CHEM 4461/CHEM 5461
INSTRUMENTAL ANALYSIS (Fall 2012)
LECTURE & LABORATORY COURSE SYLLABUS

Instructor: Dr. Kevin A. Schug
CPB 358
817-272-3541
kschug@uta.edu (contact relating to miscellaneous items by email preferred)

Office hours: T,Th 11 am – 12 pm noon or by appointment

Text (required): Christian, *Analytical Chemistry*, 6th Ed.

Additional recommended texts to refer to are Skoog, *Instrumental Analysis* and Harris, *Quantitative Analysis*

Class Schedule:			
	Lecture:	SH 129	Section 001: M, W 11 – 11:50 am
	Lab:	CPB 215	Section 101: M, W 1 – 4:50 pm
		CPB 215	Section 102: T, Th 1 – 4:50 pm
		CPB 215	Section 103: T, Th 8 – 11:50 am

Grading:		
	Exams/Homework	400 (4 x 100 pts.)
	Final	200
	Laboratory	400 (4 x 100 pts.)

Description and Goals of the Course: This course explores the fundamental basis of chemical analysis. It is designed to give the student a solid conceptual ground to understand how a given analytical technique works; including its limits and advantages. The emphasis is on solutions analysis and the course is roughly divided into: (i) Basic measurements and concepts; (ii) spectroscopy; (iii) chromatography; and (iv) mass spectrometry.

Tests, Finals, and Grading: At least a 10-point grading scale will be assumed (e.g. 90-100 A; 80-89 B; etc.), however the instructor reserves the right to adjust this scale to accommodate the spread of grades in the course. All exams will be given on the date specified by the instructor (approximate dates are given at the end of the syllabus). No make-up exams will be given. The comprehensive final exam will be given on the date assigned by the University. All exams (except for

the comprehensive final) will be worth 90 points. The remaining 10 points of exam score will be calculated based on homework (see below).

Homework: We will be using Sapling Learning, an on-line homework program, for homework this semester. Use of the system will cost you $29.99 for the semester. Below are directions on how to access Sapling Learning and set up your account.

1. Go to http://www.saplinglearning.com
2. If you already have a Sapling Learning account, log in, then skip to step 5.
3. If you have a Facebook account, you can use it to quickly create a Sapling Learning account. Click "create account" located under the username box, then click "Login with Facebook". The form will auto-fill with information from your Facebook account (you may need to log into Facebook in the popup window first). Choose a password and fill in the remaining information, accept the site policy agreement, and click "Create my new account". You can then skip to step 5.
4. Otherwise, click "create account" located under the username box. Supply the requested information and click "Create my new account". Check your email (and spam inbox) for a message from Sapling Learning and click on the link provided in that email to confirm your account.
5. Find your course in the expandable list (sorted by subject, term, and instructor) and click the title link.
6. Select your payment options and follow the remaining instructions.

Once you have registered and enrolled, you can log in at any time to complete or review your homework assignments.

During sign up - and throughout the term - if you have any technical problems or grading issues, send an email to support@saplinglearning.com explaining the issue. The Sapling support team is almost always more able (and more quick) to resolve issues than your instructor.

Several sets of homework will be assigned between each test period. All homework should be completed by the set due dates. The average of the grade earned on all homework sets within a testing period will form the basis for up to 10 points toward each exam score (i.e., if the average grade earned on all assigned homeworks prior to Exam 2 is 83%, then you will be awarded 8 out of

10 possible homework points on Exam 2). Ample time is given to complete all homework sets according to the availability of your schedule, but you are advised not to leave completion of homework assignments until the last minute.

Tools for Success in Lecture:

1. Attend class!!
2. Keep up with material. Read relevant chapters before lecture and formulate questions if a concept is unclear.
3. Dedicate appropriate study time. In Chemistry, you should consider spending three (3) hours studying outside of class for every one (1) hour of lecture.
4. Review your lecture notes after every class and seek to clarify any points which are unclear.
5. Complete all of the homework problems in a timely manner; ideally, following each lecture.
6. Don't procrastinate. These concepts take time and practice to sink in, so do not leave studying until the night before an exam.
7. Form a study group. Meet regularly to solve problems together and obtain help with difficult concepts. Collect contact info for each of your study group members.

Approximate Lecture Schedule: (*Subject to Change*)

Date (Day)	Material/Activity	Book Chapter(s)
8/27/12 (M)	Intro (syllabus); Role of analytical chemist	1
8/29/12 (W)	Statistics and statistical tests	3
9/3/12 (M)	NO CLASS (LABOR DAY)	---
9/5/12 (W)	Methods of quantification	Various (*)
9/10/12 (M)	Method validation	4
9/12/12 (W)	**TEST 1** (Test 1 HW due by 5 pm 9/11/12)	---
9/17/12 (M)	Interaction between matter and light	16
9/19/12 (W)	Basics of spectroscopy	16
9/24/12 (M)	Atomic spectroscopy 1	16, 17
9/26/12 (W)	Atomic spectroscopy 2	16, 17
10/1/12 (M)	Molecular spectroscopy 1	16
10/3/12 (W)	Molecular spectroscopy 2	16
10/8/12 (M)	TEST 2	---
10/10/12 (W)	Equilibrium and partitioning	18
10/15/12 (M)	Sample preparation 1	18 (*)
10/17/12 (W)	Sample preparation 2	18 (*)
10/22/12 (M)	Introduction to chromatography	19
10/24/12 (W)	Gas chromatography 1	20
10/29/12 (M)	Gas chromatography 2	20
10/31/12 (W)	Liquid chromatography 1	21
11/5/12 (M)	Liquid chromatography 2	21
11/7/12 (W)	TEST 3	---
11/12/12 (M)	Introduction to mass spectrometry	20, 21 (*)
11/14/12 (W)	Mass analyzers	20, 21 (*)
11/19/12 (M)	Atomic mass spectrometry	20, 21 (*)
11/21/12 (W)	Molecular mass spectrometry 1	20, 21 (*)
11/26/12 (M)	Molecular mass spectrometry 2	20, 21 (*)
11/28/12 (W)	TEST 4	---
12/3/12 (M)	Review week	---
12/5/12 (W)	Review week	---
12/12/12 (W)	FINAL EXAM	comprehensive

Denotations of (*) in the Book Chapter(s) column of the table indicate that significant material will be given in notes form, which is not covered in the book.

Laboratory Portion of the Course: There will be four open-inquiry laboratory experiments to complete. Three weeks (or six lab periods) are allotted to each experiment. Groups (2 – 3 persons per group) will rotate between experiments, so that each group will be working on a different lab on any given week. The schedule and laboratory experiments are at the end of this syllabus. Each lab experiment will be worth 100 pts and each lab report will be graded in five components, as below. The last three components are interconnected to a significant degree.

Requisition form…	10 pts
Pre-experiment…	10 pts.
Experimental Design…	50 pts.
Outcomes…	15 pts.
Presentation and Style…	15 pts.

Prior to beginning each experiment, you must fill out a **requisition form (10%)** to request necessary chemicals, and justify their need in the context of your designed experiment. A requisition form should be turned by each group to your TA on the Friday (by 5 pm) indicated in the lab schedule for each lab experiment. A 20% late penalty per day will be assessed for requisition forms not received by the due date and time.

A short **pre-experiment (10%)** activity will be performed by each group on each instrument to familiarize you with the instrument. This will be done as part of the first lab period assigned for each experiment. The pre-experiment report will be submitted as a separate document (maximum 1 page written, including exhibits and figures; single-spaced, 12 pt font, 1" margin) with each lab report (in cases where two pre-experiments are requested, your report may be up to two pages in length. All submissions will be electronically to your TA in Word documents. The pre-lab should be given the file name: "4461preexpt_expt #_last name_first initial.doc". Points can be deducted by the TA for any deviation from this file name format.

Each laboratory is open-inquiry. While you will have defined instructions for the pre-experiment, no experimental design is given for the actual laboratory experiment. You will need to meet with your lab group (out of class/lab), and formulate an **experimental design (50%)** for each experiment. This may also include some considerable literature searching for appropriate methods. Overall, be sure to give proper consideration as to how you will address statistical aspects

in your design, data collection, and presentation. Your experimental design must be accurately conveyed in your lab report. You are encouraged to consult with your TA and Dr. Schug as to the appropriateness of your experimental design. ***Begin the process of choosing and planning your laboratory experiment well before you attend lab. The time in lab should be spent doing your experiment. If you are not prepared, you will not have time to finish. Even the pre-experiment may take a little background research (e.g., refer to old lab manual) in order to perform effectively. Once an experiment has been chosen and completed by one group in a section, it cannot be chosen by another group in that same section.***

IMPORTANT: If you think you need to order any chemicals, propose your order to your TA, as soon as possible. Know a source (manufacturer), catalog number, price, and amount available/needed for each proposed order. Be able to justify your desire to purchase the chemical. In many cases, various chemicals can be obtained in-house, however, keep in mind that there are limits associated with how long it takes for ordered chemicals to arrive. USE REQUISITION FORM.

The **outcomes (20%)** of the experiment and how they are reported in your lab report are important, but they do not dictate the majority of your grade. In other words, if things do not work out as hoped, then you can still get a reasonable grade if the experimental design and presentation of the work are sound.

Presentation and style (20%) will be assessed for each lab report. All lab reports should be prepared in accordance with a submission to Journal of American Chemical Society Communications (http://pubs.acs.org/page/jacsat/submission/authors.html) . A template for the article submission can be found here: http://pubs.acs.org/page/jacsat/submission/jacsat_templates.html.
No table of contents graphics, keywords, author addresses, associated content (supporting information), or acknowledgements are needed. The submission is limited to three (3) template formatted pages total, including figures, tables, and references. You must include at least five (5) primary literature references to support your experiment and report. A maximum of four (4) tables and/or figures can be included in the manuscript. Significant care should be given to readability of text and exhibits, as significant penalties will be incurred if grammar, clarity, or format of the submission are compromised. Make sure that the tables and figures you choose are properly chosen representations of your experimental outcomes. Refer to published articles for inspiration and guidance on clarity and presentation.

IMPORTANT: Individual reports from each group member should be submitted electronically in Word format to your TA, on the Friday following the week when the lab was completed. The dates when lab reports are due are listed in red on the laboratory schedule (below). The report should have the filename: "4461report_expt #_last name_first initial.doc". Points can be deducted by the TA for any deviation from this file name format. ***Partners in groups can work together to design the experiments and collect the data, but each group member must prepare their own report, and consequently, figures and tables should not be identical. Deviations from this practice will be assessed for evidences of academic dishonesty.*** Late policy: For lab reports and pre-experiment reports, each will be assessed a 10% penalty per day that either is received after the due date.

Laboratory Schedule and TAs

	Days Times	TA (email)	Office Phone	Office Hours
101	MW 1 – 4:50pm	Choyce Weatherly (choyce.weatherly@mavs.uta.edu)	CRB 309 2-3834	TTh 1 – 3 pm
102	TTh 1 – 4:50 pm	Evelyn Wang (evelyn.wang@mavs.uta.edu)	CPB 233 2-0618	M 12:30 – 4:30 pm
103	TTh 8 – 11:50 am	Kai-Ling Huang (kai-ling.huang@mavs.uta.edu)	CRB 314 2-5436	MW 10 am – 12 pm

Week	Expt 1 (GC)	Expt 2 (Spec)	Expt 3 (HPLC)	Expt 4 (Materials)
8/27 – **8/31**	Group 1	Group 2	Group 3	Group 4
9/3 – 9/7				
9/10 – 9/14				
9/17 – **9/21**				
9/24 – **9/28**	Group 4	Group 1	Group 2	Group 3
10/1 – 10/5				
10/8 – **10/12**				
10/15 – **10/19**	Group 3	Group 4	Group 1	Group 2
10/22 – 10/26				
10/29 – **11/2**				
11/5 – **11/9**	Group 2	Group 3	Group 4	Group 1
11/12 – 11/16				
11/19 – 11/23				
11/26 – 11/30				
12/3 – **12/5** (W)	Final lab report due Wedn., 12/5			

Specific dates highlighted in red indicate due dates for pre-labs and reports. On **9/28**, your 1st lab pre-lab and report are due; on **10/19**, your 2nd; etc.

Underlined dates indicate due dates for requisition forms (Template provided) to be submitted to your TA. This form requires you to indicate proposed chemicals needed and justify your choices (experimental plan, in brief). The form must be approved by your TA before you can begin the experiment.

On the first day of the first week (8/27, 8/28), lab check-in and group assignment will be carried out.

Data storage will be either by CD±R (write once, read many), or by a dedicated, EMPTY flash drive at the discretion of the TA. ***Any flash drive will have your initials on it and must be verified virus free by the TA before each lab it is used. Absolutely no exceptions allowed.*** The penalty will impact your grade for the experiment you're performing in the form of a 10% deduction after grading if you use a drive without TA verification. Current infection rates for student flash drives for viruses/Trojans/malware in general is running over 60%. Please download Microsoft Forefront Endpoint Protection/Antivirus from the UTA website and install it on your home computers if you do not have a current, active antivirus package. Use it to test your hard and flash drives weekly.

Mandatory Online Safety Training: Students registered for this course must complete the University's required "Lab Safety Training" prior to entering the lab and undertaking any activities. Students will be notified via MavMail when their online training is available. Once notified, students should complete the required module as soon as possible, but no later than their first lab meeting. Until all required Lab Safety Training is completed, a student will not be given access to lab facilities, will not be able to participate in any lab activities, and will earn a grade of zero for any uncompleted work.

1. You should have received an email from the UTA Compliance Department. Click on the link in the email (or navigate to https://training.uta.edu for the login page)
2. Log on using your network log-on ID and password (what you use to access email). If you do not know your NetID or need to reset your password, visit http://oit.uta.edu/cs/accounts/student/netid/netid.html.
3. The available courses for completion will be listed. For Chemistry 4461/5461, complete the course entitled 'Student Lab Safety Training'
4. If you did not receive the training email and you have not already completed the training you will need to contact the training helpline (817-272-5100) or email compliance@uta.edu.
5. Students who have not completed the training by census date may be dropped from the lab (and consequently any linked lecture).

Once completed, Lab Safety Training is valid for the remainder of the same academic year (i.e. through next August) for all courses that include a lab. If a student enrolls in a lab course in a subsequent academic year, he/she must complete the required training again.

All questions/problems with online training should be directed to the University Compliance Services Training Helpline at 817-272-5100 or by emailing compliance@uta.edu.

Laboratory Experiments

Experiments: Each experiment lists a pre-experiment, which should be the first thing completed when you enter the laboratory to start a new experiment. Where multiple experiments are listed, your group may choose which laboratory experiment under each category that they wish to pursue, after the pre-experiment is completed. You should meet, discuss, and research your chosen experiment, well in advance of convening in the laboratory to perform it. Time is limited, and if you do not finish the experiment, no concessions can be made. *All of your group members must be present to perform a given experiment, unless prior approval is obtained from the TA or Dr. Schug.*

As a general reference, the old Chem 4461/5461 lab manual can be found here: https://mavspace.uta.edu:443/kschug/4461%205461%20lab%20manual%20F%2009/CHEM%204461%20Student%20Manual%20F2009_rev20090825.pdf

Experiment 1:
Gas Chromatography and Gas Chromatography – Mass Spectrometry

Pre-experiment

[Perform all pre-experiment analysis using manual injection]

Goal: Use butane to determine the dead time, in triplicate. Develop a temperature-programmed GC method to baseline separate a mixture of benzene, toluene, and xylene in less than 5 minutes and identify each component.

Procedure: Make sure the instrument is on, the carrier gas is flowing, and the computer is responding. Follow the procedure on the front of the GC to condition the instrument to be ready for use.

There is also a small manual in a folder with instructions for using the software. Each student will make three manual injections of butane to determine the dead time by using an isothermal separation method. Insert the syringe needle into the outlet of a butane lighter and press the gas release button on the lighter (**Do not strike the flint**). Withdraw the plunger on the syringe each time to the same amount, be it 1ul or 3 ul. Insert syringe, inject, withdraw syringe, and start run. Record the retention time of the peak for each repetition and calculate the

average and standard deviation for each individual and for the group as a whole. This is the dead time for the column under this method.

Prepare a mixture of benzene, toluene, and xylene with a solvent of your choice. Reach the goal by using a temperature gradient separation program and changes in flow rate. [Hint: How does GC detect things? How do you decide the temperature you want? How does flow rate affect the result?] Identify each component.

In your pre-experiment report, calculate the capacity factor for each compound, and the selectivity and resolution for each pair of compounds. Also include a copy of a representative chromatogram and details of your method. Be sure to report the results of your dead-time determination.

GC and GC-MS Experiments

GC.1) Determine and compare the relative levels of aliphatic hydrocarbons in three different varieties (octane rating, supplier, etc.) of gasoline.

GC.2) Characterize the retention of isopropanol, acetone, methyl-*tert*-butyl ether, benzene, and acetophenone using Kovats retention index.

GC.3) Compare the content of limonene in the peels of three different citrus fruits.

GC.4) Determine and compare the ethanol content of 3 different kinds of beer (i.e. lager, stout, IPA) by GC.

GC.5) Determine and compare the relative levels of ethyl alcohol in three different brands of mouth wash liquid by using gas chromatography.

GC.6) Design and carry out a selected ion monitoring experiment for the quantitative determination of methyl-*tert*-butyl ether and benzene in gasoline. Compare the content for two different gasoline samples.

GC.7) Develop a method to qualitatively assign and compare the presence of different terpenes and terpenoids in the peels of two different citrus fruits.

GC.8) Determine the identity of plasticizer components leached from a chloroform extract of three different plastic containers.

GC.9) Characterize and compare the hydrocarbon (linear, branched, aromatic, etc.) content of 3 liquid fuels (e.g. Coleman fuel, tiki torch fuel, kerosene, gasoline, and castor oil)

GC.10) Characterize and compare the polyaromatic hydrocarbon content of two samples of coal tar (e.g., from two different types of pavement).

Experiment 2:
Spectroscopy (UV-Vis, Fluorescence, Flame AA)

[You are expected to perform the pre-experiment for two out of the three spectroscopy instruments. Once ANY Spectroscopy experiment has been performed by ANY group in ANY class section, it may not be performed by any other group in any other section.]

UV-Vis spectroscopy; microarray plate reader

There is a very good manual with the instrument. Use it.

Pre-Experiment: Obtain one 96-well plate (you should use this through your pre-experiment and experiment until you run out of unused wells; each well should be used once; do not try to wash and re-use; keep careful track of the wells you used). Make a serial dilution (at least 9 points) of a 500 ppm solution of ethyl paraben in 50/50 water/methanol (v/v). Report a linear calibration curve based on the response of the compound at its maximum absorbance wavelength. Include a drawing (that you created) of a standard double-beam UV-vis spectrophotometer in your pre-experiment report. List three separate applications from the primary literature (provide references) for use of a UV-Vis microplate reader.

UV Experiments

UV.1) Develop and fully validate a method for the determination of acetophenone in contaminated well water. Full validation includes comprehensive determination of linearity, precision, accuracy, limit of detection, and limit of quantification. Be sure to research and include concessions for potential interferences.

UV.2) Determine and compare the levels of phosphate in three local bodies of water. In your report, speculate as to the origin of these compounds.

UV.3) Compare the relative levels of NADH and NAD+ in two different cell types or in two differing species of bacteria.

UV.4) Develop a UV-spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets.

UV.5) Using a UV-Vis microplate reader, determine the amount of food dye present in three types of hard candies (e.g. jolly ranchers). Also report the wavelength of the dyes present in the candies and make a qualitative comparison. Some hard candies may contain two or more food dyes.

Fluorescence Spectroscopy

Pre-Experiment: There are 2 online manuals on the computer plus a helper manual at the instrument. Make a stock solution of 100 ppm quinine in 0.5% sulfuric acid. Develop a 7 point calibration curve from a series of dilutions of the stock solution. Ensure the calibration curve is in linear response range of the instrument. The maximum detector value is 1000 AU. Keep the response of the standards below 10 % of this value.

System Configuration: Set slit bandpass to 2.5 nm, and the integration time to 0.5 sec. Repeat with slit bandpass of 10.0 nm. Report any differences, limitations of the two slit widths.

Prepare and report a calibration curve using the primary and secondary excitation and emission wavelengths (be sure to report the values used.) How do you find the excitation wavelengths to use? Include a drawing (that you created) of a standard fluorescence spectrophotometer in your pre-experiment report.

Fluorescence Experiments

FL.1) Determine the fluorescence properties (absorption/emission profiles, quantum yields, and limits of detection) for a series of biocompatible, photoluminescent polymers.

FL.2) Using fluorescence spectroscopy, determine and compare the levels of vitamin B2 in four varieties (varying manufacturers, milk-fat %, etc.) of commercial milk samples.

FL.3) Use fluorescence spectroscopy to determine the rate constant (kq) for oxidative quenching (electron transfer (eT) out to an electron acceptor), reductive quenching (eT in from an electron donor), and energy transfer (ET to a dye or other compound) for excited Ru(Phen)3 in solution. This analysis is best done using a Stern-Volmer plot. Potential quencher reagents to investigate are as follows:

Oxidative quenching: methyl viologen, Cu2+, or Fe3+
Reductive quenching: ascorbate ion (pH 5)
Energy Transfer: nickel (II) acetylacetonate (in MeOH)

FL.4) Develop a fluorometric method to quantify the amount of chlorophyll in a series of samples of cultured or natural algae.

FL.5) Determine and compare absorption/emission profiles using UV-Vis and fluorescence spectroscopy for chlorophyll extracted from three different plants (Spinach, Tree, Grass, etc.).

FL.6) Determine the rate of lactate production from pyruvate and the enzyme lactate dehydrogenase by fluorometric spectroscopy.

FL.7) Use fluorescence spectroscopy to determine and compare concentration levels on riboflavin in a variety of vitamin pills

Flame Atomic Absorption Spectroscopy

Pre-Experiment: There is a folder containing startup information for the AA at the instrument. Prepare a set of calibration standards (minimum 7 different concentrations) ranging from 1.0 to 100 ppm (~15 mL each) Fe in 0.10 M HCl from a Fe compound in the lab. Generate a 1000ppm stock solution. Also prepare a blank of 0.10 M HCl (no Fe). Run and report a calibration curve for Fe in triplicate. Use the blank-corrected response on the x-axis. Include a diagram

(that you created) for a flame AA instrument in your pre-lab report. Include a paragraph comparing and contrasting (advantages and disadvantages) flame AA, graphite furnace AA, and inductively-coupled plasma – atomic emission spectroscopy, as methods for determining metals in solution.

Flame AA Experiments

AA.1) Using flame atomic absorption spectroscopy, determine and compare the concentration of iron in four varieties of cereal flakes. Extrapolate your measurements to report whether the manufacturer's claimed content of iron is accurate.

AA.2) Using flame atomic absorption spectroscopy, determine and compare the amount of copper in three varieties of mineral supplements.

AA.3) Determine and compare the levels of sodium in three different microwave popcorn varieties.

AA.4) Determine the copper content in different age U.S. coins of all common denominations by AA (e.g. compare dimes from pre- and post-1965; pennies from pre- and post-1982).

AA.5) Using flame atomic absorption spectroscopy, determine the amount of zinc present in a Cold-Eeze lozenge and compare with listed ingredients.

AA.6) Using flame atomic absorption spectroscopy, determine and compare the amount of copper, iron, magnesium, and zinc in each group members hair.

AA.7) Using flame atomic adsorption spectroscopy, determine and compare the amounts of zinc and lead in three different soil samples.

Experiment 3:
High Performance Liquid Chromatography, Liquid Chromatography – Mass Spectrometry, and Mass Spectrometry

[You should perform both pre-experiments listed]

Pre-Experiment 1 (HPLC-UV)

Goal: Develop a gradient reversed phase HPLC method for the separation of four parabens (methyl-, ethyl-, propyl- and buryl-paraben), using a C-18 column and a methanol/water mobile phase, in less than 7 minutes.

Procedure: Make sure the instrument is on, the two solvent reservoirs are filled and degassed, the computer is responding. Record the dimensions, the make and model, the type of the stationary phase, and the type of packing for the column installed in the chromatograph.
There is a small manual at the HPLC with software instructions.

Obtain a stock solution, which contains methyl-paraben, ethyl-paraben, propyl-paraben and butyl-paraben, each at a concentration of 200 ppm, in 50/50 methanol/water from your TA. Set the flow rate of the column to value suggested by TA (This is column dependent). Set the solvent ratio of the mobile phase to 99% methanol to 1% water, and turn the pump on. Set the analysis time to 8 minutes and the detector wavelength to 254 nm. Allow at least 5 column volumes of solvent to pass through the column before beginning the analysis. Note the pressure of the system and make sure it is stable prior to the run. Place sample in auto sampler. Note position in tray. Record the chromatogram and the peak analysis. Repeat this analysis sequentially for different mobile phase compositions (ex. 90/10, 80/20, 70/30, etc) until you have an idea how mobile phase affects the result, and you get baseline separation of the compounds. Remember to allow sufficient time for equilibration at each new mobile phase. These are isocratic separation methods.

Set up the instrument to perform a gradient solvent program separation method. Start with an initial mobile phase composition of 50/50 methanol/water, a hold at that composition for 1 min, an increase to 99/1 methanol/water in 6 minutes and a 1 minute hold at the final composition. Inject the 200 ppm mixture and record a chromatogram. Modify the solvent gradient to create a method for base line separation ($R>1.5$) of all components to reach the goal.

In your pre-experiment report, calculate the capacity factor for each compound and the selectivity and resolution for each pair of compounds. Also include a copy of your chromatogram and details of your method.

Pre-Experiment 2 (LC-UV-MS)

Obtain a mixture of naphthol, phenol, benzoic acid, and benzylamine from your TA. Perform a separation (C18) of the mixture using two different mobile phase compositions: A) 70/30 methanol / 10 mM aqueous ammonium acetate; B) 70/30 methanol / 0.5% acetic acid in water. Compare UV and MS (positive and negative ionization mode) responses and peak shapes for each of the four analytes under the two conditions.

HPLC, LC-MS, and MS Experiments

LC.1) Develop a HPLC-UV separation method for a mixture of 8 hormone and endocrine disruptor compounds, and assign the identity of each peak in the chromatogram. Apply your method to determine the identity and concentration of a contaminated water sample.

LC.2) Develop an HPLC-UV method for separation and detection of anthocyanins from freeze-dried berries (at least three varieties). Compare and contrast the number and relative amount of anthocyanins in each of the berries tested. Using information from the literature on anthocyanins, attempt to assign the identity of each anthocyanin detected in each variety of berry you tested.

LC.3) Students need to develop an analytical HPLC method for separating and quantifying caffeine and vitamin B6 in four different brands of energy drinks (Red Bull, Monster, Rock Star, etc.). Students need to be able to show a complete validation that includes linearity, precision, accuracy, limit of detection, and as well as limit of quantification

LC.4) Develop a method to determine pesticide content in three different varieties of tomatoes by HPLC. Target herbicides would be urea herbicides (tebuthiuron and diuron) and triazine herbicides (simazine, atrazine, and ametryn).

LC.5) Develop a HPLC-UV method for the extraction and separation of phenolic compounds, mainly flavanols and phenolic acids, from a variety of tea leaves (at least 3). Using a primary reference source, try to determine the identity of each phenolic detected and compare the relative levels of each phenolic in each tea.

LC.6) Develop and apply an HPLC-UV method for the detection of preservatives such as benzoic or sorbic acids in three different types of food items. (Possible food items include soft drinks, canned fruit/vegetables, sauces, jams/jellys, or dried fruit.)

LC.7) Use LC-MS to identify the presence of acetaminophen, acetylsalicylic acid, ibuprofen, and caffeine in three different commercial analgesic tablets. Perform in-source CID the analytes and assign the observed fragments.

LC.8) Systematically evaluate and report the effectiveness of selected ion monitoring vs. multiple different scan ranges (i.e., increasing magnitude of m/z scan ranges) on sensitivity and linear range for the LC-MS determination of quinine in tonic water.

LC.9) Design a flow injection analysis experiment to comprehensively evaluate the concentration dependent ionization suppression or enhancement effects of ammonium acetate, ammonium formate, acetic acid, and formic acid on the response of butyl amine in a 50/50 water/methanol solution.

Experiment 4:
Materials and Thermal Analysis

Differential Scanning Calorimetry (DSC)

Pre-Experiment

Goal: Develop a method of determining the melting point of a compound, and measure various values associated with the melting point using the analysis package.

Instrument Prep: There is a basic manual provided for this instrument. Please use it to properly set up this instrument. Be sure to turn on the Nitrogen gas prior to any use of the instrument. Not doing this can damage the instrument electronics. Before loading a sample, be sure that the internal temperature reading in software reads ≤ 40 °C.

Procedure: Determine the melting point of a small piece of Indium metal. Carefully cut a piece of Indium from the provided sample, trying to keep the weight between 3 and 7 mg using the analytical scale (analytical in this case means accurate to below 1mg).

Perform an experiment heating the metal from ambient to 200 °C at 10 degrees per minute. Using the analysis software described in the manual, measure the melting point and the amount of energy required per mg to melt the metal.

Now do the same for Zinc, going from ambient to 500 °C and report the melting point and amount of energy required per mg to melt the metal.

There are other transitions a DSC can identify. Please research these in your quest to find experiments for this instrument and mention them in the pre-lab, along with the presentation of results from the above experiments.

DSC Experiments

DSC.1) Measure the relative enthalpy of unfolding of the following proteins: bovine serum albumin, cytochrome c, hemoglobin, and myoglobin (or other appropriate inexpensive proteins).

DSC.2) Determine and compare the heat capacities, glass transition temperatures, and melting temperatures for a series of polystyrene polymer standards, ranging in molecular weight from 2,500 to 50,000 Da.

DSC.3) Using DSC, evaluate the thermal events associated with the thermal degradation of aspirin. Particularly, investigate the effects of multiple heating and cooling cycles on the particular temperatures associated with these events.

DSC.4) Using TGA/DSC, study and compare thermal events associated with heating/cooling of three different varieties (e.g. densities) of commercial polyethylene polymers (e.g. milk jugs, juice bottles, etc.)

DSC.5) Compare and contrast the thermal events associated heating/cooling three different commercial plastic products produced from different polymers (e.g. PE, PP, PET, PC, etc.)

Thermogravimetric Analysis

Pre-experiment

Turn on Nitrogen Gas and ensure gas is flowing before using the instrument! Not doing this can damage the instrument electronics

Goal: Obtain thermograms for the dehydration of copper sulfate pentahydrate and determine the weight percent of one molecule of H_2O by using formula mass.

Procedure:

Before loading a sample, be sure that the internal temperature reading in software reads ≤ 40 °C. Open the furnace; insert the protection plate so that you don't drop the pan into the furnace. **[Those pans are very expensive, if you drop them, you fail the experiment and you pay for the damage!]** Carefully put a secondary pan on the loading tray and close the furnace. Press Auto Zero button on the front of the TGA and wait for the instrument to complete the tare cycle. When it's balanced, open the furnace and take out the secondary pan to load your sample (enough to make a single uniform layer at the bottom of the pan) Carefully return the pan to the loading tray and make sure to align the pan in the middle of the tray. Close the furnace.

Perform an experiment of heating the sample from ambient to 300 °C at 5 degrees per minute and then another experiment with fresh sample going from ambient to 300 °C at 25 degrees per minute. **Do not begin another run until the furnace has cooled completely!** For each transition in the thermogram, determine and report the total and individual changes in the weight percent of your analyte. Determine and report the onset temperature for each transition. Include a copy of your thermogram with details of your method in your pre-experiment report.

TGA Experiments

TGA.1) Develop and validate a method to differentiate calcium carbonate from calcium sulfate dehydrate.

TGA.2) Perform a "proximate analysis" for three different types of coal, and compare their properties.

TGA.3) Obtain four different samples of textiles and compare their thermal properties using TGA and DSC. Present the report for this analysis in the context of potential use of the method for forensics investigations.

TGA.4) Determine and compare the mass of hydrocarbon grafted onto silica phases used in HPLC column packings.

TGA.5) Obtain three different starch products and compare their thermal properties using TGA and DSC. Discuss how their properties relate to their use in food preparation.

Other Important Items

Dropping: When dropping the course, YOU are responsible to see that the proper paperwork is filed with the Department. Failure to do so will result in a grade of "F".

Drop for non-payment of tuition: If you are dropped from this class for non-payment of tuition, you may secure an Enrollment Loan through the Bursar's Office. You may not continue to attend class until your enrollment loan is applied to outstanding tuition fees.

Grade Replacement: Students enrolling in this course with the intention to replacing a previous grade earned in the same course must declare their intention to do so at the registrar's office by Census Date of the same semester in which they are enrolled.

Pass/Fail: If P or F is a grade option in this class and you intend to take this class for a pass/fail grade instead of a letter grade, you MUST inform the instructor, through the necessary paperwork, before the Census Date.

Bomb Threat Policy: In the event of a bomb threat to a specific facility, University Police will evaluate the threat. If required, exams may be moved to an alternate location, but they will NOT be postponed. UT-Arlington will prosecute those phoning in bomb threats to the fullest extent of the law.

Americans with Disabilities Act: The University of Texas at Arlington is on record as being committed to both the spirit and letter of federal equal opportunity legislation; reference Public Law 93112-The Rehabilitation Act of 1973 as amended. With the passage of new federal legislation entitled American with Disabilities Act-(ADA), pursuant to section 504 of The Rehabilitation Act, there is renewed focus on providing this population with the same opportunities enjoyed by all citizens.

As faculty members, we are required by law to provide "reasonable accommodation" to students with disabilities, so as not to discriminate on the basis of that disability. Student responsibility primarily rests with informing faculty at the beginning of the semester and in providing *authorized* documentation through designated administrative channels.

Student Support Services: UT Arlington provides a variety of resources and programs designed to help students develop academic skills, deal with personal situations, and better understand concepts and information related to their courses. Resources include tutoring, major-based learning centers, developmental education, advising and mentoring, personal counseling, and federally funded programs. For individualized referrals, students may visit the reception desk at University College (Ransom Hall), call the Maverick Resource Hotline at 817-272-6107, send a message to resources@uta.edu, or view the information at www.uta.edu/resources.
Electronic Communication: UT Arlington has adopted MavMail as its official means to communicate with students about important deadlines and events, as well as to transact university-related business regarding financial aid, tuition, grades, graduation, etc. All students are assigned a MavMail account and are responsible for checking the inbox regularly. There is no additional charge to students for using this account, which remains active even after graduation.

Information about activating and using MavMail is available at http://www.uta.edu/oit/cs/email/mavmail.php.

Student Feedback Survey: At the end of each term, students enrolled in classes categorized as lecture, seminar, or laboratory shall be directed to complete a Student Feedback Survey (SFS). Instructions on how to access the SFS for this course will be sent directly to each student through MavMail approximately 10 days before the end of the term. Each student's feedback enters the SFS database anonymously and is aggregated with that of other students enrolled in the course. UT Arlington's effort to solicit, gather, tabulate, and publish student feedback is required by state law; students are strongly urged to participate. For more information, visit http://www.uta.edu/sfs.

Academic Integrity: All students enrolled in this course are expected to adhere to the UT Arlington Honor Code:

I pledge, on my honor, to uphold UT Arlington's tradition of academic integrity, a tradition that values hard work and honest effort in the pursuit of academic excellence.

I promise that I will submit only work that I personally create or contribute to group collaborations, and I will appropriately reference any work from other sources. I will follow the highest standards of integrity and uphold the spirit of the Honor Code.