

Chem 208

Analytical Chemistry

Laboratory Manual

Fall - 2006

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This manual belongs to

Table of Contents

Table of Contents.....	2
Acknowledgement.....	4
Lab Philosophy.....	5
The Laboratory Notebook.....	7
CRITERIA FOR GRADING THE LABORATORY NOTEBOOK.....	9
Lab Report Policies.....	11
Instructions for Comprehensive Reports.....	12
Laboratory Techniques.....	15
Reagents.....	15
Primary Standards.....	15
Drying at Elevated Temperatures.....	16
Cleaning Glassware.....	16
Chromic Acid Cleaning Solutions.....	17
Cleaning Volumetric Glassware.....	17
Working Surfaces.....	17
Quantitative Transfer.....	18
Reading Instrument Scales.....	18
Weighing Out Samples and Using Weighing Bottles.....	19
Rules for Use of Analytical Balances.....	20
Heating and Concentrating Solutions.....	20
Handling Stock Solutions.....	20
Control Charts.....	21
Volumetric Glassware.....	21
Flasks, Burets and Pipets.....	21
Graduated Cylinders.....	22
Graduated Measuring Pipets.....	22
Volumetric Transfer Pipets.....	22
Absolute Calibration of a Pipet.....	23
Use of a Volumetric Flask for Solution Preparation.....	23
Burets.....	24
Use of a Buret to Carry Out a Titration.....	24
Visual Indicators.....	26
Determination of an Indicator Blank.....	26
Experiment 1 - ACID - BASE TITRATION.....	28
Lab 1 - Check-in, Buret Reading, Sampling solids.....	28
Lab 2-3 Acid-Base Titrations using Visual Indicators.....	29
I. Standardization of 0.1000 M NaOH.....	29
II. Validation of titration procedure using a Standard Reference Material and Determination of an Unknown KHP.....	30
REPORT.....	31
Experiment 2- Potentiometric Titration of Weak Polyprotic Acids.....	32
Experiment 3- Determination of Calcium by EDTA Titration.....	35
Report.....	37
Rotation Experiment 4 - Liquid Chromatographic Determination of Caffeine, Theobromine, and Vanillin in Chocolate By the Internal Standard Method.....	38
Introduction.....	38
Background information on HPLC.....	39
Method Validation.....	40
Preparation for Analysis.....	40
Operation of the Hitachi HPLC.....	42
AutoSampler Operation.....	43
I. STUDIES OF MOBILE PHASE EFFECTS.....	43
II. Preparation of Chocolate Samples.....	43

III. Analysis of Samples	44
IV. Calculations	44
Shutting down the HPLC (Manual system)	45
Report	45
Rotation Experiment 4 - Determination Of Vanillin By Spectrophotometry Comparison of Solvent Extraction and Solid Phase Extraction	46
Solvent Extraction Procedure	47
Method Development and SPE of vanillin	48
Preparation Of Standard Solutions	50
Rotation Experiment 5 - Direct Potentiometric Determinations:	52
Using Ion Selective Electrodes and Calibration Curves	52
Introduction	52
I. Evaluation of the Fluoride ISE	53
II. Analysis of a Fluoride Unknown	54
III. Analysis of F ⁻ in a Real Sample	54
IV. Determination of aluminum fluoride complex ion ratio using continuous variation method (Job's Method)	54
Report	55
Rotation Experiment 6 -Gas Chromatographic Determination of Fatty Acids in Oils Using the Internal Standard Method of Quantitation.....	56
Rotation Experiment 6 -Gas Chromatographic Determination of Fatty Acids in Oils Using the Internal Standard Method of Quantitation.....	56
Introduction	56
Operation of the GC.....	58
Methylation of Fatty Acids.....	59
Analysis of Fatty Acid Samples and Unknowns.....	60
Report	60
Composition of Common Fats and Oils.....	61
Appendix 1 - ChromPerfect Data System Setup.....	62

Acknowledgement

This manual represents almost 30 years of development of experiments. The majority of the experimental development was done by David. E. Henderson. However, the experiments rely heavily on the literature of Analytical Chemistry as noted in each specific experiment. Janet Morrison and William Church have both made significant contributions to the revision, testing, and improvement of both the writing and the experimental design.

Lab Philosophy

The fundamental assumption made of you as you begin Chem 208L is that you are an adult person with a scientific curiosity ready to learn analytical chemistry. This may not be a valid assumption in all cases, but if this is not your approach to this course, then try to pretend that it is. There is much work to be done. This may be one of the most demanding courses you will take at Trinity. However, it is also one of the most useful courses for anyone planning to do science at any level.

What you can learn from this course is extraordinary. This course and Chem 312 that follows are ultimately practical to all aspects of scientific careers. The lab skills which you will learn and refine are the most "marketable" part of your degree. The informed skepticism that you should learn to apply to the data you obtain is an essential approach for all scientists. At the completion of Chem 208 and 312 you will have hands on experience to all but a small fraction of the instruments and methods used in the various aspects of chemistry. Chem 208 will introduce the most commonly used methods and instruments and prepare you for work, summer jobs, and research in chemical laboratories.

Labs (READ THIS PART TWICE)

You will find that detailed instructions for experiments are limited to specific techniques that are not presented in the text. Many details of solution preparation are left to you to figure out. All of these tasks are within your ability with a little thought. This is done on purpose to force you to spend time thinking about the labs and to learn to plan experiments. My goal and that of the TA's is to force you to do most of this preparation before you arrive at the lab. Blackboard Quizzes are provided to assess your preparation. You will not be allowed into the lab without completing the assigned Quizzes. If you take shortcuts and do not adequately prepare for lab, I can almost guarantee you will not have sufficient time to complete the experiments and that no additional time will be allowed. If you come to lab well prepared, you can expect to leave by 4:00 in most cases (this has been demonstrated by well prepared students over the years). Labs will close promptly at 5:00. Therefore, there is a 33% margin of extra time built in. Normally, no further time will be allowed for experimental work.

A proper approach to the lab is to read the experiment and references before you come to lab. You should then reread the experiment and establish a plan of approach to the experiment in detail. Such a plan will detail each step to be taken in the order you feel is the most efficient. Frequently, the most efficient or even necessary order of the experiment will be different than the order of topics in this manual. Your plan of approach must include the weights and volumes of reagents and solutions to be used at each step in the procedure. You know what glassware you have and we will attempt to inform you of the specific reagents available for the experiments. The plan of approach should also include preparation of data tables for the data you will be taking. This will lead to better organization of your notebook and faster work in the lab. YOU

WILL BE ASKED TO SHOW YOUR PLAN OF APPROACH AND DATA TABLES AS A PREREQUISITE FOR ENTRY INTO THE LAB.

As you progress through the semester, you will find the labs are more sophisticated (and the data you get will in general be worse, so don't panic) and require more preparation. The reporting requirements also become more demanding. You will have several opportunities this term to design parts of your own experiments, each time offering more options and greater sophistication.

I hope that, by being forewarned of the expectations of the lab you will not panic when things go wrong in the lab. Changes in procedure are much more easily managed when you understand (translated as not just memorized or transcribed from handout to notebook) what is really going on. Such changes will occur due to a variety of reason ranging from the perversity of the instructor to mistakes in the preparation of solutions. Stay loose!! As you will learn, even procedures printed in the chemical literature do not always work for everyone. This is where you must learn to be skeptical and think about what you are doing. If it doesn't work, figure out why and fix it. Double check your own calculations and those presented in the manual.

Do not hesitate to ask for help at any point in the process. If you don't understand what you are doing before lab, make a point of asking questions in class before the lab or find the instructor before lab. This will insure that you are ready for the lab and will not have to wait until the instructor has gotten things going for the prepared students. When you arrive unprepared in the lab, you go to the bottom of the priority list for help. When you ask for help before lab, you are my top priority.

Finally, a few comments on cooperation between scientists (and students) are in order. No person in science can work in a vacuum (figuratively speaking). The reason for scientific meetings and the journal literature is to provide a means for the sharing of ideas and results. Out of this interaction, new ideas are born. The same can be said for your work in this course. The interactions you have with other students can be very valuable for learning the material. However, the same rules apply to these interactions that apply to those of any group of scientists. **ALL SCIENTIFIC INTERACTIONS MUST BE PROPERLY REFERENCED.** If you work with someone as a lab partner on an experiment, you are both expected to keep a lab notebook detailing what **you** did. The notebooks will not be identical as you will certainly divide the work and will have different styles of presenting what you have done. **All work not done by you must be referenced.** Material copied from your lab partners notebook should be specifically attributed to your partner. This in no way detracts from your own work. Also, when you do a report, if you use someone else's ideas as a part of the report, give them credit for it. And most important, **NEVER COPY ANYTHING THAT IS NOT PROPERLY REFERENCED.** The Student Handbook has some examples of proper practice under the section on plagiarism. **READ IT!** These rules apply to everything you do as a scientist or student.

The Laboratory Notebook¹

A scientific notebook should hold a permanent record of the experimental work. Consequently, the pages should be securely bound (not a spiral binding) and entries made in ink at the time work is done. The pages are numbered and the entries are dated. The format of the entries must be such that the book could be read by any scientist who is familiar with quantitative chemical work. Readability of the original data is essential. Neatness is convenient but not central. Neatness is not a grading criterion for the notebook. Too much neatness is often a sign that the information has not been actually written during the lab.

It is common practice to record data on the right hand page and to use the left hand page for preliminary readings, notes and calculations. The left hand page (back of the carbon page) is the only place you are allowed to write scratch notes. **Any evidence of recording data on scratch paper, lab manual pages, paper towels, etc. will result in a lab notebook grade of 0 for the experiment.**

Errors are a part of scientific work. Data is frequently invalid - experimental conditions may not be adequately controlled, reagents may be contaminated, instruments may not be operating correctly, scales of instruments may be incorrectly read, etc. If results have more than the anticipated variation, the first reaction may be to discard the whole thing and start over. Later more significance may be read into the data. All data - including data that is known to be invalid - remains a part of the laboratory record. To correct an entry, draw a single line through it and enter the correct value above the original value which remains readable. If the reason for the change is not obvious, an explanation should be recorded. If a large section of the work is considered invalid, a single line is drawn diagonally across the page and the reason for discarding the work stated. All entries remain readable. **Under no circumstances is a page removed from the book. To erase or block out data, or to remove a page from a notebook is considered a violation of scientific integrity.** In research, valid notebooks are the basis of determining priority of scientific discoveries and the granting of patent rights.

At the end of a determination, the work is summarized in a table which includes the data and calculated values for all trials. The pages on which the data, the calculations and the summary occur are cross referenced if the pages are not consecutive. With experience, it is possible to record essentially all data directly in this summary table. These tables, particularly if they show intermediate values in the calculations, are helpful in discovering trends and locating errors in calculations. One of the chief sources of errors in quantitative determinations is the incorrect treatment of the data. Calculations are as much a part of a determination as any measurement. **One example of each calculation with all units must be included in the notebook. Replicate calculations should not normally be included.**

The correct use of significant figures requires alertness and judgment. The most common error is the copying of all of the digits on the computer or calculator printout. The use of

¹ Portions of this material are derived from the work of Prof. A. J. Harrison and Prof. E. Weaver of Mt. Holyoke College and Prof. Susan K. Henderson of Quinnipiac College.

computers and calculators require careful attention to the correct entering of data followed by careful consideration of the number of figures to be retained in the final answer. One additional figure should be retained in all intermediate values of a calculation and the final answer reduced to the correct significance. It takes hard work to get good data. Don't invalidate the results with sloppy calculations.

Anyone can keep an accurate notebook if they put their mind to it. Here are some suggestions which may help organize the notebook so it is easier to read.

1. Plan what you are going to do before you start writing.
2. Don't try to put too much on one page. Leave plenty of space.
3. Use one end of your bench space for your notebook and the other for wet work - which depends on whether you are right or left handed and also the position of the sink.
4. When not in use, keep the notebook closed and off of the desk top. If a disaster occurs, data may be transferred. Draw a single line across the first page and cross reference the two pages.
5. Run through calculations on scrap paper first. This is quite permissible since calculations can always be repeated as long as the data are available. Show only one example calculation in your notebook to allow later verification of the method used.
6. Data and calculated values should be presented in tables. Even though instrument recording and computer printouts are attached to the reports, significant values should be included in the tables.

Tables of results are possibly the most crucial part of the notebook. A well organized student can often prepare the table prior to beginning the experiment. This saves laboratory time and indicates a thoughtful, orderly approach to the experiment. A well thought out table will simplify recording and subsequent calculation of results.

The importance of establishing a pattern of careful notebook keeping is difficult to overemphasize. Most industrial laboratories engaged in analytical chemistry are required to follow Good Laboratory Practice standards (GLP's) established by professional groups or government agencies. In some laboratories, for example, every weighing, buret reading, etc., must be witnessed and countersigned. This would be a little extreme in this course. An almost universal practice is to have each page witnessed at the end of each days work. Since **each day's work is turned in immediately to the TA's** this is comparable to having them sign the work each day.

Criteria for grading a lab notebook are quite simple to spell out in detail. The table below specifies the number of points which will be deducted from the lab notebook grade for each mistake. You should check your notebook carefully before you hand it in, or, better yet, trade with a friend and check each others notebooks. The goal is to learn to keep a complete notebook. We would like to see every notebook get a 10. However, if you are careless it is possible to get very low grades on these.

CRITERIA FOR GRADING THE LABORATORY NOTEBOOK

The laboratory notebook will be graded on a 10 point scale. The following criteria will be used by the laboratory assistants to grade the notebooks. Use them as a checklist. You should be able to get a perfect 10 every time if you are willing to pay attention to detail. It is also possible to get a negatived score if you are very careless.

<u>First page of experiment: pre-laboratory information</u>	<u>Points off</u>
Title of Experiment, Date, Name of Partner(s) -incomplete	1/2
<u>Objective or Purpose (typically one or two sentences)</u> wrong, unclear, or too long	1/2
<u>Pre-Laboratory information, data or calculations</u> Balanced reaction equations, formula weights of reagents to be used, amounts to be used, etc. - incomplete or wrong Literature Reference	1
<u>Experimental Procedure</u> Too lengthy	1/2
Copied word for word from handout	2
Incomplete	2
Reagent source not identified (manufacturer of chemical if available)	1
<u>Data and Observations</u> No unknown number included, if unknown given	3
Data not in tabular or organized form	1
Missing data	1
Required observations missing	1
<u>Waste Disposal</u> Section missing	½
<u>General Comments</u> sloppy or illegible writing or writing in pencil	1
notebook not signed and dated	1
significant figures not recorded correctly	1
units missing (whether 1 or more)	1
errors blotted out instead of being struck with a single line	1

Hand in the above materials before you leave lab penalty for lateness
2 pts/day

Brief Report due Friday following the lab except for Rotation Experiments

The brief report may be done in the notebook or on a computer. It should be reasonable neat and well organized but this is not a formal lab report. It should also be very brief and only cover the material noted below.

Results

a. Calculations	
Calculations not shown	1
Not done correctly (one or more)	1
Graph not included where required	2
coordinates of graph not labeled properly	1/2
Graph title missing	1/2
Poor design of graph paper or graphing improperly or inaccurately done	½

	<u>Points off</u>
b. Table of Results (where appropriate)	
No table	2
No standard deviation (if 3 or more replicate measurements are made)	1/2
Omission of final calculated results	2
<u>Conclusions</u>	
Omission of conclusion section	2
Omission of final result	2
No discussion of sources of error	1/2
No discussion of precision (if 3 or more replicate measurements are made)	1/2
No discussion of whether objectives were met	1/2
No discussion of discarded data, if appropriate	1/2
wrong tense, person or voice in conclusion section	1/2
<u>Penalty for Lateness</u>	2 pts/day

Lab Report Policies - Chemistry 208

Brief reports of analysis will be submitted for each experiment. For a limited number of experiments a comprehensive, technical journal style report will also be required.

The nature of the report required will be specified for each experiment at the end of the section for the experiment. Comprehensive reports will not be due until the relevant material has been covered in class. Those dates are specified in the syllabus. You will never prepare a "perfect" report and the attempt to do so can result in procrastination and the absence of all productive activity. The preparation of the Brief Report for these experiment, which is due at the end of the week the experiment is done, will help you summarize your results in preparation for writing the comprehensive report later in the term.

The penalty for late lab reports is 2 points per day late up to a maximum of 50 points. All reports more than 3 weeks late will automatically receive a grade of 50% if they are reasonably complete regardless of quality. This scale is designed to reward you for giving a good first effort as soon as you finish the experiment. If you delay, you will almost always get a lower grade.

There is always a possibility that due to illness or other factors you will need an extension on your lab report. Extensions will always be granted for any reasonable cause. **Extensions will only be granted through an e-mail request to the professor.** If you informally ask for and are granted an extension, this is only my way of saying, yes I will grant an extension if you ask through the formal procedure. When you request an extension, you must state your general reason for the extension and give a specific new due date when you will hand in the report. I can accept the proposed due date or change it. In any case, you will receive an e-mail reply stating the new due date for the lab. **You must print that message and attach it to your lab report when you hand it in for evaluation. If there is no written extension document submitted, the full late penalty will be assessed.** No open ended extensions will be granted.

Instructions for Comprehensive Reports

Guidelines for Authors – Taken from Analytical Chemistry Vol. 76, No. 1, January 1, 2004
Edited slightly for Chemistry 208- 2006 David henderson

Title

Use specific and informative titles with a high-keyword content. Avoid acronyms and subtitles.

Authorship

Give author(s)' full names, complete mailing address of the place where the work was done, and the current addresses of the author(s), if different, as a footnote. Indicate the corresponding author by an asterisk and provide e-mail addresses. (*Chem 312 list all lab partners as authors. The person submitting the paper is the Corresponding Author*)

Abstract

Abstracts (80–200 words) are required for all manuscripts and should describe briefly and clearly the purpose of the research, the principal results, and the major conclusions. Remember that the abstract will be the most widely read portion of the paper and will be used by abstracting services.

Text

Consult the publication for the general writing style. Write for the specialist. It is not necessary to include information and details or techniques that should be common knowledge to those in the field.

General organization. Indicate the breakdown among and within sections with center heads and side heads. Results and Discussion follow the Experimental Section. Keep all information pertinent to a particular section, and avoid repetition.

Introduction. The introduction should state the purpose of the investigation and must include appropriate citations of relevant, precedent work but should not include an extensive review of marginally related literature. If the manuscript describes a new method, indicate why it is preferable to older methods. If the manuscript describes an improved analysis of a substance, the competing methods must be referenced and compared. Absence of appropriate literature references can be grounds for rejection of the paper.

Experimental section. Use complete sentences (i.e., do not use outline form). Be consistent in voice and tense. For apparatus, list only devices of a specialized nature. List and describe preparation of special reagents only. Do not list those normally found in the laboratory and preparations described in standard handbooks and texts. Because procedures are intended as instructions to permit work to be repeated by others, give adequate details of critical steps. Published procedures should be cited but not described, except where the presentation involves substantial modifications. Very detailed procedures should be presented in Supporting Information.

Safety considerations. Describe all safety considerations, including any procedures that are hazardous, any reagents that are toxic, and any procedures requiring special precautions, in enough detail so that workers in the laboratory repeating the experiments can take appropriate safety measures. Procedures and references for the neutralization, deactivation, and ultimate disposal of unusual byproducts should be included.

Results and discussion. The results may be presented in tables or figures; however, many simple findings can be presented directly in the text with no need for tables or figures. The discussion should be concise and deal with the interpretation of the results. In most cases, combining results and discussion in a single section will give a clearer, more compact presentation.

Conclusions. Use the conclusion section only for interpretation and not to summarize information already presented in the text or abstract.

References. References to notes/comments and to the permanent literature should be numbered in one consecutive series by order of mention in the text. The complete list of literature citations should be placed on a separate page, double-spaced, at the end of the manuscript. Reference numbers in the text should be superscripted. The accuracy and completeness of the references are the author(s)' responsibility.

Use *Chemical Abstracts Service Source Index* abbreviations for journal names and provide publication year, volume, and page number (inclusive pagination is recommended). *Chemical Abstracts* reference information for foreign publications that are not readily available should also be supplied. List submitted articles as “in press” *only* if formally accepted for publication, and give the volume number and year, if known. Otherwise, use “submitted to” or “unpublished work” with the name of the place where the work was done and the date. Include name, affiliation, and date for “personal communications”.

Examples of the reference format:

(1) Ho, M.; Pemberton, J. E. *Anal. Chem.* **1998**, *70*, 4915–4920.

(2) Bard, A. J.; Faulker, L. R. *Electrochemical Methods*, 2nd ed.; Wiley & Sons: New York, 2001.

(3) Francesconi, K. A.; Kuehnelt, D. In *Environmental Chemistry of Arsenic*; Frankenberger, W. T., Jr., Ed.; Marcel Dekker: New York, 2002; pp 51–94.

Acknowledgment. Author(s) may acknowledge technical assistance, gifts, the source of special materials, credit for financial support, meeting presentation information, and the auspices under which work was done, including permission to publish.

Figures and tables

Do not use figures or tables that duplicate each other or material already in the text. Calibration plots will not normally be published; give the information in a table or in the text. Do not include tables or figures that have already been published.

If the use of a large number of figures is desired to illustrate a phenomenon, the figures can be published as Supporting Information. Straight-line figures are often not needed; the information they convey can be described sufficiently (and in less space) in the text.

Tables. Prepare tables in a consistent form, furnish each with an appropriate title, and number consecutively in the order of appearance in the text. Each table may be on a separate page and collated at the end of the manuscript (or for CHEM 312 they may be included where they are first referred to in the text.)

Figures. The quality of the submitted electronic files or paper originals determines the final quality of the published illustrations. Paper artwork or photographs that are sent with the paper are digitized during journal production.

Diagrams, graphs, charts, and other artwork should be printed on a high-resolution laser printer with dark black ink on high quality, white, smooth, opaque paper. Avoid thin, transparent, or textured papers such as vellum or tracing paper. Submit original artwork or a photographic print of the original; photocopies do not reproduce well. In general, bar graphs are a waste of space and are discouraged. Remember that artwork and graphs must fit a one-column (8.25 cm) or two-column (17.78 cm) format. The maximum height is 24 cm. For best results, submit illustrations in the actual size at which they should appear. If artwork must be submitted that needs to be reduced, choose a lettering size large enough to be legible after the figure is reduced. Avoid using complex textures and shading; these do not reproduce well. To show a pattern, use a simple crosshatch design. Photographs should be full-size, high-contrast prints with a smooth or glossy finish. If possible, please send photographs that are single- or double-column width to avoid reduction for printing. Avoid negatives, slides, and vugraphs. Photographs produced on a laser printer and prints cut from a printed publication do not normally give good results when printed. Do not write on the front or back of the image area of the photograph; these marks may show through when the photograph is printed. Color reproduction, if approved by the Editor, will be provided at no cost to the author(s). Color illustrations should only be submitted if essential for clear communication. A surcharge for color will be added to the standard cost of reprints.

Figure captions. On one page, include a double-spaced list of all captions and legends for illustrations. Make the legend a part of the caption instead of inserting it within the figure.

For Chem 208 – captions should be placed on each figure and figures may be either inserted in the document where first mentioned or compiled at the end of the document.

4. Supporting Information

In the interest of short, more concise, and readable articles, *Analytical Chemistry* requires author(s) to publish certain types of material in an appendix called Supporting Information (SI). This material can include additional examples of experimental and theoretical figures that are similar in form to figures in the article, novel algorithms, extensive tabular data (e.g., numerical values for the data in important figures in the manuscript and databases in comparative or theoretical studies of detailed kinetics or proteomics data), extensive figures connected with computational modeling, analytical and spectral characterization data for new compounds, and extensive instrument and circuit diagrams. *Analytical Chemistry* especially encourages author(s) to include figures or data in SI that are similar to those in the manuscript so that the manuscript is not repetitive, yet all information is preserved. Such figures should be cross-referenced between the two documents; in particular, author(s) are encouraged to reference SI figures and tables (Figure S-2, Table S-1, etc.) in the primary article to ensure that the reader is aware of their presence. Like the primary manuscript, SI is subject to peer review. The first page of SI should be a cover page (labeled page S-1) that lists the author(s)' names and affiliations, the title of the primary article, and an abstract that describes the nature of the materials therein and/or a table of contents. Then, as needed, SI should include any further discussion germane to the primary research article or novel SI material, such as video clips or other imagery; any expanded description of experimental procedures; any supplementary experimental or theoretical results, given as figures or tables with legends and captions that contain the same level of detail as the primary research

manuscript and that convey the significance of the result; and supplementary references for either the primary article or the SI. The material should be provided in a form suitable for immediate reproduction, because no galley proof will be provided. If SI material is submitted on paper, it should all be clipped together, separate from the primary manuscript. For electronic submissions, SI should be in an electronic file that is separate from the primary research manuscript. Page, figure, and table numbers in SI should be preceded by "S-". Color figures in SI are published at no cost to the author(s) and without editorial restrictions. Captions to figures and tables should appear on the same page as the figure or table and should provide full details, just as in the primary research article.

5. Nomenclature

Nomenclature should conform to current American usage. Insofar as possible, author(s) should use systematic names similar to those used by the International Union of Pure and Applied Chemistry and the Chemical Abstracts Service. *Chemical Abstracts* (CA) nomenclature rules are described in Appendix IV of the *Chemical Abstracts Index Guide*. For CA nomenclature advice, consult the Manager of Nomenclature Services, Chemical Abstracts Service, P.O. Box 3012, Columbus, OH 43210-0012. A name-generation service is available for a fee through CAS Client Services, 2540 Olentangy River Rd., P.O. Box 3343, Columbus, OH 43210-0334; 614-447-3870; fax 614-447-3747; answers@cas.org. Avoid trivial names. Well-known symbols and formulas may be used if ambiguity is unlikely. Define trade names and abbreviations at point of first use. Use SI units of measurement (with acceptable exceptions), and give dimensions for all terms. If nomenclature is specialized, as in mathematical and engineering reports, include a Nomenclature section at the end of the paper, giving definitions and dimensions for all terms. Type all equations and formulas clearly, and number all equations in consecutive order. General information about ACS publications is given in *The ACS Style Guide* (1997), available from Oxford University Press, Order Department, 201 Evans Rd., Cary, NC 27513. Updated instructions are available at the *Analytical Chemistry* home page at <http://pubs.acs.org/ac>.

Laboratory Techniques²

There are essentially two criteria for judging a technique –

- Does it work?
- Is there a less laborious technique which works equally well?

In a few cases, the scientific world frowns on perfectly adequate procedures. An analogous situation is eating mashed potatoes with a knife. This section and the text provide some guidance to help you develop habits of work which are both simple and effective.

Reagents

In so far as practical, reagents will be supplied in the manufacturer's bottles. Note the analysis reported on the label. **Record the manufacturer and grade of the reagent used in your lab notebook.** This information is always required in writing a technical paper and you should get in the habit of recording it.

The purity of the reagents used is a determining factor in the results obtainable in analytical work. Consequently, every effort must be made to keep stock bottles free from contamination. Under no circumstances should material be returned to a stock bottle in a general laboratory. A large stainless steel scoopula with a handle or a porcelain spatula may be used to break up caked solids. Use some judgment as you would not want to introduce the stainless steel scoopula into a reagent used for trace metal analysis. An example from recent history was a geological analysis which was ruined because of contamination from a platinum wedding ring worn by the technician doing the sample preparation. This error received world wide publicity due to the nature and importance of the erroneous findings.

In general, solids are best poured from bottles. Rotating the bottle back and forth helps to control the rate of flow. Droppers and pipettes should never be dipped into stock bottles. Droppers from dropper bottles should not come in contact with any surface outside of the dropper bottle itself.

Primary Standards

Substances prepared for use as primary standards are so labeled. These materials are expensive and must not be used for routine procedures.

² Portions of the Laboratory Techniques in this section were originally prepared by Professor A.J. Harrison and Professor E. Weaver of Mt. Holyoke College. The version presented here has been modified and adapted to this course.

Drying at Elevated Temperatures

Drying ovens for this course are thermostated and are usually operated at 120-125°C. As the name implies they are used for drying at an elevated temperature. The efficiency of the drying process also depends upon the pressure of water vapor in the immediate atmosphere. Consequently a very wet object introduced into an oven may actually cause another object in the same oven to pick up water. Consequently, one should not place wet glassware in an oven being used to dry analytical samples or standards. Before using the ovens, note the temperature at which they are operating. Do not change the setting on an oven without consulting the instructor.

Space is always at a premium in a drying oven. Distribute objects around the sides and back of the shelves so that all objects can be reached with tongs. Crucibles and weighing bottles should be dried in small labeled beakers covered with small ribbed watch glasses. Any object left in an oven overnight may be confiscated between 8:30 and 9:00 A.M. unless specific permission was obtained.

Two common problems encountered in "community" ovens are the loss of samples (someone else takes yours) and knocking over someone else's samples. The former problem is avoided by clearly labeling the samples. If you place them in a beaker, you can easily place a large paper sign with your name on it in the beaker with the sample. When manipulating samples in the oven, be careful not to knock over others.

Microwave ovens are replacing conventional ovens in the laboratory just as they are in the kitchen. Typically, samples can be dried in about 25% of the time it would require in a conventional oven. However, there are some significant exceptions. **Some samples melt, burn, or decompose when placed in a microwave oven for an extended time. Check with the instructor before drying a sample in the microwave oven.** It is always advisable to experiment with a small quantity of the material before entrusting your entire sample to the 'wave.

CAUTION: MATERIALS REMOVED FROM BOTH MICROWAVE OVENS AND CONVENTIONAL OVENS ARE HOT. USE TONGS OR GLOVES WHEN HANDLING THESE OBJECTS TO PREVENT BURNS.

Cleaning Glassware

The cleaning process should be as simple as possible. Rinsing with tap water and several small portions of distilled water may be adequate. For more dirty glassware, scrubbing with detergent and water should precede the rinsing. From a chemical point of view soap, detergents, etc. are dirt. Rinse very thoroughly with tap water and then at least three times with small volumes of distilled water. In a few cases special chemicals may be needed to dissolve solids or oils. The inside of a container which may come in contact with chemicals is not dried with a towel since this introduces lint. In many cases it is not necessary to dry glassware. Simply rinse with the solution to be used unless this would invalidate measurements. **Beware of drying glassware with compressed air. This may introduce oil vapor from the pump. I am not**

aware of any instance where this is appropriate in this lab. The best strategy is to keep all your glassware clean so that you have clean and dry equipment available at the start of each lab.

Chromic Acid Cleaning Solutions

A solution of potassium dichromate dissolved in concentrated sulfuric acid was traditionally used for difficult cleaning tasks. **THIS SOLUTION WILL NOT BE USED IN THIS LAB UNDER ANY CIRCUMSTANCES.** Chromium compounds are Class I carcinogens and their use is severely restricted.

Glassware which resists cleaning with lab soap and water may be cleaned with **Micro** or other special cleaning solutions.

For trace metal analysis, it is common practice to soak glassware in 50% nitric acid. When placing glassware into such a bath, it is important not to be splashed by the bath. When removing glassware, long, chemically resistant gloves must be worn. Do not use tongs for this job as they are dissolved by the bath. Glassware must be removed and rinsed in a manner that does not leave hazardous acid residues in the work area which other people may be exposed to.

Cleaning Volumetric Glassware

Volumetric glassware must be clean so that water drains from the surface without leaving droplets. It will not do this if there is the least bit of oil on the surface. Once "clean" glassware becomes dry it will usually not drain properly when water is again added. Volumetric glassware is cleaned just before use or cleaned and stored full of distilled water or other solvent. The 50 ml. burets can be scrubbed with lab soap - 1 teaspoon in a large beaker of warm water - and a buret brush. In doing this, scrub the buret in sections - about 10 cm. at a time. The 10 ml. buret is fragile and difficult to clean. It is too small for a brush and is cleaned in the same fashion as the pipet. The tip is very small and the cleaning solution should not be taken through it since it often contains undissolved pieces of soap.

Working Surfaces

Use paper towels to wipe up all spilled materials. Repeatedly wash the surface with a wet towel to remove water soluble materials including acids and bases. The working surface should be kept clean and dry. Spilled material is then quite evident and contamination can be kept at a minimum. If working materials are arranged in an organized manner, there are fewer opportunities for confusion and there is a higher probability that a determination will be carried through to completion without error.

Quantitative Transfer

Quantitative transfer is the complete transfer of a sample without loss of any kind. The techniques used are a matter of common sense - do not spill, splash, drool or abandon. Dry solids are poured or transferred with a spatula. If the surface tension of a liquid is high it should be transferred by pouring down a stirring rod. This prevents the liquid from running down the outside of the original container and also prevents splashing as the liquid enters the second container. Last traces are transferred by washing the original container and transfer equipment such as spatula, stirring rod and funnel with a miscible liquid. This can be done by a batch method using repeated small volumes of the wash liquid or by a continuous flow method using a stream of wash liquid from a wash bottle. A rubber policeman is used to facilitate the process and minimize the amount of wash solution necessary.

Transfer of material from a weighing bottle to a flask should always be done by pouring and not with a spatula.

Reading Instrument Scales

The advent of digital readouts has reduced the opportunities to read analog and vernier scales. Therefore, when you encounter the need to read instrument scales or calibrations on burets and pipets, it is especially important that you pay close attention to this process. Even with digital readouts, it is possible to make serious errors if the instrument has several modes of display (eg. Transmittance and Absorbance). Become familiar with the scale. Does it read from left to right or right to left? Top to bottom or bottom to top? Determine the significance in both units and magnitude of the largest divisions then determine the significance of the smallest divisions. The number and magnitude of the small divisions may not be the same in all ranges of the scale. With most analog scales the orientation of the operator's eye and the instrument determine the reading. If all readings occur at a fixed position on the instrument, it is only necessary that the position of the eye be the same for a set of measurements.

To read a variety of positions on a horizontal scale, the most reproducible orientation is to have the eye directly above the pointer. For a variety of positions on a vertical scale the most reproducible orientation is to have the eye at the same level as the pointer. In both cases the line of vision is at right angles to the surface bearing the scale.

If the surface of a liquid is to be read in place of a pointer, a reproducible position on the surface must be chosen. This is usually taken to be at the bottom of the meniscus if the liquid wets the glass and at the top if the liquid does not wet the glass. Calibrations which cover at least half of the circumference of the tube serve as a check on the correct eye level. The angle of reflection of light makes a significant difference in the appearance of the meniscus. The lighting can be controlled by holding a card against the back of the tube: For clear liquids a white card containing a broad dark line serves as the best means of reading the meniscus. The dark line is

raised until it just touches the bottom of the meniscus. The top of the line is then compared with the graduations on the device to determine the value.

Readings are in general made to 0.1 of the smallest calibration division. For example, the readings with a scale calibrated to 0.10 cm. are estimated to the nearest 0.01 cm. Since calibration lines have width, some convention must be established for the use of the line. If the line width itself is 0.02 cm., the top of the 3.10 line could be read as 3.09, the center as 3.10 and the bottom as 3.11. A pointer also has width. Choose a point of reference - the center, one edge, some irregularity on the pointer, etc. To record a reading as 3.1 cm. states that the value is thought to be closer to 3.1 cm. than it is to 3.0 cm. or 3.2 cm. To record a reading as 3.10 cm. indicates that the value is thought to be closer to 3.10 than to 3.09 or 3.11 cm. To record 3.1 cm. implies one of two things. It is impossible to determine the value more carefully or the operator simply chose not to read the value more carefully. In the first case, 3.1 is the correct reading. In the second, 3.1 is an approximate reading. To make an approximate reading is stupidity when the more exact value is needed. To record 3.1 when the value 3.10 has been read is unscientific - or to be more brutal, just plain sloppy.

Weighing Out Samples and Using Weighing Bottles

Your equipment includes three weighing bottles. These are small glass bottles with ground glass tops. Weighing bottles are to be used only for drying, storing, and weighing solid standards and unknowns. Weighing bottles should be numbered in pencil on the ground glass surface. Samples to be dried are placed in the weighing bottle without the stopper and placed in a beaker with a watch glass cover and a piece of paper with your name. This entire apparatus is then placed in the oven for the specified time. Upon removal from the oven, the weighing bottle is allowed to cool until it can be easily handled and then transferred to the desiccator. The weighing bottle should not be inserted until the bottle has come to room temperature in the desiccator.

The preferred method is known as weighing by difference, is to weigh the weighing bottle containing the dry sample, transfer the sample to the flask in which it is to be used, and reweigh the weighing bottle. This last weight becomes the first weight for a second sample if multiple samples are being prepared. This method assumes that the receiver flask has a large enough opening that there is minimal risk for spillage. The receiver flask need not be dry, so considerable time can be saved through not needing to dry glassware. When transferring from a weighing bottle to a volumetric flask, a powder funnel should be used to facilitate transfer.

Samples can be added to a clean dry container, often a weighing boat, which has been previously weighed or tared on the balance. Extreme care must be taken when samples are transferred from this container to insure that no material is lost. Normally, the solvent should be used to wash any residue from the boat into the container at the end of the transfer. Caution must be exercised with the common plastic boats as they can accumulate static electricity which either attracts or repels the particles of sample. **This method is not recommended for the most precise quantitative work.**

The best way to manipulate the weighing bottle is to use a band of dry paper pulled firmly around the bottle. Do not use your fingers directly on the weighing bottle as the moisture from your fingers will affect the weight. If the weighing bottle stands for several hours in the desiccator before taking the next sample, its weight should be rechecked.

Rules for Use of Analytical Balances

Weighing performed on the analytical balances shall never be done on weighing paper (or filter paper, paper towels, etc.) If sufficient precision is demanded to require the analytical balance it also requires the use of procedures which do not involve such high risk of loss during transfer. The second problem with use of paper is that it leads to dirty, and subsequently, damaged balances. Acceptable weighing containers are weighing bottles, plastic weighing boats, and glassware with sides to contain the material.

No reagent shall be added to or subtracted from a container while in the analytical balance. Remove the container to the bench top, make the addition and return to the balance. This is the most fundamental rule of the use of the balance and **students found violating this rule will be disciplined.**

Heating and Concentrating Solutions

Aqueous solutions may be heated either on a bunsen burner with wire gauze or on the hot plates. The burner is often a quicker method for rapid heating while the hot plate will provide a constant level of heat for a long time. **Solutions other than water or dilute aqueous salts should be heated in the hoods.** A watch glass should always be used during the heating of solutions both to prevent entry of extraneous material from your neighbors sample, the paint on the ceiling, etc., and to prevent loss of sample due to splattering. Ordinary watch glasses restrict the loss of vapor and are used to maintain the value of the solution. When evaporation is desirable the watch glass may be supported with three glass hooks or ribbed watch glasses may be used. These allow escape of vapors. In either case, some sample will collect on the watch glass and must be rinsed back into the container with a small volume of the solvent whenever the watch glass is removed. Boiling is generally to be avoided with samples due to the high risk of mechanical loss. When boiling is desired, boiling chips should be used whenever possible. Selection of an appropriate boiling chip requires knowledge of what may be added without causing contamination of the material to be boiled. Glass beads or chips, marble chips, silicon carbide, and many other substances have been used as boiling chips.

Handling Stock Solutions

A uniformly mixed solution may develop a concentration gradient on standing even in a closed bottle. Evaporation occurs from the surface of the liquid. Condensation takes place on

the wall of the container above the surface and the condensate flows down the wall into the solution again. Shake or mix stock solutions before use.

Control Charts

Control Charts will be used to evaluate the consistency of the procedures you are using. This is a procedure used in most routine labs. The charts will be posted in the lab and you are responsible for entering your data before you leave the lab at the end of each day.

The left side of the chart indicates the sequence of lab days numbered sequentially. There is also a place for you to enter the actual date. The right side of the chart has a column for you to enter the names of the members of your group and the numerical value to be recorded. The graph in the middle shows the trends in analytical results. The first three groups who enter data are only to fill in the values on the left and right side. After this much data is obtained, the average and standard deviation of the values will be determined and the scale for the chart will be established.

You will find the Control Chart very useful in your lab work after the first few weeks. It will provide an indication of the validity of your analytical results and will help the staff to detect any problems with standard solutions, LC columns, electrodes, etc.

Volumetric Glassware³

Flasks, Burets and Pipets

The specifications used by most manufacturers of volumetric glassware meet and usually exceed the National Institute of Standards and Technology (NIST) recommendations. Measurements of volumes larger than 10 ml. can easily be made with an accuracy of 1 or 2 parts per thousand. Smaller volumes, which are so much a part of present day chemistry, may present special problems.

Since the volume of a container depends upon the temperature, volumetric equipment is calibrated for a specified temperature - usually 20°C. The coefficient of expansion of glass is so small that calibrations for 20°C are valid over the usual range of laboratory temperatures. Even at 30°C the error is less than 0.3 ppt. Tolerances for various pieces of equipment have been recommended by NIST. For example, the tolerance for a 50 ml. flask or a 50 ml. transfer pipet is 0.05 ml. Properly used the volume is 50.00 ± 0.05 ml. This is a maximum error of 1 ppt. The tolerance for a 10 ml. transfer pipet is 0.02 ml., 2 ppt. Note that for the smaller volume the tolerance is proportionally larger although smaller in absolute magnitude.

³. This material was originally prepared by Prof. A. J. Harrison and Prof. E. Weaver of Mt. Holyoke College. The version presented here is modified for application to this course.

Graduated Cylinders

Graduate cylinders should not be used for any quantitative measurement which is part of an analytical determination in this course. They are useful for preparing stock solutions, HPLC mobile phases, and other places where the accuracy of the measurement does not have a direct contribution to the quantitative calculation. Graduated Cylinders are very crude pieces of volumetric equipment. Non-uniformity of glass at the base of the cylinder makes the measurement of small volumes of liquid in the bottom of the cylinder particularly unreliable. Small volumes can be measured with more precision as the difference between two larger volumes.

Graduated Measuring Pipets

Measuring pipets look like a buret without a stopcock. They are frequently convenient to use but should in no sense be considered as precision equipment - largely due to the difficulty in controlling the level of the liquid. **In normal use they are no better than graduate cylinders and should not be used quantitatively unless errors greater than 10% relative are expected in the results.**

Volumetric Transfer Pipets

A transfer pipet has a single calibration line on tubing of small diameter and is capable of high precision. It is, however, frequently used incorrectly and becomes a serious source of error. For this reason an operator should run enough calibration checks to attain self confidence in their technique as well as confidence in the equipment.

All transfer pipets in this lab are marked TD (To Deliver). The proper use of these pipets is as follows:

1. Fill the pipet above the calibration line using a bulb. **Mouth pipeting is not allowed and will result in expulsion from the lab.**
2. Tip the pipet to an angle to prevent leakage
3. Wipe excess liquid from the outer surface of the pipet with a clean towel or wipe.
4. Drain the pipet until the liquid level reaches the calibration line.
5. Touch the tip of the pipet to a glass surface to remove the attached drop which is probably present.
6. Tilt the pipet to carry it to the receiving vessel. This will prevent loss of sample.
7. Drain the contents into the receiving vessel. Do not force the liquid out. Let it take its time.
8. After the pipet has stopped draining for 10-20 seconds (during which time the film of liquid in the pipet will continue to drain down), touch the tip of the pipet to the edge of the receiving vessel. Do not blow out the liquid remaining in the pipet after this procedure.

You will rarely encounter a blow out pipet in a chemistry lab, but they are still common in biology labs. The blow out pipet will be labeled TC rather than TD.

Finally, it is never proper to place your mouth on a pipet. Many solvents and chemicals are toxic or carcinogenic. In a biology lab one must also contend with pathogenic substances. Always imagine you are pipetting a sample of the AIDS virus or a terrible toxin.

Absolute Calibration of a Pipet

Determine the weight of distilled water of known temperature transferred by a TD pipet. The degree to which the weights obtained in several trials agree is a check of your technique in the use of the pipet. Using the average of the weights and the temperature of the water calculate the absolute volume of the pipet. The volume delivered depends both on the surface tension and the viscosity of the liquid. Consequently the calibration value is only valid for water or dilute aqueous solutions.

Use of a Volumetric Flask for Solution Preparation

The interior wall of the flask - particularly the neck - should be checked for uniform drainage with a small volume of the solvent. If necessary, re-clean the flask. A funnel is usually used to obtain quantitative transfer of the sample. If the space between the stem of the funnel and the neck of the flask is small, air may be trapped in the flask when liquid is added. The trapped air may in turn force liquid back up the neck of the flask. This can be avoided by using a funnel with a stem that extends into the bulb of the flask or by tipping a shorter stemmed funnel so that the end of the stem touches the wall of the flask at one point. A small piece of paper between the funnel and the top of the flask will hold the funnel in this cocked position. Solids which dissolve readily in the solvent at room temperature may be added through the funnel if care is taken to wash the solid down with solvent. Solvent is added until the bulb of the flask is about 9/10 filled and a uniform solution is obtained by swinging the flask in a small circle to promote swirling of the liquid without bringing it into the neck. Mixing at this time allows volume changes which accompany dilution to take place before the solution is made up to volume. Solvent is now added to bring the solution to the calibration mark. The last few drops may be added with a dropper. **The stoppered flask is repeatedly inverted to obtain uniform mixing - at least 15 inversions, more if the solution is viscous.** Volume changes which accompany this mixing are included in experimental error. Since a uniform solution has been prepared, solution that is now removed on the stopper in no way changes the concentration of the solution.

Volumetric flasks should never be placed on a flame or hot plate to dissolve a difficult solute. This can cause permanent changes in the volume of the flask. If a solute is difficult to dissolve, carry out the dissolution in a beaker or flask and then quantitatively transfer the solution to the volumetric flask and bring to the final volume. Ultrasonic bath cleaners can be used to dissolve solutes in volumetric flasks.

The solution is prepared at some temperature - usually room temperature. The volume of the solution and consequently the concentration of the solution is dependent on the coefficient of expansion of the solution. (If all volumes are measured in glass equipment, then it is the difference between the coefficient for the solution and the coefficient for glass that is significant.) Around room temperature the coefficient of expansion for dilute aqueous solutions is about 0.024% per °C. A change of 4°C, therefore, corresponds to a change in concentration of about 1 ppt.

Burets

The calibration lines on a 50 ml. buret are at 0.10 ml. intervals. To obtain maximum precision, volumes are estimated to 0.01 ml. The calibrations on the 10 ml. micro-burets are at 0.020 ml. intervals and volumes are estimated to 0.002 ml.

When using a 50 ml buret, the standard deviation of a single buret reading is assumed to be ± 0.02 ml. The two readings required in measuring the volume of reagent transferred introduces an uncertainty of $[(0.02)^2 + (0.02)^2]^{1/2} = 0.03$ ml. Thus, volumes smaller than 20 ml, even under ideal conditions, would have an uncertainty greater than 1 ppt. Most operators prefer to work in the 40 ml. range.

Use of a Buret to Carry Out a Titration

Teflon stopcocks are commonly used in burets. The Teflon stopcock require no lubricant. Tension on the stopcock is simply increased until the fit is sufficiently secure to prevent leakage but not interfere with the easy rotation of the stopcock. **A Teflon stopcock should be stored free from tension- loosen the nut. This applies to those on burets and on other glassware such as separatory funnels.** The correct assembly of the Teflon stopcock has the white Teflon ring next to the glass and the black O-ring next to the Teflon nut which tightens the assembly. Check that assembly is correct before you attempt to use a Teflon stopcock.

All stopcock burets are designed to be operated with the left hand so that the right hand is free to agitate the reaction mixture. With the scale of the buret facing the operator, the handle of the stopcock is on the operator's right. With the base of the left hand to the left of the buret, the thumb and first two fingers encircle the buret to control the handle of the plug, the last two fingers against the left of the tip. This braced position of the hand leads to maximum control of the stopcock. It also makes it possible to keep constant pull on the plug into a secure position in the seat. This is essential with glass stopcocks to avoid leakage. If initially the position of the left hand seems awkward, make a conscious effort to develop skill. The instructor will be glad to give advice and commiserate with your difficulties. (This technique was developed for glass stopcocks to keep the stopcock from being pulled out. It is not an issue with Teflon stopcocks, but the design has not changed)

To fill the 50 ml buret rinse three times with 3-4 ml portions of the liquid to be used. Use a buret funnel so this liquid can be directed to flow over the entire interior surface. Do this in the buret stand to prevent any titrant which may spill from running down to your hand. Allow time for each portion to drain from the buret before the next is added. Fill the buret, including the tip, and replace the buret funnel with a buret cap. Never leave the buret funnel in the buret during a titration since it may add a drop of titrant during the titration. Just exactly what you do with the buret funnel is a problem. The next time it is used it can be a source of contamination due to evaporation or reaction of the solution remaining on it or to the solvent remaining in it if it has been washed. One procedure that is adequate for most reagents is to hang the funnel in the neck of the appropriate reagent bottle and cover the funnel with a watch glass.

To fill the tip of the buret, fully open the stopcock to allow the liquid to flow rapidly through the tip. If necessary, use a rubber bulb to exert pressure on the liquid and increase the rate of flow. Filling the 10 ml buret presents special problems since the diameter of the barrel is too small to allow liquids to be poured into the top. Consequently liquids are brought into the buret from the bottom. These burets may also be filled through the tip in exactly the same manner that a pipet is filled. This is the procedure most frequently used unless a large number of titrations are to be carried out. The tips of these burets are extremely fine and care should be taken not to pull solid particles into them.

CAUTION: If titrant is spilled on the outside of the buret, it must be cleaned up and the waste neutralized and disposed of in a proper manner.

Between laboratory periods, the burets are filled to the top with the solution being used or with distilled water and left in position on the buret stand in your lab bench.

Logically one should proceed to carry out an absolute calibration of the buret. In practice the quality of the burets manufactured today is so high and the technique is so straightforward this is not necessary unless unusual precision is necessary.

To proceed with the titration bring the level of the liquid of the filled buret onto the scale and read the position after allowing a short time for the film of liquid to drain down the walls. The reading is more objective if an effort is not made to set the level at zero on the scale. **You will be chastised by the instructor if you have any initial buret readings of 0.00 ml. Trying to set the buret at exactly 0.00 has been shown to increase errors.** Excess liquid on the tip of the buret is removed by washing with a stream of solvent from the wash bottle and touching the tip to a glass surface.

Using the right hand to swirl the flask and the left hand to control the stopcock, add liquid at a rapid and uniform rate. Reaction in the localized region of mixing produces an indicator change. The addition of the titrant is periodically stopped and the rapidity with which the indicator returns to its color in the first solution is observed. Using this as a guide, the addition of the titrant is continued at a gradually decreasing rate. The tip of the buret and the walls of the flask are washed down with a small volume of solvent from the wash bottle. The process of addition and rinsing is continued until the end has been located within a drop or within a fraction of a drop. After a suitable drainage period the buret is read.

Fractional drops are obtained by stopping the addition before a full drop has formed. This fractional drop is then washed into the reaction mixture. The volume of solvent added must be adequate to bring all of the reagents into the reaction mixture at the end point. Premature washing with large volumes of solvent may reduce the precision of the work. This is particularly true when the concentration of the titrant is small. "Small" cannot be specified since it depends upon the properties of the reactants and the indicator. The time that should be allowed for drainage depends upon the volume of liquid withdrawn, the rate of withdrawal and the dimensions of the buret. The fine tip has been designed to place a maximum limit on the second. The micro burets are particularly troublesome since the surface area is large in comparison to the volume. A check on the reading after a short time indicates whether adequate drainage time had been allowed.

An overrun end point is an overrun end point. In spite of this the initial addition should be continuous and reasonably rapid. There is a limit to how long attention can be focused on drop by drop addition. If the effort invested in a sample is not large, it is good to use one sample to find the approximate volume of titrant per unit of sample taken. The estimated volume of titrant for the following samples can then be approached rapidly with confidence. Very close to the end point it is good to keep a running record of buret readings (use the back side of the notebook page). This locates the end point between two successive readings and places a limit on the maximum error that could be involved in locating the end point.

Visual Indicators

The selection of an indicator may be a tricky business which depends upon knowing a great deal about the chemical reactions involved. Once an indicator has been selected some familiarity with that indicator should be acquired before making a serious attempt to carry out a titration. This can be done in a very qualitative manner - even to adding the titrant with a dropper to an approximate small sample in a beaker. Knowledge of the colors involved leads to confidence and greatly reduces the time consumed in the first titrations. If a back titration is feasible, the preliminary investigation provides an opportunity to decide which color change is preferred - color A to color B or color B to color A. It is easier to titrate to a definite color change but in some cases a suitable indicator is not available and it is necessary to match a color standard. The preliminary investigation sets up this color standard and gives experience in judging how rapidly the color changes. Work with an indicator until you are confident you can judge its behavior.

Determination of an Indicator Blank

The indicator gives the "end point," the point at which the titration is ended. Ideally this would be at the "equivalence point," the point at which chemically equivalent quantities of

reagents have been brought together. In practice the end point and the equivalence point may not coincide. The determination of an indicator blank gives some information on this point. Even when the indicator has been correctly chosen, a significant quantity of the titrant may be required to produce the indicator change or to react with contaminants in the reagents. In some cases it is possible to determine the quantity so used by running a blank - a titration that is equivalent in every way with the exception that the substance to be determined is not included in the reaction mixture. In order to determine an indicator blank that has meaning, the operator must be secure in their knowledge of the behavior of the indicator.

Experiment 1 - ACID - BASE TITRATION

Individual

(Consists of Laboratories 1, 2, and 3)

Lab 1 - Check-in, Buret Reading, Sampling solids

I. Check-in - You should open your assigned locker, remove all equipment to the bench top, and check the contents against the check-in sheet. Any broken or missing items should be replaced from the stockroom. Replace the equipment in the desk and turn in the signed inventory sheet. Clean all glassware that appears dirty.

II. Calibration of a Pipet - Weigh three dry 125 ml Erlenmeyer flasks. Using your best technique, pipet 10.00 ml of water into each flask and reweigh. Measure the temperature of the water used at the time you do the pipetting. Students in lockers 1-25 should use tap water while those in lockers above 25 should use DI water. From the weight of water and the density calculate the volume of water delivered. Prepare a table showing the weight of each trial, the water temperature, and the calculated volume for each trial. *In calculation of volume, don't forget to correct density for water temperature.*

III. Drying primary standard potassium hydrogen phthalate (KHP), KHP standard reference material and Unknown KHP

Clean and dry three weighing bottles. Place ~10 gm of KHP, the standard reference material, and your unknown in the bottles. Number the bottles with a pencil or Sharpie marker. Record the labels used in your notebook for later reference. You should not apply tape or labels to the bottles.

Place the bottles uncovered in the lab oven for 1.5 hours at 120°C or in the microwave oven for 15 minutes at full power. Bottles should be in a beaker covered with a watch glass. Put a piece of paper with your name on it in the beaker for identification.

When dry, transfer to a desiccator and allow to cool. Then place the stopper in the bottle and cover the desiccator for storage.

IV. Reading Burets - Clean your buret and set it in the buret stand. Fill the buret with water to approximately the ml equal to your locker number. Read your buret to the nearest 0.01 ml. Now read every other buret in the lab and record all values along with the locker number of the owner. Enter your data into the spreadsheet in the lab.

V. Errors in Sampling solids - You will be assigned a clean beaker for this exercise. Take your beaker to the large container of M&M's and fill it by scooping it through the sample.

1. Pour the M&M's onto a clean paper towel
2. Count the number of M&M of each color and record your results.

3. Identify any non M&M candy in your sample.
4. Given the total number of M&M's in the total sample, calculate the number of each color that should be present from your sample. Also calculate the concentration of any extraneous candy in parts per thousand.

Lab 2-3 Acid-Base Titrations using Visual Indicators

Prelab Exercise - Lab 2 Complete the exercises on Blackboard for this lab.

Introduction

Read: Chapter 7 and 12 in Text have relevant background information

Acid base titrations are one of the most precise and accurate experiments which can be carried out in the quantitative laboratory. Somewhat poorer precision is to be expected in titration of the weak acid due to the smaller break in the titration curve. The early parts of this experiment are designed to give you practice in titration while you discover the behavior of acids and bases and compare your experimental data to theoretical data. The final exercise is a laboratory practical exam in which you will titrate an unknown acid. By the time you do this you will have done a detailed analysis of the errors inherent in the method and will have predicted the quality of data you should obtain. Traditionally, we have awarded a grade of 100% for results having precision and accuracy better than 1 ppt. Throughout this experiment you should strive to master the techniques needed to obtain this level of precision. Your overall grade on the experiment will reflect three aspects of your work, precision, accuracy, and the quality of the lab notebook.

In order to obtain this precision you will need to be careful in your use and reading of volumetric glassware as described in the text and in this handout. The calibration of the pipet is particularly instructive as it will demonstrate to you the degree of caution required to obtain reproducible volumes. Read the instruction in the Laboratory Techniques section of this manual before you begin using any item of volumetric glassware, even if you have prior experience using it.

This analysis consists of three parts. In the first you prepare and standardize a solution of NaOH. In the second section you verify the precision and accuracy of your titration method using standard reference material of known composition. In the third section you apply the method to the analysis of an unknown.

I. Standardization of 0.1000 M NaOH

A. Prepare 2 l of approximately 0.1 M NaOH for standardization. Use 50% NaOH (w/w) (Density = 1.53 gm/ml) solution which has been filtered or allowed to settle (do not shake or agitate this solution). (You must calculate the amount of this solution needed.) Use water freshly prepared from the Barnstead Nanopure system to prepare the NaOH solution. The solution should be stored in a polyethylene bottle since sodium hydroxide dissolves silica from glass

containers. The slow diffusion of CO₂ through the plastic will not be a problem during the duration of the experiment. Minimize the exposure of this solution to air. **Mix the solution thoroughly before using. The solution may settle between labs, so always mix thoroughly before use.**

B. Standardization of NaOH solution.

Calculate the appropriate amount of KHP sample to use approximately 40 ml of base per titration.

Two methods may be used to conduct the standardization.

1. Absolute weights - Weigh accurately an appropriate amount of KHP into a titration flask (weigh by difference from the weighing bottle). Add sufficient water to dissolve and titrate with standard base to the phenolphthalein end point. (You may begin the titration before all of the solid is dissolved, but insure it is all dissolved at the end point.)

2. Aliquot method - Weigh accurately an appropriate amount of KHP into a volumetric flask. Add water to dissolve and finally dilute to the mark. **Mix thoroughly.** Withdraw aliquots of this solution with a pipet and transfer to titration flasks. Titrate to the phenolphthalein end point, (e.g., 250 ml flask would give 4 50 ml aliquots). Caution: KHP is not highly soluble and care must be taken to insure it is all dissolved and mixed before you pipet a sample..

The first method requires more weightings but uses sample very efficiently since none is wasted. The second method requires more care in transfer and dissolving but will give end points at the same place each time which greatly speeds titrations and evaluation of data. You can easily calculate the expected endpoint for the absolute weight method using a proportion for each sample. Take your pick. **BE SURE YOU USE PROPER WEIGHING PROCEDURE. ANY SPILLAGE OR LOSS IN TRANSFER OF THE SAMPLE MAKES ACCURATE WORK IMPOSSIBLE.** Weighing paper should never be used.

Repeat the titration until your results for three consecutive titrations agree within 2-4 ppt. You may discard initial values until your titrations become reproducible, but you may not discard any subsequent results without a definable experimental error.

THE TITRANT IN THIS EXPERIMENT CAUSES SEVERE BURNS. IF YOU SPILL IT ON YOURSELF, FLUSH WITH LARGE AMOUNTS OF WATER AND REPORT THE ACCIDENT TO THE INSTRUCTOR.

II. Validation of titration procedure using a Standard Reference Material and Determination of an Unknown KHP.

The standard reference material used to validate the titration is an impure KHP sample similar to your unknown. The exact analysis of this sample will be provided. This allows you to test your own technique and the accuracy of your standardization. The procedure for both the

reference material and unknown KHP are identical. Only the procedure for the unknown is given below. It is important that you complete the analysis and calculations for the reference material before you proceed any further than drying the unknown.

III. Determination of Purity of Unknown KHP - Lab Practical Exam.

Dry the unknown at 110-120°C for 1 hour. Use 1 gm of unknown for each titration. You may use either the aliquot method or the absolute weight method outlined above for the determination of the unknown. Titrate to the phenolphthalein endpoint as for the standardization. Obtain at least 3 titrations. (You should receive enough unknown for at least 6 trials.)

Evaluation - the accuracy and precision of your result will be graded on a non-linear sliding scale with a grade of 100 being awarded for results within 1 ppt. Penalties will be assessed for the following:

1. Need more unknown due to running out - 10%
2. Calculation error in result - 10%
3. Redo unknown after grade <70% - 10%

REPORT - Brief Report only- Fill in KHP Spreadsheet (on Blackboard/Labs) and submit by email. You may print the spreadsheet and use this for your summary data table for the Brief Report.

KHP Unknown Report spreadsheet in Blackboard. Save the report using SAVE AS with your name as the file name. Email the completed spreadsheet to the instructor. The graded spreadsheet will be returned by email. The following information is required to this report:

1. Percent KHP in the unknown sample from your calculations.
2. The final data table for the unknown titrations.
3. The standard deviation of the values.
4. The relative standard deviation of the values in ppt
5. The molarity of the NaOH solution
6. The value obtained for the reference material and the relative standard deviation.

NOTE: A minimum of 3 determinations must be included in each result and 4 is preferable. To obtain an "A" for this experiment the analysis must have both accuracy and precision better than +2 ppt. (.2% relative.)

Waste Disposal-

Titrated solutions can be discarded down the drain.

Excess titrant can be disposed of down the drain.

Do not dispose of the standardized NaOH as this is needed for later experiments.

Experiment 2- Potentiometric Titration of Weak Polyprotic Acids

Done in Pairs

Read: Text Chapter 12-5 and 12-9

Prelab Exercise

Complete the exercises in Blackboard

Introduction

Potentiometric (pH meter) titrations are often used instead of visual indicators. This is particularly true for titrations of weak or polyprotic acids and bases where breaks may be less sharp or occur in regions lacking good visual indicators. Potentiometric titrations can also be automated through the use of a computer controlled buret. Furthermore, analysis of the titration curve can yield information about the identity of the acid (from its pK_A) as well as its concentration.

You will be assigned a pure weak acid. You will titrate a known amount of the acid with your standard NaOH solution and obtain a detailed graph of pH vs volume of titrant. You will then compare the actual graph with a theoretical graph you will calculate using a detailed polyprotic acid spreadsheet. By adjusting the parameters of the program you will attempt to obtain the best fit of theory with experiment. Titrations are done in groups of two. Data analysis and curve fitting is to be done individually. When a detailed theoretical description of an experiment is possible, fitting experimental data with theory can allow determination of equilibrium constants under actual lab conditions (conditional equilibrium constants).

Several mathematical methods have been proposed to make it easier to find the end point of a potentiometric titration. These are mathematical alternatives to the preparation of a high accuracy graph and the use of a compass to bisect the endpoint. Probably the most common one is the use of first and second derivatives (discrete derivative). The first derivative in its simplest sense is the value of dpH/dV .

$$\frac{dpH}{dV} = \frac{pH_x - pH_{x-1}}{V_x - V_{x-1}}$$

The value of the derivative is the slope of a tangent to the titration at the midpoint between V_{x-1} and V_x . The volume point which corresponds to this derivative is the midpoint between the two volumes.

$$V_{av} = (V_x + V_{x-1})/2$$

The second derivative is obtained by taking the derivative of the first derivative. When you make a spreadsheet, if it is well designed, you will only need to enter the equations above on one cell each. The rest of the spreadsheet can then be constructed by copying these equations.

I. Potentiometric Titration.

Potentiometric titrations should be carried out in a 150 ml beaker. The tip of the buret must reach far enough into the beaker that it is under the surface of the solution so that no water need be added to wash the tip during the titration. Addition of water after the start of the titration will change the pH and shift the titration curve. Remember pH is a concentration technique while visual indicators depend mostly on total quantity. A magnetic stir bar and stirrer are used to mix the solution.

Obtain an unknown weak acid from the instructor. Weigh a 2.5 gm sample then bring to the mark with water in a 250.0 ml volumetric flask. Dilute to the mark and mix thoroughly. Take a 10 ml aliquot of the sample for analysis using a pipet. Add 25 or 50 ml of water with a pipet to bring the total volume up so that the electrode and buret tip will be submerged. The aliquot size for subsequent titrations may be adjusted to obtain a complete titration using 35-45 ml. of titrant. Use only volumetric pipets for these aliquots.

The best approach to potentiometric titration is to make each addition of base until some constant pH change is observed. Typically 0.2 to 0.3 pH units of change for each addition will give a nice curve. In regions of rapid change you may find the pH drifts or becomes unstable. Wait a reasonable time (<1 min.) for stability to return. If the reading does not stabilize, take a reading and continue. In regions close to the endpoint the pH will drift due to diffusion of titrant out of the buret tip. Delay at these points will actually increase error, so do not hesitate to take a reading and move on to the next point. You should not need more than 25-30 points to obtain good data. Take data to pH 11. Repeat the titration three times.

Create a spreadsheet to compute the first and second derivative and use the second derivative data to determine the endpoint of each titration. (put each titration on a separate worksheet in the spreadsheet) Average the endpoints. You should make a summary sheet and bring the values for the three titrations to the summary page and from the endpoint, calculate the molecular weight of your acid. Use the list of diprotic acids at the end of this experiment to identify your unknown. Determine the precision of your results and the % error between the actual value of the molecular weight of the acid and the value you determined.

II. Comparison of actual and theoretical titration

Prepare a spreadsheet to compute an accurate theoretical titration curve for your weak acid. (see Text 12-9 for equations) Copy and Paste the titration data from your derivative file into this spreadsheet. Input values of the K_a 's, the concentration of the weak acid solution determined from the titration data, the concentration of the NaOH determined in the standardization. You have entered the actual volume of titrant at each pH value and the spreadsheet will calculate the theoretical value of the volume. Graph the actual and theoretical volumes of titrant on the x axis and pH on the y axis) Make a column to compute the square of the difference between the two volumes. Then add a cell to compute the sum of these deviations. This is the overall sum of the

squares of the deviation between theory and experiment. Name the sheet as initial data then make a copy of this entire sheet in your spreadsheet and name it best fit data.

On the sheet labeled best fit, adjust K_1 , K_2 , and acid concentration to obtain the best possible fit between theory and practice as demonstrated by the sum of the squares of the deviations. Changing the concentration of acid will move the actual curve to make the endpoints overlap. Changing K_1 will adjust the curve in the region before the first equivalence point. Changing K_2 will adjust the curve in the region after the first equivalence point. You may wish to expand the scale around the second equivalence point so you can see what is happening better while making adjustments. It is acceptable to drop points at the end of the titration if they deviate from theory. The errors in this region may be due to the presence of carbonate in the titrant or to sodium error in the pH electrode. In most cases the sum of squares can be reduced to less than 1.0 and certainly to less than 10.0.

Add a worksheet that summarizes the results of the optimization. It should include the reported values of K 's, the average value you found by fitting the data, and the % difference between them.

III. Report

Brief Report only. This will include printouts of the summary page from the derivative spreadsheet and the curve fitting spreadsheet and one example of the titration graph second derivative graph, and the comparison of theoretical and actual before and after parameter adjustment.

Email the two spreadsheets to the professor.

Diprotic Acid Unknowns

- Adipic
- Fumaric
- Glutaric
- Maleic
- Malic
- Malonic
- Phthalic
- Succinic
- Glycine hydrochloride

Experiment 3- Determination of Calcium by EDTA Titration⁴

Individual

Prelab Exercise

A 0.800 gm sample CaCO_3 is used to prepare 250 ml of standard Ca^{2+} solution. When a 25.00 ml aliquot of this is titrated with EDTA, the endpoint occurs at 31.24 ml. Calculate the concentration of the EDTA solution.

An unknown CaCO_3 sample is analyzed by the same procedure. A 0.2212 gm sample is dissolved in 250 ml and 25 ml aliquot is titrated with your lab partner's EDTA which is 0.01234 M. The titration required 21.57ml of EDTA. What is the % CaO in the unknown?

Prior to the lab you must determine what natural product you wish to analyze and obtain a sample for analysis.

TIP: Dry your unknowns at the start of the lab.

Introduction

Read: Text Chapter 13

This experiment involves the use of EDTA for the analysis of the calcium content of an unknown and of a natural product. The natural product can be any substance believed to contain calcium. You should not select a product for which there is no reasonable expectation of calcium content. While this test is most commonly used to determine water hardness, you should be able to analyze almost any food product as well. The use of a high pH eliminates interferences from most metals. However, it is essential that the buffer be added prior to addition of the indicator. Large concentrations of iron or copper can interfere at any pH, so avoid samples where such interferences are expected.

Procedure

Preparation and Standardization of EDTA Solution.

Weigh about 8 gm of $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ into a clean 400 ml beaker. Add 12 pellets of NaOH and 0.2 gm $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$. Dissolve and transfer to a clean 1 liter bottle. Dilute to about 1.0 liter.

Obtain dried CaCO_3 stored in the desiccator at the front of the lab. Accurately weigh about 0.8 gm in a 250 ml beaker. Dissolve the solid **completely** (clear solution) in the minimum amount of

⁴ This method actually determines total calcium and magnesium. It is not specific for calcium.

6M HCl. Heat on a hotplate to evaporate to dryness. Then dissolve the residue in deionized water. Transfer to a 250 ml volumetric and bring to the mark.

Pipet 25 ml aliquots of the primary standard Ca^{2+} solution into 250 ml titration flasks. Add 10 ml of pH 10 buffer (285 ml conc NH_4OH + 32 gm. NH_4Cl diluted to 500 ml). Add 6-10 drops of Calmagite indicator (.05% in water). Titrate with the EDTA solution. The color change for this titration is often described as wine red to blue. The best endpoint is somewhere before the solution is completely blue. This endpoint takes a bit of practice and it is most important to always titrate to the same color. Since you are standardizing the titrant with a Ca^{2+} solution, any error in selecting the endpoint should be compensated in the unknowns. This is not the case however, if the color you choose is completely blue.

Repeat until adequate precision is obtained. Calculate the molarity of the EDTA solution and the precision and estimated experimental error.

TIP: When you know approximately where the endpoint will occur, you can run to within about 5% of the endpoint quickly. Then wait 2-3 minutes before you continue to titrate. This is necessary to allow the slow equilibration of the complex and indicator equilibria.

Analysis of Unknown

Dry unknown for about 1 hour at 100°C . Prepare a solution of the unknown as you did for the Ca standard using 0.8 to 1.0 gm of unknown diluted to 250 ml. Take 25 ml aliquots with buffer as above and titrate with standardized EDTA solution. Report calcium content as % CaO in the sample, precision and variance of the method.

Analysis of Natural Product

Select a sample of interest and determine from the literature the approximate range of calcium concentrations to be expected. (For food samples see the USDA web site) For water hardness, dilute the titrant and EDTA quantitatively 1:10. Try a test titration on a 50 ml aliquot and adjust subsequent sample size to use a reasonable amount of titrant. All samples must be buffered with 10 ml of buffer.

Avoid samples which would obscure the color change of the indicator. Also beware of samples with large amounts of transition metals such as copper as these will react with the indicator to prevent an endpoint from being observed. Report % Ca or ppm Ca in your sample.

Note

The biggest problem in this experiment is failure to add sufficient buffer. If you do not obtain an endpoint in a reasonable volume of titrant, test the pH of the solution. Because you are titrating with an acid and the formation of CaEDTA releases 2 protons per mole, the solution will gradually become more acidic. It is always best to err on the side of too much buffer.

Report

Brief report only. You may print the Spreadsheet as your summary table.

Enter your results into the **Calcium Unknown Report Spreadsheet** provided on Blackboard/Lab. Save the spreadsheet on your computer using Save AS and use your name as and the experiment as the file name. Email the Spreadsheet to the professor.

Spreadsheet requires the following information: 1.

Average value of %CaO in unknown with ppt rel. std. dev.

Summary of titration data for unknown as well as amount of sample used for each titration.

Molarity of EDTA with standard deviation

Sample size, sample preparation details, and results for Ca in your natural product sample along with the expected value and the reference for this value.

Rotation Experiment 4 - Liquid Chromatographic Determination of Caffeine, Theobromine, and Vanillin in Chocolate By the Internal Standard Method

Group

References: Text, Merck Index for chemical structures and medicinal properties.

Prelab Assignment

You must meet with your lab partner and decide what chocolate samples you wish to analyze. You should have time to analyze 2-3 samples. Your samples should be chosen with some specific question in mind.

Write a brief description of the question you hope to answer during this lab and turn it in as a prelab exercise. Bring your samples to lab.

View the HPLC instruction on Blackboard and complete the Quiz for this lab.

Introduction

Read: Text 25-3

Chocolate is a popular food formulated from the extract of the cocoa bean. The final product includes sugar and other substances to produce different types of chocolate. Dark chocolate contains the highest amount of cocoa liquor (the raw extract of roasted cocoa beans (typically 50–65%). Milk chocolate contains less cocoa liquor (25–50%) and more milk solids. white chocolate is formulated from the cocoa butter alone. This is the fat portion of the cocoa liquor without the solid cocoa powder. A common additive to chocolate is vanilla. This enhances the flavor and may also have some anti-oxidant properties.

Chocolate contains several psycho-active compounds. The primary one is theobromine. It has been observed that theobromine is relatively constant in all cocoa beans ranging from 1.5 to 3%, therefore, the theobromine content of chocolate relates directly to the amount of cocoa liquor used in the formulation. The analysis of theobromine in this experiment will allow you to find out how much actual cocoa extract is in your favorite chocolate.

Theobromine has several psychological actions. It is a diuretic, a vasodilator, and a smooth muscle relaxant. It is also a cardiac stimulant. It has been shown to be quite toxic to dogs that may die if fed moderate amounts of chocolate. Fortunately, the same effect has not been observed in humans.

Caffeine is also present in chocolate. While its concentration is not as good a measure for the cocoa content as the theobromine concentration, many people are sensitive to caffeine. Thus,

the analysis of caffeine can allow one to determine how much of this substance is present in various chocolate products. Caffeine, like theobromine, is a diuretic. However, caffeine is primarily a stimulant. It works on the central nervous system, the cardiac system, and the respiratory system.

The third component in this analysis is vanillin. This is a common flavor enhancer used in a wide range of foods. Vanillin may be either natural or synthetic. The common synthetic form is ethyl vanillin while the natural form is methyl vanillin. This analysis does not distinguish between the two forms. There is no evidence that either form is harmful or beneficial.

In preparing for this experiment, you and your lab partner need to discuss what specific question you wish to answer regarding chocolate. You may want to compare the amount of cocoa liquor in each of your favorite chocolates. You may want to look at the caffeine content of several chocolates, etc. After you have decided what question you want to answer, you must purchase the samples for analysis. You will need about 10 gm of each sample. Each group must run at least 2 samples and may attempt as many as 4. **You cannot begin this experiment if you do not bring samples to lab.**

Background information on HPLC

A High Performance Liquid Chromatograph (HPLC) is an instrument which provides rapid, efficient separation and detection of mixtures based on their chemical properties. There are several modes of HPLC, specifically adsorption (normal phase), reversed phase, ion exchange, and size exclusion. This experiment will demonstrate the use of reversed phase HPLC which is the "reverse" of normal phase chromatography in that the non-polar phase is the stationary phase while the polar phase is the mobile phase. Water is normally a part of the mobile phase and this mode of HPLC is becoming increasingly popular due to the fact that many samples of interest are in aqueous solution. Thus the use of reverse phase HPLC eliminates the need to do an extraction of the sample into a non-polar solvent.

The HPLC instrument consists of four parts, a high pressure pump capable of pushing the solvent at flow rates of up to 10 ml/min through the column, an injection valve, a column packed with fine particles of the stationary phase, and a detector capable of producing an output proportional to the amount of sample in the detector cell at a given instant. The column packing for reversed phase HPLC consists of silica particles to which are chemically bonded n-C₁₈ hydrocarbon at each active site. (Other hydrocarbons may also be used but at the present time C₁₈ is the most popular.) The particle size of the packing is typically 3–10 microns diameter. The detector used in this experiment is a spectrophotometer which measures the absorbance of light by the sample. The wavelength selected must be one at which the sample absorbs strongly. 254 nm and 280 nm are probably the most commonly used, but a variable wavelength detector allows any wavelength to be chosen.

The mode of separation involves the partition of the sample between the two phases. Samples with relatively greater solubility in the aqueous mobile phase elute first while those with greater solubility in the hydrocarbon elute later. In some cases it is desirable to control the ionic

strength or pH of the mobile phase to control the ionization of samples which exhibit acid-base characteristics. Since both caffeine and theobromine are weak bases, an acidic pH will lead to the protonation of these which reduces their retention. Ionic species are much less soluble in hydrocarbons than in water. It is important to note that the solubility of ionic species in a non-polar solvent is negligible. Thus, neutral species must be present to be retained on the column.

Method Validation

This experiment employs the internal standard method for quantitation. This method is often the easiest approach since it does not require careful quantitative technique once the internal standard is added and made homogeneous.

The internal standard method is the generally the most accurate method of quantitation for chromatographic analysis. The internal standard is a substance which has chemical properties very similar to the chemicals being analyzed. It is added as early as possible in the analysis process, and it is assumed that it will pass through the various stages of sample preparation in the same manner as the compounds of interest. For this experiment we will examine two different internal standards, 3-hydroxy- benzoic acid and theophylline. The former has a chemical structure similar to that of vanillin while the latter is similar to caffeine and theobromine.

The problem with the internal standard method in this specific procedure is that it is assumed that the analytes and the standards are extracted from the fat of the chocolate to the same extent. If this is not the case, then the method will fail to give good quantitative results. A second problem arises if the response of the detector to the analytes and standards is not linear.

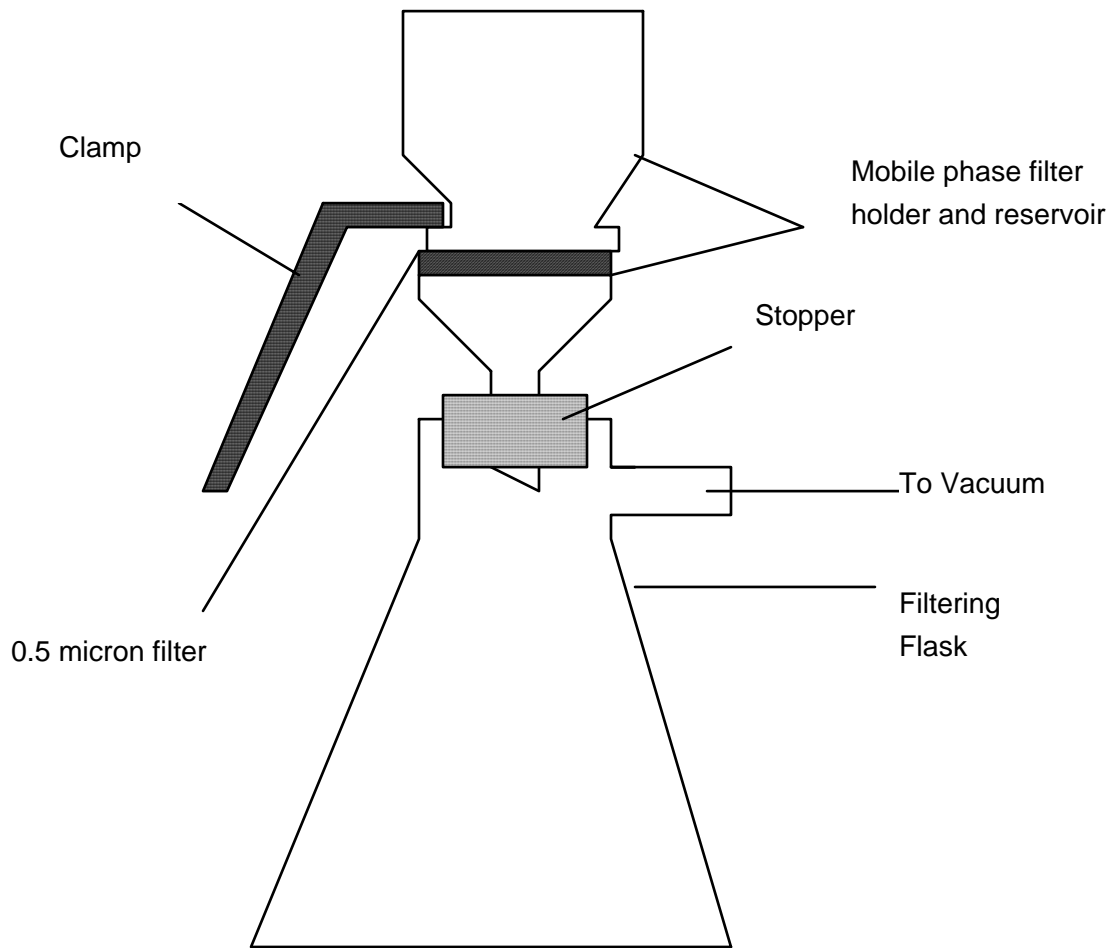
The procedure outlined below for this experiment is designed to specifically check for the two problems mentioned above to validate the analysis. Standards are run at two multiple concentration levels to confirm linearity. The extraction of analytes from the chocolate sample is repeated with the addition of a known amount of each analyte to measure the recovery of the analytes and standards from the matrix. The idea of recovery is a simple one. If you analyzed a sample of chocolate and found it to contain 5 mg of caffeine, then if you add 5 mg of caffeine to a sample of the same chocolate it should now be found to contain 10 mg of caffeine. If the analytical result is only 9 mg caffeine, then you have recovered only 4mg/5mg of caffeine or 80%. The standards of what is acceptable recovery vary depending on the overall goals of the analysis. However, the goal is always 100% recovery. (Experimental errors can lead to recoveries greater than 100% which is just as bad as less)

Preparation for Analysis

1. Mobile phase - make 500 ml of 5% acetic acid in HPLC grade deionized water (obtain from the Barnsted system in the lab). Graduate cylinder accuracy. Vacuum filter through 0.5 micron filters before use using the apparatus diagrammed below. This solution will be mixed by the pump with methanol to form the final mobile phase.

2. Prepare 300-400 ml of extracting solvent. Make this up quickly with graduate cylinders and get started extracting samples. You will need 30 ml for each sample you will analyze plus an additional 60 ml for the recovery tests. You can also use this to dilute the standard solutions you prepare. Extracting solvent is 79% water, 20% ethanol, and 1% acetic acid.

To filter mobile phases, assemble the filter apparatus on a clean filtering flask as shown below. **This apparatus is to be used only for mobile phase preparation.**



Pour the solvent into the reservoir and attach to aspirator vacuum. Use a new filter for each batch of solvent.

3. Seven standard solutions are provided (in the refrigerator). **Concentrations listed here are nominal values. Exact values will be listed on bottles and are to be used for calculations.**

1. Pure individual components. The three analytes and two internal standards are provided for determining retention time only. These five solutions are all 50-100 ppm.
2. **Internal Standard mixture** to add to chocolate samples and is used to prepare calibration standards. This solution is approximately 500 ppm theophylline and 2000 ppm 3-hydroxybenzoic acid. This solution is prepared in the extracting solvent.

3. **Recovery standard** mixture. This solution is used to prepare calibration standards and is added to all recover samples to test extraction efficiency. It contains approximately 500 ppm theobromine and caffeine and 1500 ppm vanillin. It is prepared in the extracting solvent.

Return all solutions to the refrigerator when finished.

Prepare solutions to create an internal standard calibration curve:

- Prepare three internal standard calibration samples. These are made using the internal standard mixture solution and the recovery standard above. Each solution should have the same concentration of the internal standard mixture at 50 ppm theophylline and 200 ppm 3-hydroxy benzoic acid. The analyte concentrations should be 50 ppm, 20 ppm and 5 ppm caffeine.

Operation of the Hitachi HPLC

The HPLC column is left stored in methanol. Place the solvent filter for Solvent A into your filtered solvent and turn on the pump. Bottles of methanol Solvent C and deionized water Solvent B should be already in place.

NOTE: THE ONLY BUTTONS YOU SHOULD NEED TO PRESS ON THE PUMP ARE POWER, PUMP, PRIME, A, B, C, MANUAL SET, ESCAPE, ENTER, AND THE NUMBER KEYS.

Turn on the pump and open the prime/purge valve. Place a small beaker under the valve and press the **PUMP** and **PRIME** buttons. The pump should begin to pump at maximum rate. Press the **A** button and wait until a steady stream of solvent emerges. Press the **B** button and wait again for a steady stream. Press **C** and repeat. Press **PRIME** and **PUMP** buttons again to turn off the pump.

Set up the pump to mix a mobile phase of 80% Solvent A (Acetic acid 5%) and 20% Solvent C (methanol). Set the flow rate to approximately 1 ml/min being careful that the pressure does not exceed 4500 psi. Purge the column with at least 20 ml of solvent (20 min). This is done by pressing **MANUAL SET** button and then entering the requested information on the keypad.

Turn on the detector. Set the wavelength to 280 nm. When finished purging, **Autozero** the output to 0.000 AU. Observe the baseline and continue to purge until the absorbance reading is stable. Rezero as needed.

Refer to the Appendix on the use of ChromPerfect for detailed instructions on setting up the computer for data acquisition and for quantitation.

When ready to inject your sample, fill the 50 ul syringe to approximately 40 ul without getting air bubbles in the syringe. **Be sure you have entered the sample information into the**

computer. Turn the sample valve to the LOAD position (counterclockwise). Place the needle all the way into the valve opening and inject the entire sample. DO NOT REMOVE THE SYRINGE AFTER INJECTING. This will fill the sample loop which holds exactly 10.0 ul. Rotate the valve clockwise to the inject position. OBSERVE THE PRESSURE READOUT ON THE PUMP AS YOU DO THIS. IF THE PRESSURE RISES YOU HAVE NOT TURNED THE VALVE FAR ENOUGH. The valve turns 1/6 of a turn. The valve should be turned as rapidly as possible. After you turn the valve press the inject button or start computer data acquisition manually. Remove the syringe after you complete the injection.

AutoSampler Operation

The HPLC is equipped with an autosampler (AS). Place your sample in an AS vial and insert it in the tray. Note the position of the vial. For a single run, press QuickStart and enter the vial, volume to inject and run time. The system will inject your sample. Be sure you have set up the computer with sample information before you do this. The AS will start the Computer data acquisition process.

I. STUDIES OF MOBILE PHASE EFFECTS

Begin analysis by injecting the sample containing all three analytes and the two internal standards. You should observe five distinct peaks. If the peaks are not well resolved, you should adjust the mobile phase composition to improve the separation. In general, increasing the methanol concentration will speed the elution and bring the peaks closer together. Lower methanol will cause the peaks to be retained longer on the column. You should also try a mobile phase of 85% Solvent A and 15% Solvent B(acetonitrile). Allow 20 minutes for the column to equilibrate before making an injection. If this mobile phase does not provide full separation, try one using a mix of methanol and acetonitrile. Using the optimal mobile phase, run additional chromatograms at 0.75 ml/min and 2.0 ml/min flow rates. Calculate the resolution between the two least resolved components as a function of flow rate.

After you have made any necessary adjustments to the mobile phase composition, you should determine the order of elution of the three compounds by injecting the individual standard solutions you prepared. Finally analyze the three different concentration standards. This sample is used to verify the linearity of the detector. Prepare a graph of peak area ratio vs quantity ratio for each analyte. This graph will be used for quantitation of your unknowns.

II. Preparation of Chocolate Samples

Extraction procedure

1. Chocolate samples, approximately 5 gm accurately weighed, should be broken up into small pieces before weighing and transfer to the blender.

2. Add 30 ml of extracting solvent to the blender with the chocolate.
3. Add 10.0 ml of 0.500 mg/ml 3-hydroxybenzoic acid and 0.500 mg/ml theophylline to the blender as internal standard.
4. Blend the sample in the blender for 5 minutes.
5. Filter the sample using a buchner funnel and Whatman #42 filter paper with vacuum. The filtering flask must be clean but need not be dry. You need only filter a small amount of the sample as only 1.0 ml of filtrate is required.
6. Transfer about 1 ml of filtrate to a 10 ml graduate cylinder and bring to 10.0 ml with 5% acetic acid. Keep a small sample of the filtrate for use if the initial sample is too dilute for analysis. (Note– the ability to use fairly crude volumetric technique is made possible by the presence of the internal standard. Since you are only interested in the ratio of the peaks, the exact concentration is only important to the extent that it allows you to keep the sample in the linear response range of the detector.)
7. Draw about 1 ml of the concentrated extracts into a plastic syringe and place a 0.47 micron sample filter on the syringe. Expel the sample into an autosampler vial, cap and label. Repeat with the diluted extract.

Recovery Study

8. Repeat the steps above on a second sample of the same chocolate but add 10.0 ml of the 0.5 mg/ml recovery standard solution and only 20 ml of extraction solvent to the blender with the chocolate and the internal standard solution. in step 3-7 above.

III. Analysis of Samples

Inject each sample into the HPLC at least twice to obtain reproducible peaks for analysis. This will be done on the instrument with the Autosampler and will run overnight. Data is available through the campus network for processing.

IV. Calculations

The ChromPerfect program will determine the peak areas for each peak. Examine each chromatogram and insure the baseline and other integration parameters are reasonable. If not, adjust the integration parameters to obtain accurate integrations.

Prepare a well organized and documented spreadsheet to do all calculations for this lab.

For each analyte, plot the area ratio (Area sample/Area IS) vs quantity ratio (mg sample/mg IS). Do a least squares fit and assess the linearity.

For each chocolate sample, calculate the area ratio for each analyte. Using the least squares equation, calculate the quantity ratio. Then use the known amount of IS to calculate the quantity of the analyte.

Determine the % recovery for the analytes using the recovery samples.

$$\% \text{ Recovery} = \frac{\text{mg found in spiked sample} - \text{mg found in unspiked sample}}{\text{mg added to spiked sample}} \times 100\%$$

Shutting down the HPLC (Manual system)

It is important that the HPLC be purged to remove any salts and acids which will clog the system if the solvent evaporates. Before leaving the lab, pump 15 ml of distilled water through the system. Then pump 30 ml of methanol. When this is finished the pump may be turned off along with the detector.

Report

Brief Report only. Submit your own spreadsheet (each student must prepare their own spreadsheet for this lab) Report the results of your quantitative analysis of your samples in a meaningful way. This means you must convert the concentration of caffeine, theobromine and vanillin into units which are meaningful to a user of the product analyzed. (eg. mg per serving as specified on the label.) Spreadsheet must also include % recovery.

Rotation Experiment 4 - Determination Of Vanillin By Spectrophotometry Comparison of Solvent Extraction and Solid Phase Extraction⁵

Group

Prelab Exercise

You have available solid vanillin and the following glassware:

volumetric pipets - 1.00, 5.00, 10.00, 25.0, and 50.0 ml

volumetric flasks - 25.00, 50.00, 100.00, 250.0 ml

Using only the above glassware and the 5 place balance, describe in detail how you would prepare solutions of the following concentrations from solid vanillin: 100 µg/ml, 5 µg/ml, 4 µg/ml, 2 µg/ml, and 1 µg/ml. Remember that your grade will depend on the accuracy of these solutions.

Blackboard Video on Solid Phase Extraction and Solvent Extraction and Quiz

Background

Read: Text: 23-1, 28-3

Vanillin is the common name for 3-methoxy-4-hydroxy-benzaldehyde. Besides being a very popular flavor, vanillin is also used in the synthesis of drugs, and this use now surpasses its use as a flavoring agent! The largest single use for vanillin as a starting material is for the manufacture of the antihypertensive drug called Aldomet manufactured by Merck. Aldomet is 1-3-(3,4-dihydroxy phenyl)-2-methylalanine. L-dopa is another drug made from vanilla and is used for the treatment of Parkinson's Disease. Vanillin is also used as an intermediate for several other important pharmaceutical products and, as well, has many varied uses in general industry.

Analysis Of Pure Vanilla Extract

There are no standards to tell the consumer the amount of vanillin in a sample of vanilla extract. The specification of the extract says only how many vanilla beans are soaked in a volume of alcohol. Hence it is not unreasonable to expect wide variations in quality. Two samples of commercial vanilla extract will be analyzed. The goal is to determine which contains more vanillin.

Comparison of SPE and Solvent Extraction

The goal of this experiment is to develop a Solid Phase Extraction method for analysis of vanillin in vanilla extract and to compare this method with an established solvent extraction method. The comparison of the two methods will include not only the quality of the data, but also the ease of use, time required for analysis and expense of the materials required. The

⁵ This Procedure Is Derived From "The Determination Of Vanillin In Vanilla Extract", By E.W. Ainscough and A.M. Brodie, J. Chem Ed. **1991**, 1070 - 1071.

extraction method described below is adapted from that given in the *Encyclopedia Of Industrial Chemical Analysis*. Neutral vanillin is very soluble in dichloromethane, therefore, extraction into this solvent leaves the colored material in vanilla extract in the water layer. The dichloromethane extract is then treated with dilute sodium hydroxide solution. Deprotonation of the hydroxy group occurs, and the resulting vanilate ion moves into the aqueous phase. This ion has an intense absorption maximum at 347 nm that is used to determine its concentration.

Solid Phase Extraction (SPE) is a rapidly growing sample preparation method. In SPE, a sample in a solvent is passed through a bed of a solid absorbent, similar to those used in liquid chromatography. The solvent in which the analyte is dissolved must be a weak solvent on the particular sorbent so that the analyte is completely retained on the sorbent bed. The bed is then washed with solvents to remove any weakly retained contaminants. Finally, the analyte is eluted from the bed with a small volume of a strong solvent. If there are interfering species which are also strongly retained, then this eluent must be chosen with some care. In the case of this analysis, there are no such problems. Thus, your only concern in developing the method is to insure that all of the vanillin is recovered from the sorbent.

In addition to the SPE cartridge containing the sorbent, the SPE system includes a vacuum pump and a manifold. The manifold allows multiple cartridges to be processed simultaneously. It also provides a simple means for controlling the flow rate through the bed. In the case of the Supelco manifold, this adjustment is done by turning the cartridge. Inside the manifold is a rack to hold the receiver tubes for collecting the samples.

Experimental

This experiment will require about 1 liter of 0.1 M NaOH. Combine the unused standard solutions from the first experiment. If you do not have 1 liter, you can prepare whatever additional quantity you need. It is not necessary that the solution be standardized, but only a single solution should be used by both students to prevent errors.

The standard vanillin solution (100 ug/ml in 0.1 m NaOH) must be prepared from commercially available vanillin.

One student in the group will conduct the solvent extraction. Two extractions can be performed at the same time using two sets of separatory funnels. The other student will conduct the SPE experiment. One set of analytical standards should be prepared by the group for calibration.

Solvent Extraction Procedure

CAUTION: CARE MUST BE TAKEN TO VENT THE SEPARATORY FUNNEL IN ALL EXTRACTION PROCEDURES, ESPECIALLY DURING THE INITIAL PERIOD OF AGITATION. FAILURE TO DO THIS CAN RESULT IN EXPLOSIVE EXPULSION OF THE STOPPER. YOU MUST ALWAYS BE AWARE WHERE THE TWO ENDS OF THE

FUNNEL ARE POINTED. DO NOT POINT EITHER END TOWARD YOURSELF OR ANOTHER PERSON IN THE LAB.

Add about 10 ml deionized water into a small separatory funnel, followed by exactly 1.00 ml vanilla extract. Add 20 ml dichloromethane, and shake well, releasing excess pressure from the funnel through the stopcock as required. (You should begin with a single brief shake followed by venting. dichloromethane produces a large pressure buildup when first mixed with water. After the first few ventings, you will find you can carry out vigorous shaking with only occasional venting.) Allow the aqueous and organic layers to separate. Drain the lower organic layer into a 250 ml separating funnel.

Repeat the extraction of the aqueous layer with two further 20-ml aliquots of dichloromethane and combine all the dichloromethane extracts (which should now contain the vanillin) in the large (250-ml) separating funnel. Avoid releasing any of the upper aqueous layer or the small emulsion layer between the phases when you withdraw the dichloromethane.

CAUTION: NAOH SOLUTION CAUSES SEVERE BURNS. IF YOU SPILL ANY ON YOUR SKIN, FLUSH WITH COPIOUS AMOUNTS OF WATER (10-15 MINUTES) AND REPORT THE INCIDENT TO THE INSTRUCTOR.

Carefully add 50 ml 0.1 M NaOH to the dichloromethane extract and shake well, releasing excess pressure as required. The vanillin will move into the upper aqueous layer. Drain the dichloromethane layer and any emulsions into a beaker, and run the clear aqueous layer into a 250-ml volumetric flask. Extract the dichloromethane two more times with 50-ml aliquots of 0.1 M NaOH. At this stage, keep the emulsion layer with the dichloromethane and avoid any dichloromethane in the NaOH layer.

Combine all the NaOH extracts into the 250-ml volumetric flask. Make the vanillin solution up to the mark with 0.1 M NaOH. Measure the Absorbance of this solution. If the absorbance is not optimal for your calibration curve, do a quantitative dilution using 0.1 M NaOH to obtain the most accurate possible results.

Put The Used Dichloromethane (Which Is Toxic) Into the Waste Bottle Labeled Chlorinated Organic Waste- *Not* Down The Sink. It is important not to get any water into the waste organic bottle, so make sure your waste is clean before placing it in the bottle. Disposal costs go from \$20/gal to \$100/gal if the waste has water in it.

Method Development and SPE of vanillin

The only part of the SPE method which has been checked for you is the fact that the procedure for absorbing the vanilla on the sorbent bed will in fact retain the vanillin. This was accomplished by applying the vanilla solution and analyzing the eluent by HPLC to detect any traces of vanillin not being retained.

Your task in method development is to determine how much of the eluting solvent (25% Methanol-water) is required to desorb all of the vanillin.

The procedure you will follow is to collect the eluent in 1.0 ml portions which will be analyzed spectrophotometrically for vanilla. When you know which fractions contain vanilla, you can determine the amount of eluent required to obtain 100% recovery and use that amount in your actual analysis.

SPE Method Development Procedure

1. Prepare a diluted solution of commercial vanilla by diluting 1.00 ml extract in a 10.0 ml volumetric flask with DI water.
2. Assemble the SPE Manifold with test tubes in the rack. Turn on the vacuum pump and adjust the vacuum to 20 in. on the gauge.
3. Place a C18 SPE cartridge in the manifold and condition the cartridge with 1.0 ml methanol and 1.0 ml DI water. (flow rate should be adjusted to about 1 ml/min by turning the cartridge in the manifold. CCW to increase flow) AFTER CONDITIONING- DO NOT LET THE CARTRIDGE DRY OUT AT ANY TIME. SHUT OFF FLOW BY TURNING THE CARTRIDGE CLOCKWISE.
4. Add 1.00 ml dilute vanilla extract prepared in 1 above to the cartridge and elute at about 1 ml/min.
5. Rinse cartridge with 1 ml DI water.
6. Move the cartridge to a position on the manifold with a clean TT. Add 1.0 ml of eluting solvent and elute.
7. Move cartridge to the next TT position, add 1.0 ml eluting solvent and elute. Do this part 5 times.
8. Disassemble the manifold and clean the interior of the manifold.
9. Add the contents of each test tube containing eluent to separate 10 ml volumetric flasks. Then fill to the mark with 0.10 M NaOH. Mix thoroughly.
10. Measure the absorbance of each tube as for the other vanillate ion solutions in the Spectrometer. For tubes which read higher than about 0.8 AU, do a quantitative dilution with NaOH and measure the absorbance again.
11. Prepare a graph of absorbance vs tube number and determine the volume of eluent required to elute all of the vanillin.

Applying the Method to the Analysis of Commercial Vanilla

Prepare a dilute solution of the vanilla extract you wish to analyze as you did in Step 1 of the method development. Repeat the SPE procedure for 3 separate 1.0 ml samples of the dilute vanilla extract. You may do all three at the same time on the manifold. When you reach the elution step, add the total amount of eluent you determined was necessary to elute all of the vanillin and collect this in a single tube.

13. Recover the test tubes containing the vanillin extract from the manifold and add 0.1 ml of 1.0 M NaOH for each ml of eluting solvent used. Dilute to the mark with 0.10 M NaOH. If the solution absorbance is higher than the calibration curve, prepare a quantitative dilution to insure accurate results.

Preparation Of Standard Solutions

Prepare a solution of 100 micrograms vanillin/ml in 0.1 M NaOH.
From this standard vanillin solution, prepare standard solutions of concentration 5, 4, 2, and 1 ug/ml. Your precision grade for the experiment will depend on the accuracy of these solutions, so employ your best analytical technique.

Measure the absorbances of your sample and the standard solutions using a spectrophotometer set at 347 nm. If the lowest concentration standard has an absorbance higher than your samples, then dilute one or more standards to obtain additional calibration points as needed.

Report

Group report should consist of two parts, a comparison of the two methods and the quantitative analysis of the vanilla. Each student should write the experimental section for the part of the experiment they did. Discuss the comparison of the two methods and write a single results and discussion section. No Introduction required. **One report per group.**

Comparison of SPE and Extraction

Compare Accuracy, precision, ease of use, time required for analysis, and cost of the two methods. Remember not to include the time spent in method development in the SFE experiment, only the time for the final triplicate analysis. Also take into account that you only did duplicate samples for the solvent extraction method.

Cost - SPE cartridges are \$75 per 100 (when calculating cost, do not include the cost of the method development, only the cost for a single analysis)

Methylene Chloride \$50/liter this cost includes both purchase and disposal costs. Disposal costs slightly more than purchase. Note also, if you keep the methylene chloride free from obvious water, solids, etc. the cost of disposal is reduced by 75% so a careful lab can reduce the cost to about \$30/liter.

NaOH - \$1.00/liter of 0.100 M

Methanol - \$10.00/liter

Ethanol - \$10.00/liter

Equipment costs are virtually identical and can be ignored in your analysis. Cost analysis should compare a single solvent extraction with a single SPE analysis or triplicate extractions with triplicate SPE. The only reason for doing only duplicate solvent extractions in this lab is the time involved.

Quantitative Results

Submit a graph of absorbance versus concentration for the standard solutions. Do a least squares analysis of the calibration curve and determine the R^2 to four significant figures and a plot of the residuals.

Use the equation of the best fit line to calculate the concentration of vanillin in the **original vanilla extract** in mg/ml and the total mass of vanillin in one 4 oz. bottle. Calculate the error in the analysis using the standard deviation of the calibration curve and the error between the two samples extracted.

Rotation Experiment 5 - Direct Potentiometric Determinations:

Using Ion Selective Electrodes and Calibration Curves

Group

Read: Text 15-6 through 15-9 and 19-3

Prelab Exercise

You have available a 1.0×10^{-2} M solution of fluoride and the following glassware:

volumetric pipets - 1.00, 5.00, 10.00, 25.0, and 50.0 ml

volumetric flasks - 25.00, 50.00, 100.00, 250.0 ml

Describe in detail how you would prepare solutions of the following concentrations: 1.0×10^{-7} , 3.0×10^{-7} , 1.0×10^{-6} , 1.0×10^{-5} , 1.0×10^{-4} , and 1.0×10^{-3} . Your precision grade will depend on the accuracy of the solutions you prepare.

An unknown solid contains 20.0% fluoride by weight. Describe how you would prepare a solution of 2×10^{-4} M from this solution with at least 0.5% relative accuracy. Assume you have a balance weighing to ± 0.00001 gm and the glassware above.

Blackboard Video on Ion Selective Electrodes and Quiz

You must supply a product for fluoride analysis for this lab.

Introduction

Ion selective electrodes (ISE) provide a simple and rapid means for determining the activity of a wide range of ions. In many cases the accuracy obtainable is comparable to that of simple spectrophotometry. Several types of ISE are available. Some use a solid state membrane while others use an organic liquid as the membrane across which the potential develops. Likewise, the concentration range of application and the degree of selectivity differs for each type of ISE.

In this experiment you will determine the concentration of F^- with a solid state (LaF_3) membrane electrode. The unusual characteristic of ISE's is that they respond to activity rather than concentration. Thus, any equilibrium reaction which reduces the free F^- will interfere with the determination. In addition, the electrode is selective, not specific. Interfering ions must be either eliminated or held constant. Finally, the ionic strength must be kept constant to maintain constant activity.

Fluoride is a particularly good example in that the F^- electrode also responds to OH^- . This requires measurements be made in low pH solution. However, in acidic medium, F^-

hydrolysis to HF reducing the F^- activity. The best compromise is to make readings at pH 5.5. Other interferences in the analysis of F^- are ions with which F^- forms strong complexes. Specifically Al^{3+} , Si^{4+} , and Fe^{3+} . To simplify the analysis of F^- by ISE, commercial buffer known as "Total Ionic Strength Adjustment Buffer" (TISAB) is often used. It buffers the pH at approximately 5.5, it adjusts the ionic strength to a high value to minimize variation between samples, and it complexes Al^{3+} , Si^{4+} , and Fe^{3+} to release the F^- . The commercial TISAB buffer has been shown to be less than optimal and in this lab we will use an alternative buffer proposed by Fouskaki et al.⁶ This buffer leads to faster response and makes it possible to work at concentrations as low as 10^{-7} M. Two forms of this buffer are provided. For all analytical use, the buffer containing CDTA is used to prevent interference from metal ions. A second buffer without the added complexing agent is available for the study of the stoichiometry of the Al – F complex.

This experiment consists of four parts; the evaluation of the response of the ISE by preparation of a calibration curve, the analysis of an unknown, the analysis of a "real" sample, and the determination of the ratio of aluminum and fluoride in the interfering complex using the method of continuous variation (see text reference 19-3).

Reagents - 1.0×10^{-2} M NaF solution, MES-CDTA Buffer, MES Buffer 1.0×10^{-2} M Al^{3+} (prepared from $Al_2SO_4)_3$)

Begin drying fluoride unknown at beginning of lab period!!

I. Evaluation of the Fluoride ISE

Prepare a series of standard solutions from 1.0×10^{-7} M to 1.0×10^{-3} M F^- . Use only volumetric pipets and volumetric flasks for high accuracy in this preparation. When preparing each standard, add 2 ml of MES-CDTA buffer per 100 ml of sample before you dilute the standard to volume. Graduated pipet accuracy is adequate for the addition of the buffer. You will be evaluated on the precision of these standards so do them carefully.

Place the F^- ISE and reference electrode into each solution (some F^- electrodes have built in reference electrode). Add a magnetic stir bar and place on a stirrer. Place the digital pH meter in the mV scale and allow the reading to stabilize. This may require up to a minute. Stirring the solution will hasten the process. Record the final value of mV for each solution.

Plot the graph of mV vs. $\log [F^-]$ and explain the shape of the plot. Compare the actual slope to the theoretical value expected. You may delete one or two values which deviates significantly from linearity at the low end of the curve in determining the slope but show all data in any graphs you prepare.

⁶ Morpholinoethanesulfonic acid-based buffer system for improved detection limit and stability of the fluoride ion selective electrode. M. Fouskaki, S. Sotiropoulou, M. Kořc'ı, N.A. Chaniotakis, *Analytica Chimica Acta* 478 (2003) 77–84.

II. Analysis of a Fluoride Unknown

You will receive a solid unknown which contains somewhere between 10-50% fluoride by weight. Dry the sample for 1 hour at 110 degrees and store in the desiccator until ready for use. **(Do not dry in the microwave)**. Prepare a sample of suitable concentration to fall near the middle of the calibration curve. The closer you get to the middle the lower the uncertainty and hence the better the precision of the measurement will be. Add MES-CDTA buffer at the rate of 2 ml/100 ml sample when you dilute the unknown. If further dilution is required, maintain the MES-CDTA concentration for each dilution.

Report the % fluoride by weight in your original unknown and the precision obtained from least squares analysis in ppt.

Note: When you calculate the std. dev. of the unknown concentration (S_c) using least squares, the value is in the same units as the value of the independent variable, namely $\log[F^-]$. You cannot simply write $\text{Log}[F^-] \pm S_c$ since subtraction of logs is division. Add and subtract the value of S_c from $\text{Log } \mu\text{g/ml}$ and take the inverse log of each. This will give you the value of $[F^-]$ plus one std. dev. and minus one std. dev.

III. Analysis of F^- in a Real Sample

You may select any sample for analysis for which there is a reasonable expectation of the presence of F^- . There are a wide variety of dental preparations containing F^- including tooth paste and some special mouth wash products. Hartford municipal water is fortified to 1.0 ppm fluoride and does not make an interesting sample. Some natural F^- is present in well and surface water. Tea also may contain some fluoride. If you wish to analyze fluoride in tea, then brew it with deionized water or correct for fluoride in water used for preparation as a blank. Select a product for analysis and have it approved by the instructor (you get points here for originality and for not doing the same product as everyone else in the class).

Measure an appropriate amount of sample and prepare a solution as appropriate for the calibration curve prepared above. Add 2 ml of MES-CTDA solution to 100 ml of sample. Calculate the $[F^-]$ from the calibration curve and report this and the concentration of F^- in the original product.

Typical samples: Toothpaste - Weigh 200 mg of paste and boil with about 50 ml of water for 2 minutes. Cool, transfer to a 100 ml volumetric flask and dilute to the mark.

Fluoride drops - Dilute 1.0 ml of sample to 100 ml. Take a 25 ml aliquot and dilute this to 100 ml.

IV. Determination of aluminum fluoride complex ion ratio using continuous variation method (Job's Method).

Prepare 6 solutions as follows using graduated pipets. Each solution is diluted to 100 ml in a volumetric flask.

1×10^{-4} M F^- 1 ml of fluoride stock (1×10^{-2} M) + 2 ml MES buffer

0.9×10^{-5} M F with 0.1×10^{-5} M Al 0.90 ml fluoride + 0.1 ml Al stock + 2 ml MES buffer

0.8×10^{-5} M F with 0.2×10^{-5} M Al 0.80 ml fluoride + 0.2 ml Al stock + 2 ml MES buffer

0.4×10^{-5} M F with 0.6×10^{-5} M Al 0.40 ml fluoride + 0.60 ml Al stock + 2 ml MES buffer

0.3×10^{-5} M F with 0.7×10^{-5} M Al 0.30 ml fluoride + 0.70 ml Al stock + 2 ml MES buffer

0.2×10^{-5} M F with 0.8×10^{-5} M Al 0.20 ml fluoride + 0.80 ml Al stock + 2 ml MES buffer

Measure the potential of each solution using the fluoride ISE. Use the calibration curve previously obtained to determine the free fluoride concentration in each solution. Plot the free fluoride as a function of mole fraction and determine the ratio of fluoride to aluminum in the complex. The data should produce two intersecting straight lines. Draw a straight line through the first two or three points and through the last two or three points and find the mole ratio at the intersection.

Report

Submit a full technical report on this experiment. Include a calibration curve for $\log[F^-]$ vs mV. Plot the data and least squares fit and the R^2 to 4 significant figures. Is the graph linear? Discuss any non-linearity and any problems you observed in doing the experiment. What is the relationship of the slope to the theoretical, predicted value? Plot the residuals (% error vs $\log[F^-]$). If you determine that there is significant deviation from linearity in your data, you may delete points in the nonlinear portion and obtain a new curve fit.

Determine the percent NaF in your unknown and the % or ppm fluoride in your product sample using the best method for your data. This may involve using the entire curve or a limited portion of the curve which brackets your samples. Determine the precision of each measurement using equation 5-14 on p 87 in the text. Remember that the standard deviation you get is for $\log(F^-)$ and you will need to add and subtract this from the unknown $\log(F^-)$ and take anti-logs to get the error in concentration. Discuss the degree of accuracy and precision obtainable with the ISE technique.

Report the results of your determination of the stoichiometry of the complex between aluminum and fluoride and compare this with results reported in the chemical literature. (You will have to look this up.) The method you are using, Job's method should also be looked up in the text or the literature so you will understand it better.

Rotation Experiment 6 -Gas Chromatographic Determination of Fatty Acids in Oils Using the Internal Standard Method of Quantitation

Group

References: Fatty Acid References: Metcalfe, *Anal. Chem.*, V. **33**, p. 363; Metcalfe, *Anal. Chem.*, V. **38**, p. 514. Text: Chapter 24 for general background. Blackboard handout chapter has more details.

Prelab Assignment

You must meet with your lab partner and decide what fat samples you wish to analyze. You should have time to analyze 2-3 samples. Your samples should be chosen with some specific question in mind.

Write a brief description of the question you hope to answer during this lab and turn it in as a prelab exercise. Bring your samples to lab.

View the Video on the GC on Blackboard and take the associated Quiz

Introduction

Gas Chromatography (GC) is a widely used technique for separation of complex mixtures of volatile compounds. In Gas Liquid Partition Chromatography (sometimes called GLC or simply lumped with other types GC) the chemical equilibrium between the sample in the vapor phase and in solution in a stationary liquid phase present in the column is exploited to produce a separation is affected only on the basis of volatility.

Many compounds of interest do not possess sufficient volatility to be passed through a GC column. Examples of these are sugars, amino acids, metals and large fatty acids. However, it is often possible to react these compounds with reagents to produce new compounds or derivatives which may be analyzed by GC. One of the most common derivatization procedures is the replacement of strongly hydrogen bonding hydrogens of acids or sugars with methyl groups. The decrease in hydrogen bonding of the methyl esters then allows sufficient volatility for analysis.

In this experiment you will carry out a quantitative analysis of an unknown synthetic mixture of fatty acids and of the fatty acids in a cooking oil or other fatty food product. You will be able to determine both what acids are present and how much of each is present. For the cooking oil this will allow you to determine the types of oil (soy, olive, cottonseed, etc.) which were used to make up the oil and the amount of unsaturated and polyunsaturated fats which are present. Due to the current interest in unsaturated fats in the diet you may wish to use some high fat component in your diet for this analysis. The analysis of fat in foods is designed to determine the type of fat and to quantitatively measure the relative amounts of each fat. However, it is not an analysis of the total fat quantity in a food product due to the time required to carry out a

complete extraction. (If you would like to do a total fat analysis, the professor can arrange for you to carry this out before the day of the lab.)

The quantitative procedure you will use is the most accurate method available for this type of analysis. It allows quantitation through the addition of an "internal standard" to your samples. The assumptions involved in this procedure are that the standard reacts and is detected in the GC in a manner identical to the sample. Your internal standard will be heptadecanoic acid (C₁₇ saturated fatty acid). This acid is chemically identical to the C₁₆, C₁₈, and C₂₀ fatty acids present in most oils but it is not found in any naturally occurring oils.

To apply the internal standard procedure one simply adds a weighed quantity of the standard to the weighed sample and carries it through the methylation and GC procedures. Recent experience with this method has indicated that the overall system response to various FAME's (Fatty Acid Methyl Esters--that is, fatty acids which have been derivatized to more volatile and less polar methyl ester species) varies due to non-linearities in the injection process. Therefore, you will run standard mixtures of saturated and unsaturated FAME's containing the internal standard and determine the response ratios at various concentrations.

The mg of each FAME is calculated for a sample and the percent composition for each FAME is obtained by dividing the mg FAME/total mg found. This calculation takes the total of all GC peaks as 100% and determines each FAME relative to that total.

The % Recovery can then be determined using the equation

$$\% \text{ Recovery} = \frac{\text{total mg FAME found}}{\text{Total mg fat used}}$$

For samples which are essentially 100% fat (the unknown, oils, butter, fats extracted from chips, etc.) the % recovery is a fairly good measure of the success of the experimental procedure. For these samples the result should be close to 100% recovery. Since each peak may contain either a positive or negative error, the actual % recovery can be both <100% or >100%. For samples which contain non-fat components, the % recovery will be approximately the % fat of the sample if the experiment is done well.

The column on which you will conduct the analysis is a fused silica tube coated with a thin layer of a polymer. The best separation is obtained when the polymer has a specific affinity for unsaturated fatty acids and allows them to be separated from the saturated acids. The most difficult pair to resolve is methyl stearate (Octadecanoic acid methyl ester abbreviated 18:0) and methyl oleate (Octadecenoic acid methyl ester abbreviated 18:1). You should be able to obtain at least a partial separation of these two esters. The column in one of the instruments can even separate cis and trans isomers of fats. All natural fats are present as the cis isomer, but the partial hydrogenation of fats produces trans acids. Note: There are several isomers of polyunsaturated fats and you may obtain more peaks for food oils than with the standards. If you have several peaks at the retention time around 18:2, you may assume they are isomers for the purposes of this lab. However, you could obtain additional standards or use GC-MS for further identification.

The GC can be operated in two ways, at one temperature (isothermal) or using a temperature program. When separating a few components the former is preferred but for more complex separations the latter is needed to increase the speed of the process and improve the separation. Temperature programming provides better separation of volatile compounds and more rapid elution of less volatile compounds. It also increases the overall efficiency of the separation for capillary columns by decreasing sample overloading. This topic will be discussed in more detail in the lecture. (see notes on General Chromatography)

Operation of the GC

The instrument requires a warmup time of about 30 min. During this time you should prepare your samples. As soon as the instrument is warm, one person should begin using the GC while the other finishes sample prep.

1. Turn on gas tanks - Main valve and shutoff valve. Do not adjust the regulator pressure. He should be 52 psi, H₂ should be 20 psi, Air should be 30 psi.
2. Open the column oven and record the specifications of the column you will be using. Turn on the GC. Switch on right side at the back.
3. Set temperatures - All injectors and detectors 250 deg. (Press Inj A, 250 Enter, etc.) Set oven temperature to 180 deg.
4. Measure flow rate through each column. Insert the probe of the flow meter into the column. Record the column flow rate. Also measure and record the flow rate through the split vent. The ratio of the column flow to the split vent flow determines the fraction of your sample which actually goes to the column. The value should be between 0.03 and 0.10. If not, ask the instructor to adjust it.
5. Turn on H₂ and Air valves for each detector (top left side). Wait a few minutes then press ignite buttons. You should hear a small pop. Confirm the flames are lit using a watchglass over the vent. Look for condensation on the cool glass. If not present, relight.
6. Set the Range to 0 and the ATTN to 2. Check the DET control to insure the detectors are both ON.
7. The data system is connected to SIG 1. You select the column and detector you will use by pressing SIG 1 and then either A or B and ENTER.
8. Before each run, press the ZERO button and then ENTER to effect the ZERO operation.
9. Check that PURGE A and B are both ON. The Timetable Events list should be programmed to turn off the Purge B valve at time 0.0 and to turn it on at time 1.0. This is necessary for samples to be properly injected.

Methylation of Fatty Acids

Note, each student should methylate their own unknown and food sample.

Two samples should be carried through this procedure, a synthetic unknown mixture obtained from the instructor and a sample of food, oil or margarine which you must provide. As your oil sample you may wish to try the fats in a junk food such as potato chips or corn chips. For these samples it will be necessary to extract some of the fat with a small quantity of CH_2Cl_2 from a sample of the product. The product should indicate the grams of fat in a weighed serving, and this will allow you to select an appropriate sample size. Extract the sample, filter to remove solids, and transfer to a beaker. Remove the solvent by heating gently on a hotplate.

Weigh accurately using the 5 place balance about 15-20 mg of fat into a 50 ml Erlenmeyer flask. Weigh accurately about 3-6 mg of C_{17} internal standard into the same flask.

Prepare 0.5 M NaOH by placing 3 NaOH pellets in 50 ml. of methanol in a dry beaker or flask. Swirl until the pellets are mostly dissolved. They will not dissolve completely. Use the solution as is. DO NOT store the solution as it is not stable for more than a few hours.

Add 10 ml of 0.5 N methanolic NaOH solution to samples and heat on hot plate until all fat has gone into solution (about 5 minutes). If methanol evaporates you will have a white mass. This is fine, just add more methanol. You may also observe the formation of a gelatinous residue during this process which is the saponified fat. If residual fat particles remain unreacted add another portion of NaOH solution and heat again. You should not need to heat more than 10 minutes maximum.

Add 5 ml of BF_3 -methanol solution (10-15% BF_3) and boil for 2 minutes. You may well have a white mess at this point. Ignore this fact. However, all of the original sample should be gone at this point. If not, add more NaOH solution and BF_3 .

Cool the flask and add about 25 ml of CH_2Cl_2 to the flask and swirl thoroughly to dissolve and extract any methyl esters which may have precipitated. Transfer to separatory funnel and add 75 ml water. Withdraw the CH_2Cl_2 layer and store in a small flask or beaker. Gravity Filter the solution, if cloudy, through Whatman 1PS paper.

Place 1 ml of each extract in an autosampler vial, cap and label it. Prepare a dilute extract by adding 1 drop of your extract to an AS vial and add 1 ml of CH_2Cl_2 . Cap and label this vial.

CAUTIONS: ALCOHOLIC NaOH IS HIGHLY CAUSTIC AND CAN CAUSE SEVERE BURNS. IF YOU SPILL THIS SOLUTION ON YOUR SKIN, FLUSH IMMEDIATELY WITH WATER AND HAVE A PARTNER INFORM THE INSTRUCTOR.

THE ENTIRE METHYLATION PROCEDURE MUST BE CARRIED OUT IN THE HOODS. BF_3 IS TOXIC AND THE VAPORS SHOULD NOT BE INHALED.

Analysis of Fatty Acid Samples and Unknowns

Inject each sample and the three quantitative standards and obtain chromatograms. Using the Analysis program, observe the integration. Change parameters as needed until you include all of the area of each peak in the integration. Print a report for each run with a chromatogram and peak table. (Peak tables can be output as Excel Spreadsheets) Concentrated and dilute samples are prepared to allow choice of the sample with the best peak shapes and retention times for analysis.

For each analyte, plot the area ratio (Area sample/Area C_{17}IS) vs quantity ratio (mg sample/mg C_{17}IS). Do a least squares fit and assess the linearity.

For each fat sample, calculate the area ratio for each analyte. Using the least squares equation, calculate the quantity ratio. Then use the known amount of C_{17}IS to calculate the quantity of the analyte. (Think Spreadsheet)

Report

Brief report only - This report can consist entirely of well constructed and annotated spreadsheet and the answers to the questions posed below. Each student must construct their own spreadsheet and write their own answers.

Tabulate the retention times obtained for the FAME's for runs done under various conditions. What is the effect of varying the temperature and of temperature programming? Evaluate the advantages and disadvantages of temperature programming from your experience.

Identify the fatty acids in your unknown and in your oil sample based on retention times. Use retention times from the most dilute sample.

Spreadsheet

For each fat in your unknown and food sample, prepare an internal standard calibration curve. (Recommend that you put each on a separate page of your spreadsheet and then summarize the slope and intercept in a table on a summary page)

On the summary page, identify each fat in the unknown and food sample by their retention time and calculate the mg of each fat and the % of that fat in the total sample.

Determine the % recovery of FAMES in your sample and unknown.

Use the tables of composition of various oils which follows to estimate the source of the oils in your food product (not for unknown). Indicate this with food sample in your summary. Print the summary page and attach it with your answers to the questions and hand in. Email your spreadsheet to the professor.

Composition of Common Fats and Oils

	10:0	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	
Olive Oil												
CA			----		6.9		2.3	84.4	4.6		0.1	
Italy			1.2		9.2		2.0	83.1	3.9		0.2	
Spain			----		9.4		1.4	80.5	6.9		0.2	
Range			0.1- 1.2		7- 16		1- 3	65- 85	4- 15		0.1- 0.3	
Soy			0- 0.5		7- 11	<1	2- 6	15- 33	43- 56	5- 11	3	0.3-
Peanut			<1		6- 9	<1.7	3- 6	53- 71	13- 27			2- 4
Corn			<1.7		8- 12	0.2- 1.6	2- 5	19- 49	34- 62	<3		<0.2
Sunflower					3- 6		1- 3	14- 43	44- 75			0.6- 4
Cottonseed			0.5- 1.5		20- 23		1- 3	23- 35	42- 54			0.2- 1.
Coconut	6- 10	44- 52		13- 19		8- 11	<1	1- 3	5- 8	<2.5		<0.4
Palm Kernel	3- 7	40- 52	14- 17			7- 9	<1	1- 3	13- 19		0.5- 2	<1
Safflower			tr.		3- 6		1- 4	13- 21	73- 79	tr.		<0.2
Butterfat	1.7- 3.2	2.2- 4.5	5.4- 14.6	.6- 1.6	26- 41	2.8- 5.7	6- 11	18.7- 33		0.9- 3.7		1- 3
Linseed					4- 7	2- 5	12- 34	17- 24		35- 60		<1
Lard			1- 4		20- 28		4- 14	41- 51	2- 15			

Appendix 1 - ChromPerfect Data System Setup.

The computer data acquisition program consists of several separate programs. One can move between them in the **Windows** environment by holding down the ALT key and using the TAB key to toggle between programs. You will use Acquisition, Calibration, Method, and Analysis programs from the Chrom Perfect menu.

1. Go to the **Method** function and retrieve the method file having the name of the column type installed in the GC or Chocolate for the HPLC. This will contain the basic information for the analysis. You will need to specify the name of your calibration file. You can make up a name for this file at this point and you will create the file later. It is in this file that you select the range for the chromatographic display for the real time chromatogram. Finally, you can enter the two integration parameters in this file once you have determined them. These are peak width and Threshold. Set the Peak width to 0.04 and the Threshold to 2. In Misc, make sure the quantitation is set for Internal Standard.

2. Go to the **Acquisition** function and click on the Method block for your instrument. Select the Method file you have just created from the Directory list. Identify your sample, the names of the users, and select a file name for your runs. You should use only a single file name for the day but should change the information about the sample before each run. You must also input the amount of internal standard in each sample you are going to run before you start the run. This and the sample identification should be entered for each run.

You are now ready to inject samples. **You must press the START button on the GC before you inject each sample. On the HPLC press the MARK button on the Detector before you turn the injection valve to start the data system.** The run will stop at the end of the specified program time as specified in the Method file. Run a sample which contains all of the components to be analyzed. Observe the chromatogram and determine whether you are satisfied with the separation and time. If you need more resolution of early peaks, you can reduce the temperature in GC or reduce the strong solvent in HPLC. Your goal is to obtain a peak for each component in the sample which is resolved from all others.

To display the chromatogram after a run ends, enter the **Analysis** program and select the name of your data file from the File Open Directory list. The chromatogram will appear and can be printed along with a report as needed. You may expand any part of the chromatogram using the left mouse button to select the area to be expanded and the right mouse button to execute the expansion. ALT DELETE will remove the expansion. You may also open several chromatograms at the same time for comparison by highlighting multiple files. These multiple runs can be displayed Stacked in separate windows or overlaid in a single window.

Examine the expanded chromatogram to insure that the peak integration (as shown by the baseline) includes the area of each peak. Adjust the Threshold and Peak width parameters in Integration Auto to obtain a satisfactory integration. Then change the parameters in the Method file so they will be applied to all chromatograms. If the baseline in an HPLC chromatogram is not correct, it may be necessary to force the baseline to zero just before the first peak. This is also done using Integration Auto and insert a set baseline now event.

When you have a satisfactory chromatogram for your standard reference sample, use File Save Area File to save the peak areas.