"When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meager and unsatisfactory kind: it may be the beginning of knowledge, but you have scarcely, in your thoughts advanced to the stage of science whatever the matter may be."

Lord Kelvin (Popular Lectures I, 73).

This is probably the most famous quote from Lord Kelvin one of the most productive physicists of the 19th century and one of the founders of thermodynamics. It should be the credo of all analytical chemists. The quantitative treatment of separation science is what this course is all about. In addition to trying to impart specific and pragmatic information about modern separation science one of my metagoals is to induce you to think quantitatively. Analytical chemistry is most fundamentally a quantitative science. There is no such thing as a good analytical chemist who does not think quantitatively.

**Office:** 327 Smith Hall

**Phone:** 624-0253 (I return all detailed messages! I prefer that you not just leave a number--I want you to tell me what it is about.)

**E-mail:** petecarr@umn.edu (please, please, please use email! Thanks!)

**Office Hours:** I will generally be available at 10AM before class and after 1PM after class as well as at other times. Please use email to set up if not right before or after class.

**Teaching Assistant:** None

You are responsible for reading this and all handouts given throughout the semester. See the last page of the syllabus, sign it and put in my mailbox (A5) NOW!!!! PLEASE

**Cell Phones and Related Technology:** I never thought I would have to put this in the syllabus of a graduate course but the use of cell phones, texting devices or any electronic communication devices during class is not allowed. This is grad school not junior high.

**Plagiarism:** There will be several writing assignments during the semester. It is your responsibility to understand what plagiarism is and the universities policy concerning plagiarism. Be assured that I will report any incidents. It is my obligation as a faculty member to do so. Any work based on plagiarism will receive a grade of zero and will result in a letter in your graduate file. Plagiarism is fundamentally dishonest. It is stealing the work of others. Dishonesty is utterly incompatible with scientific ethics.

**INTRODUCTION**
Title: Advanced Analytical Chemistry-I (4 credits)

**Brief Description:** This course has one main focus: the fundamental mass transport and phase transfer thermodynamic aspects of separation science especially chromatography. It will include a description of gas and high performance liquid chromatography. We will not explore the application of these techniques to specific analytical problems; however, the **basic principles** and **practical approaches** to how analytical methods are developed will be discussed. We will touch briefly on issues related to detection and quantitation, but I will **emphasize what takes place inside the chromatographic column or separation device.** Modern electrophoretic techniques will also be explored.

**Prerequisites:** A good basic **working** knowledge of undergraduate physical chemistry, especially thermodynamics, is **absolutely required.** Even if you are relatively **confident of your understanding** it would be a good idea to review from the get go what **free energy and chemical potential** are all about, the basics of phase equilibrium, definition of standard states (I have found that very few understand this concept and fewer appreciate its importance), activity and activity coefficients, Henry’s and Raoult’s laws, diffusion of gases and liquids, ionic conductivity and mobility and electrochemical equilibrium. In addition, familiarity with the language of ordinary and partial (multi-variable) differential equations is assumed. **Although you will not be required to solve any such equations,** you will need this background to follow their application to chromatographic problems. I suggest you review this topic immediately. A good book is E. Kreyszig, *Advanced Engineering Mathematics.* Other good places are the classic books on the mathematics of diffusion such as Crank and Nicholson or Carlslaw and Jaegger. Several of these books are on the web. **Last, I assume that you have taken and mastered the content of undergraduate courses in both quantitative analysis and instrumental analysis.**

**Course Books and Notes:** There is no good book on chromatography available at the graduate level. Reference 2 below is what I used many years ago, but it is now out of date and out of print; however, you can get used copies through Amazon.com. References 1 and 3 are excellent; they only deal with very broad principles and the dynamics of chromatography. Sadly they don’t deal with the molecular basis of chromatography. Reference 4 is just superb but it does not treat detectors, quantitation or intermolecular interactions. Reference 5 is the classic book on solving diffusion and mass transfer problems. All electrochemists and chromatographers need to have some idea of what is available in this book. **You must take a look at pages 1-10.** If you can’t get it at the library then ask me for my copy.

Because no one book really feels right to me I have written course notes that correspond closely to my lectures. They are about 650 pages long. The department won’t let me give them to you on paper but I will provide them to you via a class Google Drive. **You MUST read these notes BEFORE class. I have indicated in the lecture guide the date when each section should be FINISHED.** Note the folks in the computer aided instruction room don’t want you to print them on their equipment but it is a very good idea to have a printed copy. Also I have compiled a set of “**Classic Chromatography Papers**” (see the class Google Drive). A number of these papers, which I call the “**Essential Papers**”, are assigned readings (see list of lectures) and you will be held responsible for reading these papers. **A section of each mid-semester exam and some of the periodic quizzes will focus on these “Essentials”.**
Reference 6 is one of my favorite books on Chemical Thermodynamics. I like it because it treats thermodynamics in a **practical, down to earth fashion** solving real chemical problems and not as a branch of metaphysics and mathematics. My comments on book 7 speak for themselves.

**Important Books on Separation Science:**

1. J. C. Giddings, *Dynamics of Chromatography*, Dekker, New York, 1965. **If you read only one book from cover to cover about chromatography in your whole life, it should be this one. No one can be considered an expert on chromatography until they have mastered this book.**


7. H. C. Berg, *Random Walks in Biology*, Princeton University Press, Princeton New Jersey, expanded edition 1983. **Absolutely wonderful book.** I am ashamed to admit that I only discovered this book a few years ago. It is a short paperback. It sets forth a great deal of the basic principles of what diffusion is all about but in the context of biology. I was amazed at how important diffusion is in biology. Chromatography does not work at all without diffusion but nor would biological cells. If you are going to any graduate work in separation science or a biological science **buy this book.** It is a bargain. The first two chapters are included in the Addendum to Chromatography (#10) Classics. **READ THIS NOW!** It will take a while for it to percolate through your brain.

**COURSE GOAL:** My chief goal in the course is to provide you with the background necessary to **readily interpret** a current fundamental paper in separation science.

**One-Minute Papers:** After class every day, you will write a brief statement as to what you feel is the **TAKE HOME MESSAGE** of the day’s presentation. It should not be a grocery list of the topics touched upon. Also the **email must start with a question about the lecture.** In order to ask a question which demonstrates some understanding or insight you must have thoroughly reviewed the material and read the relevant lecture notes. Therefore **I will not accept emails**
from mobile devices. The question may well be something that you could not clarify by simply reading the notes in greater depth. It could be something such as “Well you said thus and so does this mean that XYZ is true?” You should write the email the same evening after a lecture having thoroughly reviewed your daily notes and the relevant course notes but it must be received before the next class. It is quite easy to tell when an email was written without having read my notes. I can’t cover all of my notes in lecture but my notes anticipate many questions that I receive by email.

These will all be read but not graded; 5% of the total course credits will be assigned to those who take more than 75% of the available opportunities to write these one-minute papers. I urge you to do this for many reasons. They need not be long. They should focus on the key ideas. They must not simply be a list of topics like “Today we talked about extra column broadening. It comes from the injector, detector and the connection tubing.” Rather “Excessive extra column effects can completely ruin a separation. The relative importance of extra column broadening in GC and LC was described. It can be made negligible by using very small volumes of sample, small and fast detectors and narrow connection tubes.”

Grades and Goals:

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<td>Mid Semester Exam - 2</td>
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<td>Final Exam (including take-home part if any)</td>
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<td>Quizzes (see some examples below)</td>
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Note the two mid-semester exams will be given in the evening so that you can have 2 hours to work on them (this is a 4 credit course). There will be one or two “projects” assigned this semester that will constitute 15% of your total grade. One will be based on the use of an HPLC simulator which has gone through several generations. It rather accurately simulates a real HPLC under reversed phase chromatography (RPC) conditions. This will take place around the time of lecture 7 or 8. It will entail your doing a number of “experiments” with the simulator concerning the basics of LC, writing a brief report and an oral presentation of that report to me. The second project will involve a critical comparison of two or three closely related papers and or topics. That will take place about 2/3rd of the way through the lectures. Again there will be a brief written critical report on the papers which will be graded. There may be a third project. It will be announced in class well ahead of time. I will make assignments and give answers to various problem sets but these will not be graded. You are strongly advised to work on these problem sets as many of the questions will be used on exams. Many of the questions have been used on quizzes and exams

Grades in this class are entirely non-competitive. I would be delighted to assign all A’s. In general, the grades will be as follows:
A  >  85  
B  >  75  
C  >  65

According to the newly adopted policy of the University Senate, all course syllabi are to present the University’s standards for uniform grading.

A achievement that is outstanding relative to the level necessary to meet course requirements. Extra-ordinary performance on all requirements—meets and exceeds all.

B achievement that is significantly above the level necessary to meet course requirements. Meets all requirements, exceeds some.

C achievement that meets the course requirements in every respect.

D achievement that is worthy of credit even though it fails to meet fully the course requirements.

S achievement that is satisfactory, which is equivalent to a C- or better.

F (or N) Represents failure (or no credit) and signifies that the work was either (1) completed but at a level of achievement that is not worthy of credit or (2) was not completed and there was no agreement between the instructor and the student that the student would be awarded an I.

Exams: There will be a mid-semester exam scheduled for sometime after week 4 of the semester (see exam schedule below). It will be an evening exam of approximately 2 hours duration. A second mid-quarter will be scheduled after week 8 (see exam schedule below). The date of the final is given based on the lecture schedule. In general my exams are not simple “plug and chugs”. When you understand a principle you can use it to solve problems. Simply being familiar with a concept is not the same as understanding it; you must be able to apply it. I will provide “cheat sheets” for the exams. You can enhance them. You may use programmable calculator on exams, but not a computer. The exams must be written in dark blue or black ink! (I cannot read—nor will I grade exams written in pencil or light ink.) The in-class part of the final exam will be three hours long. Please budget your time appropriately. The take-home exam (if any) would be announced well ahead of time in class. More details will be made available shortly.

Quizzes, etc.: There will be many (7-10) short quizzes. I generally find a fairly good correlation between the total points scored on the quizzes and the final total points even though quizzes are worth only a tiny fraction (5%) of the total final grade. I suspect this is because students who study every day and keep up with the course material do well on quizzes and the same factor promotes learning so that they do well on the exams and final exam in contrast to crash studying for mid-semester and final exams which results in little learning.
**Problem Sets:** There are numerous exercises at the end of sections of my notes. **In fact you gain a lot by reading these questions before reading my notes,** You should keep up with these as the course progresses. I strongly encourage you to form groups to work these problems. However, I will periodically handout a problem set. These will be handed in and returned. Questions based on and related to the ESSENTIAL PAPERS will also appear on the mid-semester and final exams.

**Difficulties:** I need to know by the end of week #1 any problems you might have with completing the exams or assignments. I will be far more receptive and flexible if I can accommodate you ahead of time than if you assume that I will be able to accommodate you after the fact. Contrary to the old US Army saying, it is **NOT easier to beg forgiveness than ask permission.**
The Use and Need for Derivations in Chemistry

In this course we will do a fair number of detailed derivations. I think perhaps some people don’t really understand why they are done.

**DERIVATION**—Definition: (mathematics) The operation of deducing one function from another according to some fixed law.

The above definition is as good as any place to start. Usually in the physical sciences, the idea of a derivation is similar to the above in spirit. However, our route is to relate one or more simpler concepts to one another to rigorously understand a more complex situation. Thus we may combine several physical laws with some degree of mathematical gymnastics and arrive at an equation which describes our system.

Why do we do derivations?

There are many reasons why derivations are used in teaching. The following spring to mind:

1. A derivation provides a framework in which all of the assumptions, limitations of knowledge, physical descriptions and mathematical approximations involved in the description of a system can be presented in an orderly fashion. This is the primary reason I believe that derivations are important. There is a sign in my office that says “A number without a standard deviation is nothing”. Exactly the same is true of an equation that is simply memorized and whose underlying assumptions are unknown.
2. We need the answer to help us understand what is happening or what we expect to happen.
3. Sometimes a derivation is the most succinct way to present a concept.
4. A derivation of a simple system is a warm-up exercise for the real thing; i.e., it provides the basis for tackling more complex phenomena.
5. Understanding a derivation in detail is an excellent way to hone one’s critical thinking skills. Indeed one of my metagoals in this course is to take you through some critical thinking exercises. Doing derivations is one form of a critical thinking exercise. Here is another critical thinking exercise. Compare and contrast reversed phases liquid chromatography and capillary zone electrophoresis from both a qualitative and quantitative perspective in terms of theory and practice.

By and large, when a result is derived rather than simply stated, it means that the concepts involved are as important as the final result, and as is often the case, the derivation is more important than the result.

As an example, consider the equation for the gas-liquid partition coefficient, that is, the equilibrium constant for partitioning of an infinitely dilute solute (2) between the gas phase and a liquid stationary phase:
If all you want is to get the information across, then all that needs be done is to write the equation. However, we will derive it in class.

To derive this equation, one makes use of two physical laws: the ideal gas equation and Henry's law of gas solubility in a liquid. In addition, one makes an approximation, consistent with the fact that Henry's law will only be accurate in dilute solution, for the sake of algebraic simplicity.

**IDEAL GAS EQUATION.**

\[ P^*V = n^*R^*T \]

Mathematically, we reorganize this so that we have the gas phase concentration

\[ C_g = \frac{n}{V} = \frac{P}{(R^*T)} \]

Surely all chemists recognize that the ideal gas equation is approximate, but is approached exactly at low pressure and high temperature. Deviations at ambient temperatures and pressures are only a few percent.

**HENRY'S LAW.**

At this point we begin to see the real problem with the result. Henry's law, otherwise known as the law of infinitely dilute non-electrolytes, states that the partial pressure of a vapor above a solution of that substance will be proportional to the mole fraction of the solute in that solution. That is all the good Dr. Henry tells us, i.e., it is purely empirical.

\[ P_2 = K_H^*X_2 \]

By thermodynamics, we relate the Henry's law constant to the saturated vapor pressure of the pure substance, and by **choice of the pure substance as the standard state**, to the activity coefficient.

\[ K_H = \gamma^*P^o \]

Since the partition coefficient (K no subscript) is in concentration units, we convert the mole fraction of the solute to its concentration:

\[ X_2 = \frac{n_2}{n_2 + n_s} \]

because the solution is very dilute, for Henry's law to hold it does no physical harm to our description and algebraically simplifies the result to neglect the moles of solute (n_2 relative to the moles of solvent n_s in the denominator).
\[ C_{2,1} = \frac{n_2}{V_S} = X_2 \cdot \frac{n_S}{V_S} \]

Recognizing the definition of molar volume \(V = V_S/n_S\), we arrive at the desired result. It is now clear that the above result is approximate. It clearly can only be applied when deviations from Henry's law are small.

**Sample Quizzes**

**QUIZ #1:**

1. Plot to scale a chromatogram which contains equal amounts of all solutes whose \(k'\) values are 1, 2 and 3. Assume zonal chromatography with equal plates for all species.

2. Plot to scale a frontal chromatogram of a mixture of three species whose \(k'\) values are 1, 2 and 3.

**QUIZ #2:**

1. Can \(N\) be determined from knowledge of peak area, height and retention time? How?

2. Suppose you have a tailed peak. Will \(N\) that is determined from the peak width at half-height \((w_{1/2})\) be less than, greater than or equal to \(N\) determined from peak area and peak height?

3. Assume that the baseline is totally flat and noise free. Is it better to quantitate based on peak area or height? Explain.

**QUIZ #3:**

1.a. Describe three things that happen to the height, location and valley points as two peaks merge. That is, as \(R\) gets smaller describe what happens to the observed height of the peaks, their location and the valley between the two peaks.

b. For equal size peaks at what value of \(R\) do separate peak maxima disappear.

c. For equal size peaks at what value of \(R\) does one achieve baseline separation?

**Books on Chemical Thermodynamics and Related Subjects**

Chromatography and separation science relies heavily of basic thermodynamics an area of chemistry which many students are woefully under prepared. There may be more text books and monographs on this subject than any other area in chemistry. The wisest remark I ever heard about the subject was from my teaching assistant who ran physical chemistry lab when I took in many years ago. This wise fellow said: “The first time I took thermo as part of the first course in Physical Chemistry as an undergrad I really did not understand it at all. The second time I took it in grad school it was a course in straight Thermodynamics and I thought I was catching on. Now that I am really working in the field I know I
don’t get it at all.” This is simultaneously a wonderfully simple and profoundly subtle field of amazing utility.

**Fundamental Thermodynamics Books:**


   This is *the* classic book on thermodynamics for chemists. While it is rigorous it is not highly mathematical and much of the discussion focuses on applications to chemical systems.


   Available in paperback, excellent, somewhat mathematical but still chemically oriented.


   A tougher read but applies thermodynamics to experimental data so it is a very useful book to the chemists.


   A marvelous, readable introduction to thermodynamics and the meaning of the second law. It is intended for a junior/senior year chemistry major course.


   This is a superb book both philosophical and practical. **It is written by an experimentalist for experimentalists.** The book is very rigorous and thorough. The notation is difficult. Not a good book to start with but a superb book to get into the nitty-gritty and understand the subject.


   A very elementary short exposition for advanced first year undergraduates.

**Applied Thermodynamics:**


   This is a superb book on all aspects of solutions for the practical chemist and chemical engineer. The authors use thermodynamics and elementary statistical mechanics to develop a number of models of fluid systems especially mixtures.


This is a very, very useful book on practical applications of thermodynamics. It is a superb reference that is encyclopedic in scope and detail. It is a real how to type book. Treats all aspects of phase equilibrium thermodynamics.


A very fine book. Very much from an engineering perspective. Strong emphasis on fluid phase equilibrium.

**Liquids and Mixtures:**


A very readable introduction. **Good place to start.**


An elementary level discussion. Quite readable.


A superb summary of state of knowledge at time of printing. Encyclopedic. A somewhat harder read.


Useful treatment of subject, readable, covers a number of modern but simple solution models in some detail.


A very general but introductory presentation of modern theories of liquids and some solutions.

A general very readable book on issues related to solubility. Prof. Grant was a very well regarded professor of pharmaceutics here at the U of M.

Here are some concepts that will be relied upon and referred to repeatedly. You need to achieve oneness with them:

**General Key Chromatographic Concepts**

1. Both the retention volume and retention factor are thermodynamic quantities when measured from the first moment (center of mass). That is, they do not vary with flow rate.

2. Peak broadening is due primarily to differences in lateral (radial) solute velocities.

3. Fast diffusion in the lateral (radial) direction decreases peak width; diffusion in the axial (longitudinal) direction increases peak width.

4. \(<x>^2 = 2Dt\). The mean square distance a molecule can move by diffusion is equal to 2 times its diffusion coefficient times the time allowed for diffusion. The average distance a molecule moves by diffusion is zero.

5. Diffusion coefficients in gases are about three orders of magnitude faster than diffusion coefficients in liquids.

6. The viscosity of gases is about three orders of magnitude smaller than liquids.

7. In GC k’ decreases as column temperature is raised. GC column is held at temperatures below the normal boiling point of the solute.

8. In LC k’ decreases as the fraction of strong solvent in the mobile phase increases.

9. Peak width is proportional to the square root of the column length.

10. Peak separation is proportional to the first power of the column length.

11. To a crude first approximation it is reasonable to assume that all species have the same plate count (N) and HETP.

12. Diffusion coefficients increase as temperature is increased and viscosity decreases as temperature is increased.

13. HETP increases in proportion to the particle diameter or (particle diameter)^2 depending on the specific broadening mechanism.

14. Under analytically useful conditions the HETP increases as linear velocity is increased.
15. The maximum peak capacity increases with plate count and with $k'_{\text{last}}$.

16. The peak capacity of a two dimensional separation is the product of the peak capacity of the two methods.
Common Misconceptions About Separations

I have seen the following errors made time and time again:

1. Diffusion and viscosity have no control over retention in chromatography. The **retention characteristics are purely thermodynamic** in character and are not related to dynamical characteristics.

2. Interaction of analytes with the wall in CE is extremely deleterious. These induce the need for radial diffusion which is so slow that the peak width is destroyed.

3. Alcohols are not acids in water.

4. The “A” term cannot be negative. This would sharpen not broaden a peak.

5. Cationic solutes “tail” in RPLC due to interaction with ionized SiOH groups of the silica support.

**If you really understand separation science you will be able to answer these questions.**

Think about these questions as the course progresses:

1. A sample containing two solutes having retention volumes of 1.0 and 2.0 ml is injected into a column operated at a flow rate of 1 ml/min. Ignore the effect of broadening. Show the elution pattern **at the entrance** after elution is conducted for 0.5 minutes in the forward direction and then the **direction of flow is reversed**. Explain your answer in detail.

2. A single sample is injected into a column and the peak width is recorded. The same sample is injected again eluted exactly half way through the column and the flow reversed. The peak width at the entrance is measured. Ignore extra column broadening effects. Which peak width will be larger? Explain your answer in detail. There is no one correct answer to this problem. Your assumptions are critical.

3. A mixture is injected into a column operated so that no heat can enter or leave the column (i.e. the column is adiabatic). Furthermore assume that the flow rate and pressure drop is so low that essentially no work is done. Even under these conditions the chromatographic column will be able to separate the sample. Is the second law of thermodynamics violated? Explain in detail. There is an interesting short paper by G. H. Stewart (Sepn. Sci. and Tech., 13, 201 (1978)) that does a nice job explaining this issue and compares chromatography to a classical separation method like distillation.

The Language of Chromatography

Chromatography has generated its own language. As is the case, with the English language, the vocabulary is huge and the number of concepts involved is great because it has drawn from many other sciences (analytical, physical, organic and biochemistry) as well as technologies like chemical engineering. An extensive glossary of terms commonly thrown about
by chromatographers will be provided. Despite the fact that I hate it the word “chromatograph” is now acceptably used as both a noun and a verb (according to IUPAC). Thus, it is now officially OK to say “I chromatographed the mixture.” However, the word chromatography is a noun NOT an adjective thus the device packed with a stationary phase through which a liquid is flowing is a “chromatographic column” not a “chromatography column.”
I have read the entire syllabus.

Name

Please turn in to my mail box (A5).