the Examine button. Drag the cursor across the graph until it gets to the point where the slope changes and read the F. Pt when it is displayed.
6. To print the graph click Temp axis label. Check Run 1 and Latest boxes.

You can label the two curves by using the Make Annotation from the Analyze menu and dragging to the appropriate line.

Data Analysis:

1. Find the ΔT between pure lauric acid, T_1 and the solution, T_2
2. Calculate the molality of the solution m, from the relationship $\Delta T = K_f m$.
   K_f for lauric acid is 3.9 ˚C-kg/mol
3. Calculate the GMM of the solute from the moles calculated in #2 and the number of grams of solute.
4. Obtain the true value and calculate the percent error.

Conclusion:

1. Discuss possible sources of error in this investigation

2. Boiling point elevation is another colligative property of solutions. Discuss why salt is more useful than sugar in melting ice on the road. (This has nothing to do with taste, attracting flies or anything like that. It has to do with particles.)

3. Write a conclusive statement about this investigation.

Investigation: Finding Molecular Weight from Boiling Point Elevation

This assignment has no instructions other than the tasks outlined in the purpose.

Purpose:
Design an experiment to measure the Boiling Point Elevation of water when a measured amount of salt is added.
Use distilled water.
Calculate the GMM of the salt.

Use either $CuCl_2$ or $Al_2(SO_4)_3$.
Use 100. g of water.

Hint: Remember, the molality in this case is of the total number of ions/ kg of solvent
Hint: Work fast. Remember, as the water boils, it leaves the beaker. Maybe you ought to weigh it after it cools to see how much you lose.
The Boiling Point Constant for water in 0.52˚C kg/mol

Each group writes a lab report.
Time allotted:
Experiment - 2 days
Lab report - 2 days

Investigation: TESTING FOR LEAD IN SOIL

Purpose:

To learn the techniques needed to prepare and test samples of soil for lead.

PROBLEM: Use the testing apparatus to distinguish quantitative differences in the amount of lead.

Use purchased topsoil, which is presumably lead-free. We will call this "domestic" soil. This will serve as our control. We will compare the testing procedure with soil which we have sampled from the environment, "wild" soil.

Preliminary activity:

A. Weigh an empty 50 mL centrifuge tube. Add between 0.250 and 0.500 mg of soil. Weigh again to get exact weight.
B. Add 5 mL of 25% HNO_3, stir thoroughly.
C. Add water to the sonicator so that it covers the level of liquid in the centrifuge tube. Run the sonicator for 30 minutes.
D. Add distilled water to the 50 mL mark on the tube.

E. Shake the tube vigorously and allow the soil to settle. Decant the supernatant liquid into a clean 5 mL screw cap sample bottle.

F. Add one Paceprep tablet and crush with a NEW crushing/stirring rod until the tablet completely dissolves.

Testing Procedure:

Each sample is assigned a project ID number.

Press on. The HOME SCREEN is displayed, showing project ID number. Scroll down using the V until the word SOIL is shown on the top line. Press ENTER. The screen will display the current calibration code, which should match the code on the electrode package. The system checks itself, and may warn if the battery is low.

Enter the correct sample ID number. Press enter when the correct six digit number has been entered.

At the prompt INSERT ELECTRODE, attach the electrode to the cap of the testing apparatus and make sure it is properly plugged into the instrument. Immerse the electrode into the sample vial. Do not disturb the instrument or tube holder during the test.
The reading for Lead in soil will appear for 30 seconds. (It can be retrieved by pressing ON and > repeatedly.

(< means [Pb] is too low to read, > means [Pb] is too high to read.)

Repeat using samples taken from the environment.

REMEMBER, THE REASON WE ARE DOING THIS IS THAT LEAD IS A POISONOUS, POLLUTANT CHEMICAL. HANDLE IT CAREFULLY. FOLLOW THE RULES:
****DO NOT TOUCH IT WITH YOUR BARE HANDS.**
**** WASH YOUR HANDS CAREFULLY, EVEN IF YOU DID NOT TOUCH THE CHEMICAL**
**** DO NOT PUT YOUR FINGERS NEAR YOUR MOUTH**
**** NO FOOD NEARBY!!!**

-

SAMPLING PROCEDURE:
*** *DON'T USE GLASS OR METAL CONTAINERS OR SHOVELS, as glass and metal may contain lead.** Plastic sandwich bags are good, but make sure the writing is on the outside. Plastic spoons are good. **AVOID ANYTHING THAT HAS BEEN DYED, WRITTEN ON, PRINTED ON ETC.**, as dyes and paints may contain lead and contaminate your sample.

Select the site and mark a circle of about one meter in diameter. Remove four or five teaspoons <u>from the surface</u> of different places in your circle. Your samples should not come from deeper than 2 - 3 inches. Make sure you mix well!! Make sure each sample is labeled as to the site from which it was taken and the date.
MAKE SURE YOU ASK PERMISSION BEFORE YOU START TO LOOK THROUGH SOMEONE'S PROPERTY!!!

Conclusion:

Discussion points:

a. occurrence and causes of lead pollution
b. common sources of lead pollution/poisoning
c. how people get lead poisoning
d. physiological effects of lead poisoning
e. who are the people most affected by lead poisoning
f. what can be done to help people who have lead poisoning
g. some methods to get rid of lead in soil

Please cite any sources of information.

Investigation: Titrations - Strong acid vs. Strong base

Comments:

Titration is an analytical process.

When you titrate you add measured amounts of one solution to measured amounts of another. You do this to find the equivalence point which is the point at which the moles of reactant in one of the solutions is equal to the moles of reactant in the other. Usually you do this to find the concentration (Molarity) of one of the solutions, when you know the concentration of the other.

The formula is $M_1V_1 = M_2V_2$

When any strong base is added to any strong acid, the net ionic reaction is

$H^+(aq) + OH^-(aq) \rightarrow HOH(l)$

with the H^+ coming from the acid and the OH^- coming from the base.

When the moles of added H^+ = moles of added OH^- you are at the equivalence point of your titration. This is true for any acid or any base, weak or strong. When you titrate a strong acid and a strong base, the ions that are not H^+ and OH^- are spectators (do not interact with the water), and the pH will be 7.

To summarize:

When the acid and base are both strong the following are true:

The pH of the solution is 7.0 at equivalence

The moles of H^+ = moles of OH^-. All other ions in the solution are said to be spectator ions.

If you are titrating a weak acid or a weak base, the ions that are not H^+ and OH^-, **DO** interact with the water. Therefore, the pH will not be 7 at the equivalence point. This will be addressed in the next experiment.

Investigation: Titrations - Strong acid vs. Strong base

Purpose:
To learn how to use the computer to titrate
To titrate HCl against NaOH, draw a titration curve, and have the curve to use as a standard.

Procedure:
A. Set up computer -
Open Logger Pro
1. Double click on File
   Double click Probes and Sensors
   Slide right to pH System
   Select pH titration

2.Graph appears. It should have pH as the y axis, vol (mL) as the x axis.
Click any number on the scale to reset from default values. Set pH to start at 0 and vol to go to 25 mL. **Do not try to change the display after this until you are finished collecting data. You can change scale later, but it's a bad idea to try to alter them during the titration.**

3. At the top of the graph there is a box that says **COLLECT**.

B. Measure exactly 10 mL 0.1 M HCl into the beaker using a pipette.

1. Place the pH electrode into the HCl. Click COLLECT. W hen you press it, two boxes will appear: - STOP and KEEP
   This will start the experiment.
IF YOU PRESS STOP AT ANY TIME, YOU CANNOT RESUME THE EXPERIMENT, AND MUST START ALL OVER AGAIN.

2. Watch the pH at the side of the graph. When it is stable, click KEEP, or press enter.

3. A new box appears that says OK, and next to it a space to type in the Volume of NaOH added. Type in 0. Click OK or press enter

4. KEEP appears. Click. This will register a point on the graph. (If the pH is too low, it may not appear on the graph. Don't worry, the range can be changed later to make it appear.)

5. Add 0.5 mL of 0.1 M NaOH to the HCl. Place the electrode in the solution. Click KEEP, or press return. Type in volume of added NaOH, 0.5 mL return- draws point.
The pH electrode will remain in the solution. It does NOT need to be rinsed between additions of base. You can use it to stir the solution (CAREFULLY!!!).

6. Add another 0.5 mL of 0.1 M NaOH to the HCl. Place the electrode in the solution. Click START; Watch the pH at the bottom of the graph. When it is stable, click KEEP, or press return. -OK appears, type in TOTAL volume of added NaOH, 1.0 mL. KEEP or return- draws point. You must read the total Volume of NaOH and record it, or your points will all be at the same place on the x axis.

C. Continue to add 0.5 mL increments of the NaOH solution to the HCl solution. YOU MUST KEEP A RUNNING TOTAL OF VOLUME ADDED.
In fact, the actual amount of NaOH added is not as important as the fact that you add an accurate number to the total. Don't worry if you do not add exactly 0.5 mL, but make sure you keep an accurate count of the actual mL that was added.

D. Measure the pH after each addition. The graph will be drawn as you go along. There is minimal change, then a steep change, then slow change again. Do not stop adding NaOH until the top horizontal line has been established. This is past the equivalence point.

E. File menu - title graph, print

F. Windows menu - data table, File menu - print data table

DATA ANALYSIS

1. Calculate the $[H^+]$ in the original solution. You know the [OH-], since it was bought prepared. It is 0.1 M.

There are two ways of doing this.
a. Using the pH of the acid before any base was added:
pH = _____ (from experimental measurement)
$[H^+]$ = _____ M
b. Using the equivalence point of the reaction - when $[H^+] = [OH^-]$
Since this is a strong acid and a strong base, the pH when the [H+] = $[OH^-]$ will be 7.
Use the graph to find the volume of OH- when the pH is 7. The volume of H+ is 10 mL.
$V_aM_a = V_bM_b$

2. a. Are the two values calculated in Q#1 the same? b. What might be a reason for any disparity between them? c. Which value do you think is the most accurate? d. Why?

3. a. What is the pH of a solution that contains 9.5 mL of 0.1 M NaOH and 10 mL of 0.1 M HCl?

b. What is the pH of a solution that contains 10.5 mL of NaOH and 10 mL of 0.1 M HCl?

4. Select a chemical indicator that would be accurate for this reaction if you were not measuring pH electronically.

Investigation: Weak Acids, Buffers

OVERVIEW:

Strong acids and bases dissociate or ionize completely. Strong acids Weak acids dissociate only partially and weak bases remove some H+ from water molecules, leaving OH-ions. Buffers are mixtures of two sets of particles, one of which can neutralize small amounts of acid; the other can neutralize small amounts of base. One of the most common buffer types consists of a weak acid and a salt of that same acid.

Pure Vitamin C is Ascorbic acid, $H_2C_6H_6O_6$. Only one H^+ ionizes to any appreciable amount, so we will write it HAsc for short. (As if it has only one H). The non chewable tablet contains 250 mg of ascorbic acid which is a weak acid, ionized partially. It tastes very sour. Kiddies hate it.

Investigation: Part I - Titration of Weak acid vs. strong base

WHAT YOU ARE MEASURING

In a strong acid solution, all the molecules dissociate so:

$[H^+]$= Initial M, $[H^+]$ -->pH

In a weak acid solution, only some of the molecules dissociate so:

$[H^+]$ 2 = (Initial M x Ka), $[H^+]$ --> pH.

If you know pH, you can find $[H^+]$ and use it to calculate Ka. If you don't know pH or $[H^+]$, you must know K_a and use it to calculate the $[H^+]$. (You always know what you started with, unless you're really out of it!!!)

PURPOSE:

To titrate the Vitamin C tablet

To calculate Ka for Ascorbic Acid

To compare equivalence points found by calculation and from the pH titration graph.

DIRECTIONS:

A. **Preliminary activities:**

a. Data

Calculate the GMM of Ascorbic acid, $H_2C_6H_6O_6$

What is the mass of ascorbic acid in each tablet? (See label)

How many moles of ascorbic acid are there in each tablet?

How many tablets do you need to make 100 mL of approximately 0.1M HAsc?

What is the exact M of the HAsc solution?

b. Prepare approximately 0.1M Ascorbic acid solution

c. Use a mortar and pestle to crush the number of tablets calculated above and place in a 100 mL volumetric flask.
d. Wash as much of the powder from the mortar into the flask as you can.
e. Add water to the 100 mL mark.
There will be a lot of undissolved material from the tablet. All of the Ascorbic acid should be dissolved. If there is floating material, make sure it is floating above the volume line on the neck of the flask.
0.1 M NaOH Solution is used as purchased.

B. Measure exactly 10 mL of 0.1 M ascorbic acid solution into a 30 mL beaker. MAKE SURE YOU GET A pH VALUE FOR THE ACID BEFORE YOU START ADDING THE BASE!!! You will need it to find Ka. Add NaOH in 0.5 mL or smaller increments. (Remember that the Volume of NaOH is the total volume added.) Continue until there is a top plateau on the graph

C. Graph the pH vs mL of NaOH added. Print the graph.

Data Analysis:

1. Calculate the initial [H+] present using the pH you measured before you added any NaOH.
pH = [H+] =

2. Calculate Ka. Use the [H+] for pure Vitamin C.

$$K_a = \frac{[H^+]\,[HC_6H_6O_6^-1]}{[H_2C_6H_6O_6]} = \frac{[H^+]2}{\text{Init M} - X} \sim \frac{[H^+]2}{\text{Init M}}$$

3. Obtain the true value for the K_a of Ascorbic acid. Calculate the % error for this part of the experiment.

$$\frac{\text{experimental - true}}{\text{true}} \times 100$$

4. Using the true value for K_a, find the Kb ($K_aK_b = K_w$)

5. The equivalence point occurs when $V_aM_a = V_bM_b$. Calculate Volume of base at the equivalence point.

6. Using the graph, read the pH at the equivalence point. (What is the pH when the [NaOH] is equal to the volume you calculated? It should occur in the middle of the steepest part of the graph. It will not be 7.)

7. Calculate the pH at the equivalence point.
   K_b = [OH-1]2 / [Asc-1]
   The [Asc-1] = $M_a V_a$/Vt

8. Compare the two values of equivalent point pH's.

Part II -Titration of Buffered weak acid vs. strong base

Purpose: To titrate the chewable Vitamin C tablet

Chewable Vitamin C contains a mixture of ascorbic acid and sodium ascorbate, Na2C6H6O6 (Na Asc) . Their total weight is the number of mg indicated on the label. Since the Ascorbate ion is present along with molecular Ascorbic acid there is only a very small amount of H+ present in the solution of the chewable tablet. This makes it taste far less sour than the plain ascorbic acid. It will turn into ascorbic acid when it meets the HCl in your tummy. The chewable tablets are BUFFERS. There is additional material present in the buffer - flavoring, sugar etc.

Procedure: Make a solution that is close to 0.1M buffered ascorbic acid. The recipe is the same as it was in Part I.
Titrate it versus the NaOH solution that you used in the first part.

Data Analysis:

In a buffer, you have a mixture of a weak acid and its salt. Therefore there is less H+ in solution, either as molecules, or dissociated. The only way you can tell the $[H^+]$ that you started with is by finding it from the pH.

$$K_a = \frac{[H^+]\ (\text{init salt})}{(\text{init acid})}$$

and

$$[H^+] = K_a \times \frac{(\text{init acid})}{(\text{init salt})}$$

1. Use the pH to find $[H^+]$. Use the Ka and the $[H^+]$ to find the ratio of Asc to HAsc. This is the best you can do with the information available.

2. Since you cannot know how much of the original tablet was HAsc, (the rest was Na Asc), you cannot use $V_aM_a = V_bM_b$ to calculate the equivalence point of the buffer. Use the graph. The midpoint of the steepest climb is the equivalence point. Estimate the volume Na OH from the graph, and the pH at that point also.

Part IV - Conclusion/ Discussion

1. Write equations for the following:
a. Net ionic for the formation of ascorbic acid from the ascorbate ion and stomach acid (HCl)
b. Net ionic for the formation of ascorbic acid from sodium ascorbate and water.

2. Compare the three titrations - Strong acid, weak acid, and buffered acid with respect to the following:
a. Shape of the graphs
b. pH at initial point
c. pH at equivalence
(One of the best ways to do this is to superimpose two of the graphs and hold them up to the light.)

3. Discuss possible sources of error.
Include mention of the following:
What effect the "flavoring" might have on the experimental results
Accuracy of the pH electrode
Accuracy of the initial Molarities

Mention human and systemic errors.
Remember: If you have not mentioned something as part of your procedure, you cannot blame it for being part of your error.

Chromatography Activity Using Organic Molecules

Introduction

The separation of simple and complex mixtures into their components, as well as the isolation of the individual components, has become an important physical method of separation in all branches of chemistry. Examples are from the research laboratory preparing new chemical compounds to the quality control laboratory testing to assure compliance with federal regulations. The different types of chromatography in common use are paper, thin-layer (TLC), column, and gas-liquid (GC, VPC, GLC).

All of the chromatographic procedures involve the interaction of a mobile phase (either a gas or a liquid) and a stationary phase (either a liquid or a solid). The four basic types are categorized in the following table:

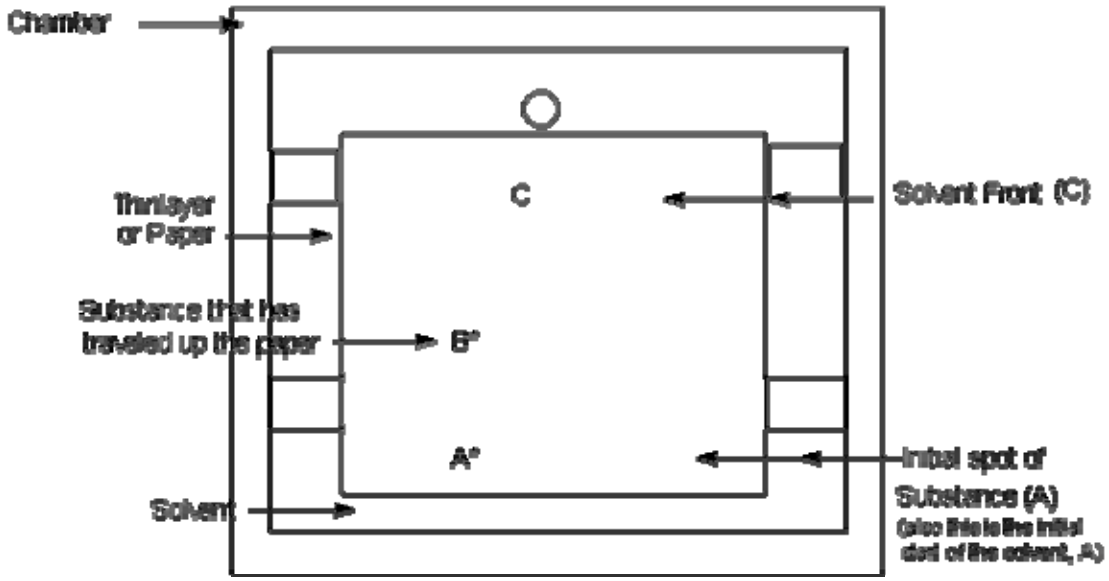
TYPE	MOBILE	STATIONARY	PRINCIPLE OF SEPARATION	USES
column	liquid	solid	adsorption	Preparative scale separations
Thin-layer	liquid	solid	adsorption	Qualitative analysis & small scale separations
Gas-liquid (GLC)	gas	liquid	partition	Qualitative & Qualitative Analysis
Paper	liquid	liquid	partition	Qualitative & Qualitative analysis of polar and ionic compounds

$$R_f = \frac{B - A}{C - A} = \frac{\text{distance spot moved}}{\text{distance solvent moved}}$$

When the stationary phase is a solid, one is dealing with the equilibrium between the adsorption of the compound on the solid surface by adhesive forces (intermolecular bonding between the substance and the molecules of the solid phase) and its solution in the liquid phase (also intermolecular bonding but between the substance and the molecules of the liquid phase). The liquid is passing over the surface of the solid phase in one direction. In column chromatography the liquid normally moves from top to bottom (making use of gravity). In thin-layer (TLC) the liquid moves against gravity by moving by capillary action between grains or particles of the solid phase.

When the stationary phase is a liquid, we are dealing with partition equilibrium between the two fluids (the stationary liquid phase and either the liquid or gas of the mobile phase). Here we are dealing with the relative strength of intermolecular bonding with the substance with each of the fluids in the liquid or gas phase. In gas-liquid chromatography (GLC) the stationary phase is a liquid (silicon oil, carbowax, etc.) that is coating an inert solid that is packed in a tube (glass, stainless steel or copper). In paper chromatography the liquid stationary phase is generally water that is adsorbed onto the fibers of the paper. In GLC the mobile phase is a gas (generally He is used although any inert gas will do). While in paper chromatography, it is another liquid that has different physical properties and constants from those of the stationary phase, even though the liquids themselves may be mutually soluble in one another.

In paper and TLC, you determine the Rf value of a substance under certain experimental conditions. The Rf value is the decimal value of the distance that a substance has moved (from its starting point to its final point after a fixed period of time) divided by the decimal value of the distance the solvent has moved. The solvent is measured from the same starting point as that of the substance, to the solvent fronts (the furthest the solvent has moved).


Chamber
Thinlayer or Paper
Substance that has traveled up the paper
Solvent
C
B°
A°
Solvent Front (C)
Initial spot of Substance (A)
(figure 1)

In GLC the measurement is of the retention time of the substance. This is the time between injection of the sample into the GLC and the time it passes the detector. Today most detectors are coupled with a strip chart recorder and one can measure the time as a function of distance the pen has traveled from injection of the sample until a deflection peak is recorded. A graphic of the GLC function is given in Figure 2.

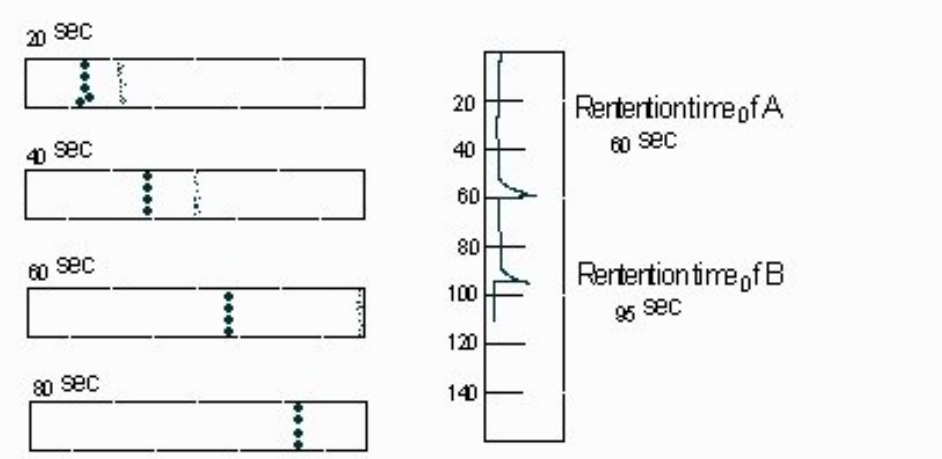


Figure (2)

Under the same conditions of column temperature and carrier gas (He) flow the retention time for a substance will be the same.

Equipment

GLC, 4" x 8" piece of chromatographic paper, ruler, chromatographic chamber, stapler, capillary tubes.

Chemicals

Student - amino acids: **leucine, proline, histidine, alanine, unknowns (paper)**

G.C. unknowns: **Hexane, methanol, n-propanol (flammable)**

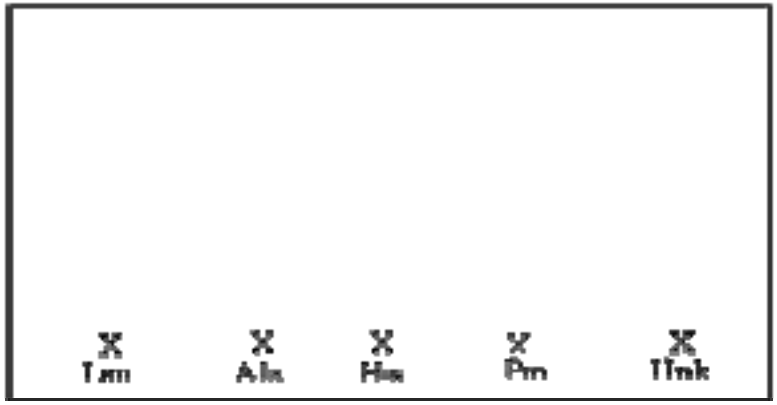
Spill/Disposal

Amino acids: ***Spill/Disposal: A***
G.C. unknowns: small amounts (< 5mls) ***Spill/Disposal: A***
developing solvent: ***Spill/Disposal: A***

IN HOODS
developing solvent (acetic acid, iso-propanol) + ninhydrin (already in chromatography chambers)

Procedure

Part I. Separation of Amino Acids by Paper Chromatography
1. Take a piece (4" x 8") of chromatography paper and draw a light pencil line 3 cm from the longer edge. Place an X at 3 cm, 6 cm, 9 cm, 12 cm and 15 cm. Handle the paper as little as possible. When handling it, hold it on the edges. Under the first X place the letters Leu (this is where the known amino acid, leucine, will be applied to the paper), under the second X place the letters Ala (for alanine), under the third X place the letters His (for histidine), under the fourth X place the letters Pro (for proline), under the last X place the number or single letter of the unknown you are assigned by your lab instructor. All of these marks must be done in graphite (or lead) pencil. Do not use ink. (See Figure 3)



(figure 3)

2. You may want to practice the spotting of a solution on paper. Practice with water and a paper towel (or a piece of filter paper). Do not waste the chromatographic paper for practicing. Place a capillary tube in the water. When you remove the capillary, you will see a small amount of solution on the inside of the capillary even when you don't stopper the upper part of the tube. Now gently touch the end of the capillary with the solution to the paper allowing only a small amount of the liquid to wet the paper. What you want is a small spot of solution on the paper--about 2mm (.2 cm in diameter). If you want more material put on the paper, you should wait until the first spot has dried before applying a second spot of the same material. When you feel you are ready, place a small spot of the leucine solution on the X that you have labeled Leu. Do the same for the remaining X's. Put a spot of the solution for which the X is labeled. Do not use the same capillary for each--you will contaminate each spot with the previous substance. Use a different capillary for each solution (or use the capillary that was present in the solution).

3. Allow the spots to dry. Carefully put the paper into the chromatography chambers that will be located in the hoods. The starting line should not be below the level of the solvent in the chromatography chamber. The developing solution can be hazardous so do not remove chromatography chambers from the hoods. The developing solution will already be in the chamber. Allow the chromatogram to develop at least one hour. The amino acids will move up the paper by capillary action at different rates depending on their relative solubilities in the developing solvent and the water on the cellulose of the paper.

4. After the solvent has risen a sufficient amount, remove the paper and mark with a pencil line to the farthest position on the paper reached by the solvent. Immediately take the paper to the drying chambers also located in a separate hood. Gently dry the paper with the heat guns provided.

5. Heat the developed paper for two to three minutes with the heat guns. The amino acids react with the ninhydrin (in the developing solution) spots. Once the spots are developed sufficiently, remove the chromatography paper from the drying chamber. Circle the spots and measure the distance each has traveled compared to the distance moved by the solvent. Record this on the data sheet. These are the Rf values. Also note the color of the spots.

6. From the R_f values of the known amino acid solutions and the R_f values of the spots of your unknown, what amino acids were present in the unknown?

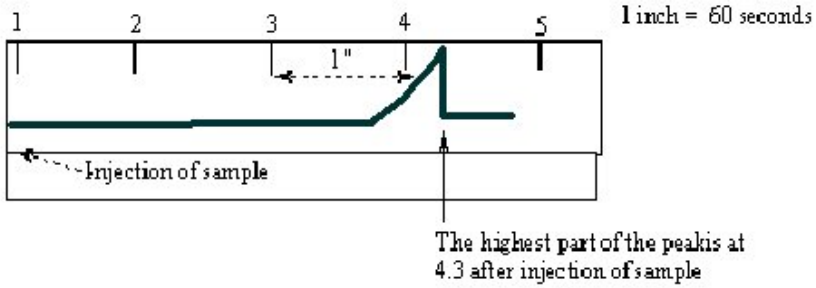
Part II. Qualitative Analysis Using the A Column

1. You will be doing a gas chromatograph of a sample of a mixture that is prepared from the following compounds: hexane, methanol and n-propanol. Your instructor will run samples of the individual pure compounds.

2. The instrument conditions are:
Temperature: 115° C
Carrier gas flow: about 60 ml/min at column exit
Detector Current: 200 mA (He carrier gas)

It is important that you do not change the current, gas flow, or temperature during the experiment. Not only will it affect your results but it could cause serious damage to the instrument. Also do not touch the metal plate, where you inject your sample. Both plates--the **A** and **B** column--will be only a few degrees below the temperature of the columns, which is 110° C, so the plates are about 100-105°C. This is hot enough to blister the skin.

3. Rinse the syringe twice with the sample to be tested. (Squirt the waste rinse into the flask provided at the GC.) Draw a third syringe of the mixture sample from the sample container (2 m L or .002 mL of sample). With the recorder running at 1"/min, inject the sample into the **A** column. Push the needle in all the way through the rubber septum. A metal stop on the syringe will prevent the needle from going in too far. With the needle in the column depress the plunger to inject the sample. Simultaneously, you will need to push the **Start** button on the recorder. The instructor will set the time on the run so that the longest retained material will have cleared the instruments. Do not do this yourself, because the time varies with the temperature.



1. You will note that the different substances take different lengths of time to exit the instrument (reach the detector).

The retention time is the time between when the sample was injected and the time of the highest part of a peak emerging (being detected by the detector). In the above example, the sample emerged 4.2". The instrument that we are now using is an integrator recorder. When the sample is injected, you need to press Start simultaneously. After the sample has run sufficiently, you will press Stop. At this time the recorder will automatically print out retention times and percent area under the curve. (The percent area is relative to the amounts of each chemical in the sample.)

Your unknown graph is then compared to the known samples and you should be able to determine what is in your unknown. Record the data. Determine the amount (in mLs) of the compound that has the highest percent in your unknown.

Chromatography - Final Data Sheet

Name:_____

DO CALCULATIONS AND HAND IN ONE COPY OF THE DATA SHEET PER GROUP WITH PAPER CHROMATOGRAPH AND GAS CHROMATOGRAPH ATTACHED.

Part I

Unknown number	**Color**
Distance solvent rose	
Distance leucine rose	
Distance alanine rose	
Distance histidine rose	
Distance proline rose	
Amino acids contained in unknown:	

Part II	
Known Sample	
Retention time	**% Composition**
Methanol	
Hexane	
n-Propanol	
Unknown sample number:	Components present in mixture
1.	
2.	
3.	

Calculate the milliliters of most abundant component of your mixture: _____ mL

Partners Name _____