Chemistry 316

Laboratory Manual for Instrumental Analysis

2003-3

Course Instructor: Dr. Paul Li

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Outline of CHEM 316-4 (2003-3) Instrumental Analysis

Instructor: Dr. Paul Li SSB-7104 tel: 291-5956 email: paulli@sfu.ca
Lecture venue: AQ5005 Lab (in C7017): Starts from Sep 15 Monday (1:30- 5:20 pm)
Office hour: Tues and Thurs 11:30 am-12:30 pm (preferably with appointments)
Lab TA: Jacky Chou (wlchou@sfu.ca)
Textbook: Skoog DA, Holler FJ, Nieman TA, Principles of Instrumental Analysis, 5th edition, Saunders, 1998.
Grading: Lab (35%), Mid-term exam (Oct 9, 20%), Final exam (Dec 6, 45%)

Lecture contents (see separate handouts):

1. Molecular spectroscopy (Mol) 2. Atomic spectroscopy (AS)
3. Gas chromatography (GC) 4. chromatographic separation (Sepn)
5. Liquid chromatography (LC) 6.Capillary electrophoresis (CE)

Experiments (lab experiments start on Sep 15):

1. Literature study (5)
2. Molecular Spectroscopy (12)
3. Atomic Spectroscopy (8)
4. Gas Chromatography (6)
5. Liquid Chromatography (4)

The lecture materials have been taken from the textbook, and from the following references:
R1: QD79I5I52 1981 Instrumental Methods of Analysis 6 th ed
R2: QD75E9 1985 Instrumental Methods of Chemical Analysis 4th ed
R3: QD79I5S58 1985 Principles of Instrumental Analysis 3rd ed
R4: QD75.3E58 1995 Environmental Analytical Chemistry 1st ed
R5: QD271S83 1996 Chromatographic Methods 5th ed
R6: QD79I5S39 1984 Chemistry Experiments for instrumental method
R7: QD75.4S8M54 2000 Statistics and chemometrics for analytical chemistry 4th Ed.
(check library reserves for these references, solutions to problems and sample exam papers)

Teaching schedule:

Sep 2 Intro	Sep 4 Std/Cal		Oct 7 Rev	Oct 9 **Mid-term**	AS/Lum lab	Nov 11 CE	Nov 13 CE	AS/Lum/GC /LC lab
Sep 9 Mol	Sep11 Mol	Literature study	Oct 14 Sepn	Oct 16 Sepn	AS/Lum/GC lab	Nov 18 CE	Nov 20 Projects	AS/Lum/GC /LC lab
Sep 16 Mol	Sep 18 Mol	Mol lab starts on Sep 15	Oct 21 GC	Oct 23 GC	AS/Lum/GC lab	Nov 25 Rev	Nov 27 Rev	
Sep 23 AS	Sep 25 AS	Lum lab	Oct 28 GC	Oct 30 LC	AS/Lum/GC/LC lab			
Sep 30 AS	Oct 2 Sepn	AS/Lum lab	Nov 4 LC	Nov 6 LC	AS/Lum/GC/LC lab			

Expectations for Chem 316

Name (optional): _____

(Please tear this page off and hand it in)

1. Why do you take this course?
2. What instrumental techniques do you want to learn?
3. Do you want to learn more techniques, or to learn less, but in-depth?
4. Will you attend lectures regularly?
5. How much time do you expect to spend to study this course?
6. Will you consider dropping this course if this is not the right one for you? Or do you have no choice?
7. What lab skills do you like to learn in the lab class?
8. Do you want to finish the lecture of a certain technique before starting the lab for it?
9. Does the mid-term exam for C316 clash with your other mid-term exams?
10. What career will you pursue after you graduate? Will this course be relevant to your future career?
11. Do you want email your questions, concerns and suggestions to me?
12. What teaching ability do you expect of your instructor?
13. What are your other expectations or questions about this course?
14. Will you pursue an undergraduate project or directed study to learn more about modern analytical chemistry?

(Please use the other side if space is not enough for you)

Overview

This laboratory course includes literature study and experimental work. Experiments will most likely start after the theoretical principles are covered in lectures. Lab experiments will start on Sep. 15. The lab course requirements are summarized as follows:

1. Literature review (report due on in week 3 during the first lab session)
2. Molecular Spectroscopy (UV-vis absorption, Rayleigh scattering, Raman spectroscopy, fluorescence emission and excitation spectra, quantum efficiency, quenching, detection limit, external standard calibration)
3. Atomic Spectroscopy (sampling/nebulization, burner height, atomic emission, atomic absorption, standard addition/external standard calibration, chemical interferences)
4. Gas Chromatography (elution order, isothermal elution, van Deemter plot, temperature programming, internal standard calibration)
5. Liquid Chromatography (reverse-phase LC, isocratic elution, gradient elution, standard addition/internal standard calibration)

Pre-laboratory Preparation

Read/review lecture notes and textbook for theory. Read the lab procedures carefully. If possible, watch carefully while other students perform similar experiments before you do. Always think why I should do this experiment, and why such steps are necessary in the procedure.

Lab grouping

There will be 5 groups (A-E) of 2-3 students performing 14 experiments, i.e. 5 Mol. experiments, 4 AS experiments, 3 GC and 2 LC experiments. Each group will be responsible for sample preparation, experimental work and lab report of a particular experiment using an instrumental technique. Tentative grouping will be based on the class list. Suggest changes in grouping, if any, to me.

3	**Mol**	A1	B3	A2																		
4	**Mol**				C4	D5																
5	**Mol**	E1	A3	E2			**AS**	B1	C2	A3												
6	**Mol**				B4	E3	**AS**				E4	D2										
7	**Mol**	D1		D2		C5	**AS**	A1	E3	B2			**GC**	C1	A2			E3				
8	**Mol**				A4	B5	**AS**				B4	C1	**GC**		C3	D1	D2					
9	**Mol**	C1	D3	C2			**AS**	A2	A4	D3			**GC**	E2					**LC**	B1	E2	B2
10	**Mol**				E4	A5	**AS**			C4	D4	E2	**GC**			A1	B2	B3	**LC**	D1	C2	
11	**Mol**	B1	C3	B2			**AS**	D1		C3			**GC**	E1	A3				**LC**	A2	E1	D2
12	**Mol**				E5	D4	**AS**				B3	E1	**GC**			D3	B1	C2	**LC**	C1	A1	

Please check if your group do all 14 experiments and do them during either the first 2-h or last 2-h of each lab session.

Report

The report will contain experimental objectives, tabulation of raw data, observations, data analysis and calculations, graph plotting (if any), answers to questions. The report can be either typewritten or legibly written. Each group will hand in one report, hopefully completed in the lab. The due date of the lab report is one

week after the lab is completed. Please hand in the reports to the TA in the lab. The last lab reports will be handed to me in the Tues or Thurs class for the Mon and Thurs groups, respectively.

Grading Scheme: Individual grades will be assigned. The grade will be based on the individual's performance and diligency in the laboratory, plus the reports which include experimental observations, data analysis and conclusion. Students may score higher than the others in the same group.

Introduction, figure of merits, and Calibration

Selection of analytical methods

- Speed, ease & convenience, operator skill needed, availability of equipment, cost.
- Figures of merit: to evaluate performance of instruments (Table 1-3)

1. Sensitivity:

$$S = m \cdot c + S_{bl}$$

Calibration sensitivity (IUPAC): slope of calibration curve (m) at the concentration of interest, with units.

Analytical sensitivity: takes into account of precision, $\gamma = m/s_s$; dimensionless; γ is higher for lower s_s even if m is not improved.

2. Detection limit: (see Eg. 1-1 and additional notes)

$$S_m = \overline{S_{bl}} + 3s_{bl}$$

$$c_m = \frac{S_m - \overline{S_{bl}}}{m} = \frac{3s_{bl}}{m}$$

S_{bl} is a good estimate for $\overline{S_{bl}}$ and $S_{y/x}$ for s_{bl}.

3. Dynamic range: from LOQ to LOL which is at least 2 orders of magnitude, e.g. from 10^{-4} to 10^{-2} M (Fig 1-7).

LOQ = 10 s_{bl}/m c.f. $c_m = 3\ s_{bl}/m$

Calibration (see C215 notes)

1. External standard
2. Standard addition
3. Internal standard
4. Linear regression and associated uncertainties

Exercises:

1-5, 1-6, 1-8, 1-9, 1-10, 1-11.

Literature review and research proposal

Objectives: To understand how modern analytical chemistry instrumentation is applied for chemical analysis in environmental, biochemical, forensic, pharmaceutical, and other related fields.

Read the attached article which is extracted from Anal. Chem. 73, 2001, 678A. Then, identify the samples, the analytes and the techniques used for chemical analysis in the first forensic case of the trampled teenager. Provide brief descriptions about your choices.

Hand in the report (in less than 500 words) in week 3 during the first lab session. Each student will hand in his/her own report.

Notes to the technician

Molecular spectroscopy (Sep 15 - Nov 20)

Apparatus
Two fluorescence cuvettes (one quartz and one plastic) and one absorption cuvette
fluorescence spectrometer
UV-vis spectrometer (Needed during the first 2 h during week 3, 5, 7, 9, 11. Please contact John)
pH meter
twenty 50-mL volumetric flasks
Two 250-mL volumetric flasks
One 100-mL volumetric flask
One 1-L volumetric flask

Materials
1 M H_2SO_4 (50 ml x 8 x 10 = 4 L)
quinine sulfate solid (150 mg x 4 x 10 = 6 g)
fluorescein, sodium salt (100 mg x 10 = 1 g)
5,000 ppm sodium fluoride (50 mL x 10 = 500 mL)
5,000 ppm sodium chloride (50 mL x 10 = 500 mL)
5,000 ppm sodium bromide (50 mL x 10 = 500 mL)
tonic water (bottled, not canned)
6 M NaOH (10 mL x 10 = 100 mL)

Atomic spectroscopy (Sep 29 – Nov 20)

Apparatus

1. Perkin Elmer 1100B Atomic Absorption Spectrophotometer
2. Calcium/magnesium hollow cathode lamps.
3. Sixteen 100-mL flasks
4. Five 50-mL flasks
5. Two 250-mL flasks
6. Two 100-mL plastic bottles
7. One 100-mL measuring cylinder

Chemicals

1. 1,000 ppm (or μg/mL) solution of Ca (50 mL x 10 = 500 mL)
2. 1,000 ppm solution of Mg (50 mL x 10 = 500 mL)
3. 10,000 ppm solution of K (50 mL x 10 = 500 mL)
4. 10,000 ppm solution of Sr (50 mL x 10 = 500 mL)
5. 0.1 M EDTA (100 mL x 10 = 1000 mL)
8. 1 M H_3PO_4 (25 mL x 10 = 250 mL)

Gas Chromatography (Oct 13 – Nov 20)

(a) Individual standard solution of benzene, toluene, ethylbenzene and xylenes in hexane at a concentration of 1000 ppm. Each contains 1,000 ppm bromobenzene.
(b) 4 calibration standards of a mixture of benzene, toluene, ethylbenzene and xylenes (BTEX) in hexane at a level of 100, 500, 1000 and 2000 ppm (μL/L).

Each standard mixture also contains 1,000 ppm bromobenzene as the internal standard.
(c) A 94-octane gasoline sample is prepared in 10,000 ppm by volume in hexane. This sample also contains bromobenzene as the internal standard at 1,000 ppm.

Liquid Chromatography (Oct 27 – Nov 20)

Materials

- HPLC-grade acetonitrile
- An acidic aqueous buffer (contains K_2HPO_4 adjusted by H_3PO_4 to pH 3.10)
- 100-ppm of phthalic acid (50 mL x 10 = 500 mL)
- 100-ppm of salicylic acid (50 mL x 10 = 500 mL)
- 100-ppm of benzoic acid (50 mL x 10 = 500 mL)
- 100-ppm of p-nitrophenyl acetic acid (100 mL x 10 = 1000 mL)
- seven 50-mL volumetric flasks
- four 10-mL graduated pipette

Molecular Spectroscopy

Pre-Laboratory

1. Find the excitation and emission wavelengths of quinine sulfate and fluorescein
2. Find the chemical structures and pK_a of quinine sulfate and fluorescein
3. Read the method for the determination of detection limit.
4. Determine how to prepare the quinine sulfate solutions of various concentrations. What should be used to make the dilution?
5. Determine how to prepare the quinine sulfate solutions containing various concentrations of sodium halides.
6. Determine how to prepare a 250-mL solution of 100 μM fluorescein

This experiment is comprised of 5 sections as follows:

1. Absorption and fluorescence (excitation and emission) spectra
2. Determination of quinine sulfate in tonic water using fluorescence spectroscopy
3. Quenching interference in fluorescence spectroscopy
4. Rayleigh and Raman scattering at low fluorescent intensity
5. Effect of pH on quantum yield of fluorescein

Materials/Chemicals

Apparatus

Fluorescence cuvette and absorption cuvette. (Examine their physical differences) For quinine's work, the fluorescence cuvette is made of quartz and is very expensive. For fluorescein's work, the cuvette is made of plastic (Why?).
pH meter
UV-vis spectrometer, fluorescence spectrometer

Materials

1 M H_2SO_4
quinine sulfate solid (To prepare a 100 μg/mL (or ppm) solution, dissolve 120.7 mg of quinine sulfate dihydrate in distilled water and transfer the solution to a 1-L flask; add 50 mL 1M H_2SO_4, and dilute to the mark with water) (prepare fresh daily and protect from light)
fluorescein, sodium salt
5,000 ppm each of sodium fluoride, sodium chloride and sodium bromide
tonic water
6 M NaOH
twenty 50-mL volumetric flasks
Two 250-mL volumetric flasks
One 100-mL volumetric flask
One 1-L volumetric flask

References:

Skoog/Holler/Nieman, Principles of Instrumental Analysis, 5th edition, 1998, Saunders College Publishing (or Skoog/Leary, Principles of Instrumental Analysis, 4 th edition, 1992, Saunders College Publishing, Florida)

1.J. R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum Press, New York, 1983.
2.T. C. O'Haver, "The development of Luminescence Spectrometry as an Analytical Tool," J. Chem. Educ., 55, 423 (1978).

EXPERIMENTAL

1. Absorption and fluorescence (excitation and emission) spectra (seven 50-mL flasks)

(a) Prepare a 100 μg/mL quinine sulfate solution (50 mL). Then dilute it to give a 10 μg/mL quinine sulfate solution (50 mL). Perform a UV-Vis absorption scan (200-800 nm) to locate the excitation wavelengths of quinine. It is useful to initially run a blank and then the quinine standard to establish the quinine absorption wavelengths. Print out the absorption spectra.

(b) Use the 10 μg/mL quinine sulfate solution. Select an excitation wavelength, then perform an emission scan (200-800 nm).

(c) Select the emission wavelength, then perform the excitation scan.

(d) If there are more than one excitation peaks, select another excitation wavelength and repeat (b).

(e) Prepare a 0.05 M H_2SO_4 solution (500 mL) from the 1 M stock. Prepare a series of 50-mL quinine standard solutions (10, 1, 0.1, 0.01, 0.001, 0.0001 μg/mL) by sequential dilution with 0.05 M H_2SO_4 from the 100 μg/mL stock solution. Measure UV absorbances of these solutions using the absorption wavelength(s) determined from (a). Make further dilution of the standard until the most dilute solution gives an absorbance approximately that of the blank of 0.05 M H_2SO_4. Remember to shield off room light from the cuvette holder with a cover.

Q: Determine the molar absorptivity (or extinction coefficient) of quinine sulfate?
Q: Explain why there two peaks in the excitation spectrum. Which one should be selected for subsequent measurements?
Q: Compare the absorption and excitation spectra.
Q. Determine the detection limit of quinine in the UV absorption method.

2. Determination of quinine sulfate in tonic water using fluorescence spectroscopy (one 250-mL flasks)

Select the excitation and emission wavelengths that result in the highest measured fluorescent intensity.

Use the same series of quinine standard solutions (10, 1, 0.1, 0.01, 0.001, 0.0001 μg/mL) you have prepared in (1). Perform measurement of fluorescent intensity at the selected excitation and emission wavelengths. Make further dilution of the standard until the most dilute solution gives a fluorescent intensity approximately that of the blank of 0.05 M H_2SO_4.

Prepare the tonic water sample by appropriate dilutions using 0.05 M H_2SO_4 (e.g. dilute 5 mL of tonic water to 250 mL using 0.05 M H_2SO_4). Measure the fluorescence intensity three times with the sample.

Q: Plot the calibration curve and determine the detection limit of quinine.
Q: Compare this detection limit of quinine with the one obtained in UV absorption spectroscopy.
Q. Using linear regression analysis, determine the concentration of quinine in tonic water, with associated uncertainty. Set up the spreadsheet while you are waiting for the results.

3. Quenching Interference in Fluorescence Spectroscopy (one 1-L flask, five 50-mL flasks)

Prepare a 0.05 M H_2SO_4 solution (1 L) from the 1 M stock.

Prepare 10 μg/mL quinine sulfate solutions (50 mL) which contain five concentrations of sodium chloride (0.005 – 0.1 M). Transfer the solution to plastic storage bottles. Measure the fluorescence intensity of these solutions at the selected excitation and emission wavelengths.

Repeat with quinine sulfate solutions which contain five concentrations of sodium bromide.
Repeat with quinine sulfate solutions which contain five concentrations of sodium fluoride.

Q: On the same graph, plot the 3 curves of fluorescent intensity versus concentration of sodium halide.
Q: Discuss the shapes of the three curves and compare their differences.
Q: Discuss the mechanism of fluorescent quenching.

4. Rayleigh and Raman scattering at low fluorescent intensity (two 50-mL flasks)

Prepare a 0.05 M H_2SO_4 solution (50 mL) from the 1 M stock. Prepare a 100 μg/mL quinine sulfate solution (50 mL). Then dilute it to give a 50-mL solution of 0.01 μg/mL quinine sulfate in 0.05 M H_2SO_4.

Perform one excitation and two emission scans for a 50-mL solution of 0.01 μg/mL quinine sulfate. Repeat the three scans with a solution of 0.05 M H_2SO_4 alone. Slightly change the excitation wavelength to be 20 nm higher than the selected value, perform another emission scan of the quinine sulfate solution.

Q: Discuss the various features in the fluorescent spectra of a low concentration of quinine sulfate or even a blank solution.
Q: Differentiate between an Rayleigh peak and an Raman peak.
Q: Differentiate between an Raman peak and a fluorescent peak.

5.Effect of pH on quantum yield of fluorescein (one 250-mL flask, one 100-mL flask, six 50-mL flasks)

Prepare a fluorescein solution (250 mL) in water (100 μM). Dilute the 6 M NaOH solution to give 100 mL of 0.1 M NaOH solution. Do not use a glass-stoppered bottled to store NaOH solution. (Why?)

Take 25 mL of 100-μM fluorescein solution, use a dropper or burette, add a small amount of 0.1 M NaOH and measure the pH of the solution so that a series of solution with unit difference in pH is obtained (i.e. around 7, 8, 9, 10, 11, 12). Top up to 50 mL. Use the literature values of excitation and emission wavelengths for fluorescein, measure the fluorescent intensities of these solutions.

Q: Plot fluorescent intensities versus pH.
Q. Discuss the shape of the curve and the chemistry involved.

Technical notes on the use of the Spectrofluorometer

1. Turn on the lamp power supply (LPS-220). Wait for 15 sec or so, then press the "Ignite" button. (You can confirm whether the lamp is on by observing the bluish white light leaking from a hole on top of the lamp housing. You may wait for sometime and adjust the power to 75 W.
2. Turn on the Motor Drive Unit (MD-5020). You may need to turn the PMT power on and then wait for sometime and adjust the PMT voltage to 1000 V.
3. Then turn on the computer and monitor.
4. Run the instrument program "FeliX".
5. Run Acquire|Emission Scan. You may be asked to confirm the monochromator wavelength positions. Read the two numbers from the instrument and input them in the computer.
6. For an emission scan, put in the excitation wavelength (as obtained from the UV-Visible spectrophotometer), and the range (i.e. start and end) of the emission wavelengths.
7. Press "Acquire" to allow the communication between the computer and the instrument to establish.
8. Make sure the cuvette with the sample is in place. Press "Start" to see a profile of counts/sec versus wavelengths to be plotted in real-time.
9. After the scan in completed, save the file by doing File|save. Save the data in the *.txt format, and transfer the file to Excel for data manipulation and plotting.
10. You may close the file by doing File|Close.
11. To do an excitation scan, put in the emission wavelength (as obtained from the emission scan), and the range (i.e. start and end) of the excitation wavelengths. Repeat 7 and 8.
12. Repeat 9 and 10 to save your data.
13. After obtaining the optimal excitation and emission wavelengths, you do not need to do scans. You will perform various measurements using the time-based run. Select Acquire|Timebased. Put in the emission and excitation wavelengths. Put in a duration of, say, 30 sec. Press "Start" and then press "Acquire" to begin time-based measurement.
14. After the timebased run is completed, drag a window over the 30 sec period, and perform Math|Average.
15. After all experiments and make sure all data are OK, turn off the computer, then the Motor Drive, then the Lamp power supply.

Technical notes on the use of the UV-Visible Spectrophotometer

1. Turn on the power to the instrument HP8453.
2. Turn on the computer and monitor.
3. Run the instrument program "UV-vis HP8453"
4. Wait for about 30 s for the program initialization.
5. Perform a spectral scan by doing Method|Spectrum/peaks. Put in the start and stop wavelengths.
6. From the spectrum, select the wavelength at the absorption maximum.
7. To measure the absorbance of various sample solutions, do Method|Fixed wavelengths. Put in the absorption wavelength.
8. First put in the blank, and do Measure|Blank. Then put in various samples, and do Measure|Sample.
9. After all experiments and make sure all data are OK, turn off the computer and the instrument.

Flame Atomic Spectroscopy

Pre-Laboratory Preparation

1. Using the wavelength tables that are in the library or in the lab, obtain the most sensitive spectral lines for emission of the Ca atom and Ca^+ ion, and for Mg atom and Mg^+ ion.
Hints: (a) CaI: indicates a transition from the excited state to the ground state of a neutral gas phase calcium atom for emission, or vice versa for an absorption.
(b) CaII: indicates a transition from the excited state to the ground state of a singly charged calcium ion (Ca^+) for emission, or vice versa for an absorption.
2. Read the method for the determination of detection limit.
3. Review the external standard and standard addition methods.
4. Determine how to prepare the Ca and Mg solutions.
5. Determine how to prepare the Ca and Mg solutions containing various amounts of additives. Set up a table to indicate the amounts of various added components in the solutions.

This experiment is comprised of 4 sections as follows:
1. Instrumental settings (Nebulization efficiency and Burner height)
2. Wavelength scan, atomic emission and absorption
3. Chemical Interferences
4. Detection limit, external standard versus standard addition

Apparatus

9. Perkin Elmer 1100B Atomic Absorption Spectrophotometer
10. Calcium and magnesium hollow cathode lamps.
11. Sixteen 100-mL flasks
12. Five 50-mL flasks
13. Two 250-mL flasks
14. Two 100-mL plastic bottles
15. One 100-mL measuring cylinder

Chemicals

6. 1,000 ppm (or μg/mL) solutions of Ca, Mg
7. 10,000 ppm solutions of K, Sr
8. 0.1 M EDTA
16. 1 M H_3PO_4

References:

Chapters 6, 8, 9, and 10 in Skoog/Holler/Nieman, Principles of Instrumental Analysis, 5^{th} edition, 1998, Saunders College Publishing, Florida

Or chapters 4, 5, 6, 10, and 11 from Skoog/Leary, Principles of Instrumental Analysis, 4 th edition, 1992, Saunders College Publishing, Florida

Procedures:

1. Instrumental settings (one 250-mL flask, two plastic bottles)
a) Nebulization efficiency

Prepare a 10 ppm Ca solution (250 mL). Put in a measured amount (100 mL) of 10 ppm Ca solution in the plastic sample bottle. The put in a measured amount of water (50 mL) in a plastic drain container (Why plastic?). Insert the sample and drain tubes in the respective bottles. Light the flame. Let the aspiration and nebulization go on for certain time. Shut off the flame. Then measure sample and drain container volumes again. Hence determine the nebulization efficiency.

(The sample uptake-rate could be adjusted by turning the red knob on the nebulizer, But it is NOT necessary to adjust the sample uptake rate here.)

Q. Calculate the rate of delivery of Ca atoms to the flame per second.

b) Burner height

Set the wavelength at the value for CaI emission.

Aspirate the 10-ppm Ca solution, set the full range of the detector by pressing “gain”.

Then turn the flame OFF and set the zero point by pressing “autozero”.

Relight the flame, but do NOT reset autozero or gain until this section is completed.

The signal intensity fluctuates rapidly about the mean. You should record at least five readings of the signal intensity and calculate the mean and standard deviation of the five readings. The standard deviation is considered as the noise.

For each height setting, measure the emission intensity for the blank (background) and the 10 ppm Ca (signal). Repeat this experiment for a total of 5 height measurements. Measure the height using the coarse graduated scale located above the flame-burner-assembly height adjustment knob.

Note the nature of the flame while aspirating the 10 ppm solution of Ca. Record your observations.

Q: Determine the signal-to-noise ratio (S/N) and signal-to-background (S/B) ratios of a CaI emission line as a function of viewing (or burner) height.

Q: Plot S/B and S/N as a function of viewing height.

Q. Is the maximum S/N for Ca, as a function of viewing height, found at the same viewing height as the maximum S/B ratio? Why?

Q. Explain this finding based on your knowledge of various zones in a flame.

2.Wavelength scan, atomic emission and absorption (Two 100-mL flasks)

Prepare 10-ppm of Mg and Ca solutions (100 mL).

Perform the wavelength scan (±10 nm) to identify the spectral peak maxima for (a) CaI, (b) MgI, and (c) CaOH emissions. Use a scan rate of 5 nm/min.

Q: Note the wavelength at which a spectral peak occurs in each case. After manually recording the data, sketch each wavelength scan. (Sorry, there is no printer)

Q. Take a note of the slit width used in the monochromator.

Q. If there is a spectral interference in the bandpass region of analytical interest, suggest some appropriate actions that could be taken to eliminate the spectral interference.

Set the burner height to the position of maximum S/B for the CaI emission as determined in (1).

Use the appropriate solutions, measure the signals (no scans) at the appropriate wavelength for the following emission lines: (a) CaI, (b) CaII, (c) MgI, and (d) MgII.

Turn on the hollow cathode lamps for Mg/Ca. In the continuous mode, measure the signals at the appropriate wavelength of the CaI absorption line.

Repeat with the measurement of the MgI absorption line.

Q: Compare the intensities for the atomic and ionic emission lines for Ca. Do the same for Mg.

Q: Compare the signals measured in emission and absorption for Ca. Repeat for Mg.
Q: What modes (emission or absorption) and transition (atomic or ionic) provide the best measured signals for Ca, and for Mg?

3.Chemical Interferences (nine 100-mL flasks)
(a) Ionization equilibria:
Prepare 10-ppm Ca solutions (100 mL) which contain (a) no K, (b) 10 ppm K and (c) 1000 ppm K. Measure CaI emission of these solutions.

Repeat with 10-ppm Mg solutions (100 mL) which contain (a) no K, (b) 10 ppm K and (c) 1000 ppm K. Measure MgI absorption with these solutions.

Q: How does the presence of K affect the Ca and Mg signals, if at all, in an air-acetylene flame?
Q. What happens if a hotter flame, such as a nitrous-oxide/acetylene flame, is used?

(b) Formation of low volatility compounds:

Prepare the solutions (100 mL) containing 10-ppm Ca and the following substances. Measure the emission intensities at the CaI line of the following Ca solutions which contain
(i) no additives, (ii) 0.01 M H_3PO_4, (ii) 0.01 M H_3PO_4 + 1,000 ppm Sr^{2+}
(iii) 0.01 M H_3PO_4 + 0.05 M EDTA

Q: What kind of interference does H_3PO_4 produce? Describe the chemistry.
Q: Explain the chemistry of the use of a releasing agent: Sr^{2+}.
Q: Explain the chemistry of the use of a protective agent: EDTA.

9. Detection limit, external standard versus standard addition (one 250-mL flask, five 100-mL flask and five 50-mL flasks)

Prepare 100-mL Ca solutions of several concentrations (i.e. 10, 5, 1, 0.1 ppm or lower). Prepare 250 mL of an "unknown" solution containing 10-ppm Ca and 0.01 M H_3PO_4.
With the lamp off, measure the intensity of various Ca solutions using the CaI emission line.
Measure the emission intensity five times.
With the instrument settings unchanged, measure the emission intensity of the "unknown" three times.

Q. How do you estimate detection limits?
Q. Plot the calibration curve and determine the detection limit of Ca
Q: determine the amount of Ca in the unknown solution, plus its associated uncertainty.

To a 25.0-mL aliquot of the unknown, add 0.2 mL of the 1000-ppm Ca solution. Then make up a final volume of 50.0 mL using distilled water. This will increase the Ca concentration of the unknown by 4 ppm. Repeat, with 25.0-mL aliquots of the unknown and the addition of 0, 0.4, 0.6 and 0.8 of the 1000-ppm Ca solution. Measure the atomic emission of these calibration standards.

Q: Calculate the concentration of Ca added to each calibration standard.
Q: Construct the standard addition calibration curve.
Q: Use linear regression, determine the concentration of Ca in the unknown and estimate the uncertainty. Note that the formulae for estimation of uncertainty are different in the two cases of external standard and standard addition. Set up the spreadsheet while you are waiting for the results.
Q: Compare the advantages and disadvantages of the two calibration methods.

Technical notes on atomic spectroscopy

If the main power switch on the Spectrophotometer has not been turned on, do so now.
Examine the following sections:

Exhaust Hood- ensure that it is operating
Hollow Cathode Lamp (HCL) Turret- a quad mount
Hollow Cathode Lamp Focusing Lenses (between the HCL and the monochromator)
Nebulizer/Spray Chamber/Slot Burner Assembly
Sample Introduction Tube
Drain tube and the reservoir for the waste liquid that drains from the spray chamber
Oxidant and Fuel Lines that lead to the burner head.
Computer Control Console and the attached key pad.
Compressed Gas Cylinder containing the fuel, acetylene.
Compressed Air Line bringing the oxidant to the spectrophotometer.

On the Control Computer Console, the white keys on the keyboard have the following functions:

ELEMent SELECT- select an element from the table for analysis, the number that is entered corresponds to the atomic number of the element

PROGram- used to define the measurement mode (absorption or emission) for the experiment,

also used to program the instrument to calculate an analytical working curve from a set of calibration standards)

SETUP- this algorithm is used to set up various instrument parameters

In the emission mode, the bar graph indicates the intensity of the analyte emission

In the absorption mode, the bar graphs display the HCL signal intensity and the signal of the Deuterium Lamp that is used for Background Correction

CONTinuous- the algorithm displays a line graph of the signal (either absorption or emission) as a function of time

USER INDEX- this algorithm is useful for performing routine analyses; the instrument will display the results of an elemental analysis for a set of samples by using stored working curves to calculate the analyte concentrations in the sample

WAVE1SCAN- When used in the CONT mode, it allows the operator to perform a wavelength scan

RUN- execute a programmed analysis on a set of samples

GAIN- used in conjunction with the SETUP menu screen

Depressing the gain key in the setup mode causes the photomultiplier tube (PMT) high voltage to be adjusted to the optimum range and the bar graph is set to a mid-range value. You can also manually set the high voltage, V, supplied to the PMT by pressing a voltage value (from 1 to 990) and then pressing gain. The screen displays an energy value according to the following formula:

$V = 1000 - (10 \times \text{Energy})$ (i.e. Energy = 1 represents 990 V)

ATOMizer CONTRol- displays a menu screen on the bottom of the monitor that allows the fuel and oxidant mixtures of the gases being delivered to the burner assembly

SAMPLE SEQuence- an algorithm that is used to define the order of quantitative analysis for a set of samples

Operation of the Spectrophotometer

A. Lighting the Flame

A.1 Turn the orange handled to open the air valve. This valve is located on the copper piping downstream from the regulator that is used to set the delivery pressure of air to the spectrophotometer.

The delivery pressure of air should be ~ 58 psi.

A.2 Turn the lever on the gas regulator that is connected to the acetylene cylinder counter-clockwise until it rotates freely. Then slowly open the main valve that is attached to the top of the cylinder. (Opening the main valve on the acetylene cylinder with the gas regulator second stage at a preset delivery pressure could damage the gas regulator.)

If the pressure in the cylinder of acetylene is below ~180 psi, inform your laboratory instructor.

Reset the delivery pressure on the second stage regulator by turning the lever clockwise to a pressure of 14 psi. Open the line on/off valve (located on the gas line that delivers acetylene to the spectrophotometer from the regulator) by turning two complete turns counter-clockwise from the closed position.

A.3 Press the white key ATOM CONTRol and then press the gray key that activates the CHECK GASES subroutine.

Press the gray key CHECK F & O to check the flow rate and pressure of the flame fuel and oxidant.

If the spectrophotometer flashes the warning that "Fuel Pressure is to Low", again press the gray key CHECK F & O and check the delivery pressure of the acetylene at the second stage of the regulator. If necessary, adjust the delivery pressure to read 13 psi when fuel valve on the spectrophotometer is open.

Recheck the fuel and oxidant delivery flows and pressure. If there is still a fuel or oxidant delivery problem to the flame-burner-assembly, inform your laboratory instructor.

A.4 Igniting the fuel/oxidant mixture.

Get a match ready, but do not light it at this time.

Depress the red key FLAME ON/OFF button. You will immediately hear an audible click as the oxidant solenoid valve opens. The oxidant is now flowing to the flame-burner-assembly.
Light the match. After about a four second delay, a second solenoid will open with an audible click. Fuel is now flowing to the flame-burner-assembly. Move the burning match along the top surface of the burner from the edge towards the slot in the burner to ignite the fuel/oxidant mixture.

If the fuel/oxidant mixture does not light, allow the spectrophotometer to automatically shut off the gas flow before repeating this section.

If a flame is ignited, deposit the match in the glass jar located on the bench to the right of the spectrophotometer.

Once the flame has been ignited, CHECK under PROGram to ensure that the oxidant and fuel flows are
Fuel 2.5 l/min. Oxidant 8 l/min.

IF THE ACTUAL FLOWS RATES ARE DIFFERENT FROM THESE VALUES, ASK YOUR LABORATORY DEMONSTRATOR TO RESET THESE FLOWS TO THE PROPER SETTINGS.

A.5 Ensure that the nebulizer is not plugged by placing the teflon-capillary tubing into a flask of fresh distilled water. You should be able to see the water being drawn through the tubing by repeatedly dipping the tubing in and out of the liquid and watching the air bubble thus formed being crawn through the capillary tubing.

A.6 If you want to align the Hollow Cathode Lamp now, shut off the flame by pressing the red key FLAME ON/OFF.

B.1 <u>Alignment of a Hollow Cathode Lamp (HCL)</u>

Turn the Flame Off before aligning the lamp.

B.2 Insert the Hollow Cathode Lamp into one of the four mounts on the quad-turret. Plug the electrical lead of the Hollow Cathode Lamp into the socket that is labeled HCL1- please take care not to bend the electrical leads on the Hollow Cathode Lamp.

B.3 Press the white key PROGram. Select Emission mode of operating the Spectrophotometer using the gray key.

B.4 Press the white key ELEMent SELECT. Select Lamp 1 as being active using the gray key.

B.5 Press the white key SETUP. Activate the Lamp Current by pressing the gray key. Set the lamp current to 5 mA.

B.5 If the signal intensity of the Hollow Cathode Emission is off scale, press the white key GAIN.
Adjust the orientation of the Hollow Cathode Lamp using the two plastic set screws that are at the left end of the quad turret- optimize the Hollow Cathode Lamp signal intensity by adjusting (up/down) the plastic set screw that is located nearest the flame-burner-assembly first, then further optimize the emission signal intensity of the Hollow Cathode lamp by adjusting (tilt) the plastic set screw that is located furthest away from the flame-burner-assembly.

B.6 Press the gray key to activate the Wavelength subroutine. When the WAVELENGTH icon on the screen stops flashing, you may continue.

B.7 Repeat steps B.5 through B.6. These steps are repeated to arrive at the best positioning on the HCL. In addition, because the grating in the monochromator is driven by gears. Mechanical meshing of gears causes slight inaccuracies in the movement of the grating. Therefore, the wavelength on the screen of the monitor is likely not the actual wavelength of the monochromator, but a wavelength scan around the desired wavelength will allow you to find the actual wavelength. Keep this point in mind at all times.

B.8 If you are going to do emission study now, turn the Lamp Off.

C. Setting up the Instrument for an EMISSION Study

C.1 REPEAT STEP B TO SET THE MONOCHROMATOR TO THE WAVELENGTH OF INTEREST (for a neutral atom emission/absorption line).

C.2 Light the Flame.

C.3 The first screen displayed will show a Table of all of the elements that are pre-programmed. This screen can always be recalled using the white key ELEMent SELECT.

Select the element of interest (Calcium). This will set the monochromator to the default wavelength for CaI. To set other wavelengths, press the numbers and then press wavelength.

(Ensure that the Hollow Cathode Lamp Current is zero.)

C.4 Press the white key PROGram.

Select the mode to EMISSION using the gray key.

C.5 Adjustment of gain, zero and wavelength

It is useful to initially adjust the position of the slot in the burner-head of the flame-burner-assembly to the center of the exit lens leading to the monochromator.

i) Place the sample uptake capillary tubing in the reservoir containing distilled water (the blank).
ii) Press the white key SETUP to recall the bar-graph signal intensity display on the screen.
iii) Press the white key GAIN. This will cause the signal intensity of the Blank to read 50 % of full scale.
iv) Aspirate the 10 ppm Ca solution.
v) If the signal intensity for the 10 ppm Ca solution is off scale, again press the white key GAIN. This will cause spectrophotometer to adjust the signal intensity for the 10 ppm Ca solution to read 50 % of full scale.
vi) Now re-aspirate the Blank, the signal intensity of this solution should be very low on the bar graph display. (What is important at this point is that there should be a noticeable difference in the bar graph signal intensity of the Blank versus the 10 ppm Ca solution.)
vii) Press the white key CONTinuous to activate the signal trace Signal Intensity versus Time (seconds).
Determine the signal intensity of the Blank by aspirating the distilledwater solution. Press the gray key ZERO.
viii) Aspirate the solution of ~ 10 ppm Ca. Optimize the lateral and transverse position of the flame-burner-assembly for maximum signal intensity.

If the signal level is saturated (either too low or too high and noticeable by the flat AND straight signal intensity trace), the GAIN of the output electronics needs to be adjusted. Press the white key GAIN. Once the signal intensity of the Ca has stabilized, re-aspirate the Blank to ensure that the signal you are trying to optimize is a Ca emission signal and press the white key ZERO.

ix) Optimize the height of the burner.

x) Press the white key SETUP. Press the gray key WAVELENGTH while aspirating the 10 ppm Ca solution.

C.6 In order to ensure that there are no spectral interferences present, we do the following steps:

i) While in the continuous mode, press the white key WAVE1SCAN.

Scan the spectrometer over the wavelength of interest while aspirating a blank AND the test solution to ensure that there are no interferences.

Scan the wavelength of the spectrometer automatically (being sure to select the wavelength-scan rate such that the signal intensity values can be manually recorded in real time.

IT IS DECEPTIVELY EASY TO SET THE MONOCHROMATOR ONTO A BACKGROUND EMISSION STRUCTURE FROM THE FLAME, AND NOT ON AN ANALYTICAL TRANSITION. How can you be sure that you set the monochromator to view the analytical transition and not a structural background feature of the flame itself ?

C.7 To shut down in this mode, turn the flame OFF by pressing the red key FLAME ON/OFF.

D <u>Setting up the spectrophotometer for an atomic absorption study.</u>

D.1 Align the Hollow Cathode Lamp, section B and

Optimize the flame-burner-assembly position, section C.

D.2 Re-light the Flame, section A.

Press the white key PROGram. Select the mode to Absorption using the gray key.

Set the Hollow Cathode Lamp Current to its normal operating range

D.3 While aspirating the Blank (i.e. absorbance is zero), press AUTOZERO and then the white key GAIN.

D.4 To shut down in this mode, turn the Hollow Cathode Lamp Current to ZERO and turn the Flame OFF.

E. Shutting down the Perkin-Elmer 1100B Spectrophotometer.

i) Shut off the Hollow Cathode Lamp current.

ii) Prepare a solution that is ~ 5 % nitric acid in distilled water. Aspirate this solution for approximately 10 minutes. Then aspirate distilled water for 5 to 10 minutes.

iii) Shut the Flame off.

iv) Turn the main valve OFF on the acetylene tank. Also, turn the lever on the gas regulator that is connected to the cylinder of acetylene counter-clockwise until it rotates freely.

Shut off the air supply (orange handled valve).

v) Press the white key ATOM CONTROl and then press the gray key that activates the CHECK GASES subroutine.

Press the gray key CHECK F & O once to bleed the pressure of air and acetylene within the lines leading to the spectrophotometer.

vi) Turn the small valve on the acetylene line leading to the spectrophotometer to OFF.

vi) Turn the main power switch of the spectrophotometer to off.

Gas Chromatography Laboratory

Prelab preparations

Check the chemical structures and boiling points of benzene, toluene, ethyl benzene, and xylenes
Obtain the formulae to calculate N, H, and k' of an eluted species.
Review Van Deemter equation and the method of internal standard.

Overview
BTEX is a commonly used abbreviation for the compounds benzene, toluene, ethyl benzene, and xylenes. BTEX is used as an octane booster as it has an octane number greater than 100. BTEX is the component which is responsible for the majority of the toxicity of unleaded gasoline. Moreover, the relatively high water solubility of the BTEX components relative to the aliphatics in gasoline leads to contamination of ground waters whenever there is a gasoline spill.

This experiment is comprised of 3 sections as follows:
1) To study the effect of column gas flow rate on the isothermal separation of the BTEX mixture.
2) To study the effect of column temperature on the separation of a BTEX mixture.
3) To perform quantitative measurement of BTEX components in a 94-octane gasoline by the method of internal standard.

Materials

Students are provided with the following standards.
(a) Individual standard solution of benzene, toluene, ethylbenzene and xylenes in hexane at a concentration of 1000 ppm. Each contains 1,000 ppm bromobenzene.
(b) 4 calibration standards of a mixture of benzene, toluene, ethylbenzene and xylenes (BTEX) in hexane at a level of 100, 500, 1000 and 2000 ppm (μL/L).
The total concentration of xylenes, consisting of a mixture of three isomers, is a factor of three times higher. There are three structural isomers in the xylene sample, two of which cannot be resolved from one another. Each standard mixture also contains 1,000 ppm bromobenzene as the internal standard.
(c) A 94-octane gasoline sample is prepared in 10,000 ppm by volume in hexane. This sample also contains bromobenzene as the internal standard at 1,000 ppm.

Hexane is very volatile. Keep all solution vials tightly closed when not in use.

References:
Review chapters 26 & 27 of Skoog/Holler/Nieman, Principles of Instrumental Anlaysis, (5^{th} edition, 1998, Saunders College Publishing, Florida) for theory of chromatography and GC.
Review Chapter 5 of Harris, Qualitative Chemical Analysis, (5^{th} Ed., Freeman, 1999) for the internal standard procedure.

The journal article titled Injection Techniques in Capillary GC, Anal. Chem., 1994, 66(20), 1009A-1019A contains examples of significant errors that can arise at the sample injection step.

Additional information can be found in Gas Chromatography, A Practical Approach, P. J. Baugh, ed., 1993, Oxford University Press. This book covers the quantitative principles of chromatography quite well.

PROCEDURE

(1) Effect of column gas flow rate on isothermal GC separation of BTEX compounds (Van Deemter equation)

Run a sample of hexanes. Select an oven temperature of 55 °C and linear gas velocity of 30 cm/s for the run.

Meanwhile, prepare and run a 1,000 ppm standard of benzene, a 1,000 ppm standard of toluene, a 1,000 ppm standard of ethyl benzene, and a 1,000 ppm standard of the xylene isomers. Also run a sample of bromobenzene, because this compound is used as the internal standard later in this experiment. Run a 1000 ppm BTEX mixture.

Q: In this experiment, how do you identify which chromtographic peak corresponds to which compound? Suggest one method that is more reliable than this?
Q: Based on the chemical structures and boiling points of these four analytes, and knowing the general structure of the stationary phase, explain the elution order of these compounds.

Now, run the sample (1,000 ppm BTEX standard) isothermally at 55 °C at carrier gas linear velocities of 15, 20, 25, 40 and 50 cm/s.

Q: Calculate number of plates k', N and H for one compound (e.g. the component with the median k') for all runs.
Q: Perform the Van Deemter plot and find out the optimal values of H and v.
Q: Comment on the separation of compounds, particularly the (o, p, and m-) xylene isomers.

(2) Effect of column temperature and temperature programming

Set the carrier gas linear velocity to the value calculated in (1).

(a) Effect of column temperature on the isothermal GC separation of BTEX mixture.

Run a 1,000 ppm BTEX standard mixture isothermally at 35, 55 and 75 °C at the optimal carrier gas linear velocity, or 30 cm/s.

Q: Evaluate the effect of column temperature on separation, resolution, and analysis time in the light of R_s and k' values. What compounds should you selected for this evaluation?
Q: Does the elution order change at different temperatures? Confirm the identities of uncertain peaks, if any, by injecting individual standards.

(b) Effect of column temperature programming on the GC separation of the BTEX mixture

Run the 1,000 ppm BTEX standard at the optimal carrier gas initial linear velocity or 30 cm/s using a linear temperature ramp from 35 °C to 75 °C at 20 °C per minute at constant flow.

Q: Discuss the effect of column temperature programming on separation, resolution and analysis time in the light of R_s and k' values.
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(3) Quantitative analysis of BTEX-components in a gasoline sample

Run the gasoline sample that has already been spiked with the internal standard bromobenzene at 1,000 ppm. Establish a four level calibration (100 to 2,000 ppm) of BTEX standards for the following GC conditions: carrier gas initial linear velocity of 30 cm/s , and temperature program from 35 °C to 75 °C at constant flow.

Q: Calculate the peak area ratios for all individual components in BTEX using the method of internal standards.
Q: Perform linear regression analysis and determine the amount of each BTEX component in the gasoline sample. Estimate the uncertainty associated with your determination. Estimate the detection limit of your calibration.

Notes on the use of the GC Equipment (HP 5890)

1. Overview

CARRIER GAS SUPPLY

Helium is used as a carrier gas. Set at 30 psi during operation, 15 psi at shutdown. This gas can be supplied to the column in two ways: (a) constant pressure mode or (b) constant mass flow mode, see pull-down menu: Instrument|Inlet Pressure Program.

In the constant pressure mode, the pressure of the gas supply is keyed in and the value is maintained constant during an entire chromatographic run. To achieve a constant mass flow mode (initially set at a specific oven temperature), check the box for it, and put in the pressure of the gas supply which will change if the oven temperature changes.

(Optional) The flow rate can be measured using a soap bubble flowmeter. It should be suitable for measuring both low flow rates (e.g. carrier gas) and high flow rates (e.g. air for an FID)

SAMPLE INJECTOR

The injector consists of a heated glass sleeve inside a heated chamber. The top of the injector is sealed with a silicone septum. A small flow (~ 2 mL/min) of a carrier gas is directed across the top of an injector to sweep out any contaminates that might be present due to a puncture in the septum and/or air entering the injector volume via the septum. This gas is vented through the septum purge outlet. The bottom of the injector is connected to the column and the split vent. (See Fig. 1)

Sample injections can be made in the

(a) Split mode

(b) Splitless mode

In this experiment, the split mode is used. The method of split injection allows only a fraction of the total vaporized sample to enter the column. All sample vapor not injected onto the column is vented. You will set the split ratio by keying in the split flow rate under the pull-down menu: Instrument|Inlet Pressure Program.

(Optional) procedure to adjust the split ratio with the help of a soap bubble flowmeter.

1. Set He gas pressure to 30 psi at the second stage regulator.
2. Press “Purge/Valve” then “A” to display current split vent status.
3. If “OFF” is displayed, press “ON” to restore split flow through the inlet insert.
4. Adjust the total flow by turning the total flow control valve above the split vent.

Good sample injection procedures are (See Fig 2):

1. Fill the 10-μL syringe (note that a GC syringe needle is pointed) with sample and empty it to the waste about 3 times to clean the syringe.
2. Immerse the needle into the sample, and if necessary, pump the syringe by slowly aspirating and rapidly expelling the solvent to remove any air bubbles.
3. Fill the syringe to the 1-μL mark
4. Retract the plunger and pull about 4 μL of air into the syringe. NOTE the volume of sample ACTUALLY loaded into the syringe.
5. Make sure the ready signal is on. Insert the syringe needle into the injection port, depress the plunger quickly and withdraw syringe immediately. Press the “Start” button on the instrument. Do not bend the needle or the plunger!

OVEN

If the oven door is opened, the instrument will be shut off for safety reasons.

The oven remains off until switched on again by pressing “oven temp” and then “on”.

COLUMN

An HP-1 capillary column with the following dimensions is installed in the GC;
length 25 m, internal diameter of the capillary is 0.32 mm, thickness of the stationary phase (dimethylpolysiloxane) is 0.17 μm.

DETECTOR

A flame ionization detector (FID) is installed in the GC. The FID detector is supplied with three flows of gas;

H_2 ~ 30 mL/min. (~ 15 psi)
Air ~400 mL/min. (~ 34 psi)
He ~30 mL/min. (~ 30 psi)
These values are adequate for most analyses and will not be varied during this exercise.

2. GC START-UP PROCEDURE:

Because the GC requires a warm-up time of a couple of hours, the following parameters have been programmed into the central processing unit (CPU) of the GC.

Injector temperature 250 ºC
Detector temperature 250 ºC
Maximum OVEN Temperature 300 ºC

The GC METHODS for this assignment are stored in the directory (C:\HP) on the hard drive of the control computer.

Create your own subdirectory in the directory for storage of your data, giving each datafile a name unique to you and your partner under the pull-down menu:RunContr|Sample Info.

SLOWLY open the main cylinder valves for H_2 and air. The two regulators should display 15 psi and 34 psi, respectively.

Open the hydrogen, air and helium makeup valves to the FID. DO NOT FORCE THE NEEDLE VALVES, THEY ARE DELICATE.

The various flows of gases should have been adjusted as follows:
(column + makeup) to 30 mL/min.
(column + makeup + H_2) to 60 mL/min when air was off, i.e. flow of H_2 is 30 mL/min.
(column + makeup + air) to 430 mL/min when H_2 was off, i.e. flow of air is 400 mL/min.
FID are designed to operate best with a carrier flow rate of at least 20 mL/min, typical of packed column applications. Carrier flow rates less than 10 mL/min (typical capillary column applications) require capillary makeup gas to ensure a total flow rate (carrier + makeup) of at least 20 mL/min. Makeup gas is added directly to H_2 within the detector flow manifold.
FID sensitivity depends on the ratio of H_2 to carrier gas flow (or carrier + makeup gas for capillary columns). Moreover, if sample components are in high concentration, increased air flow may be necessary (up to 650 mL/min). If sample components are in low concentrations, reduced air flow rates are acceptable (375 to 425 mL/min)

Turn on the detector by pressing “DET” then “ON”. Press the "sig1" button to display the FID current. (The current should be around 1 pA).

Ignite the FID by depressing the ignite button for ~1 sec. You should hear a faint "pop" as the flame is lit, and the current should rapidly increase to ~ 15 pA and then remain fairly constant. If the current is still around 1 pA, then the FID is not on. (Waving your hand overtop the exit port of the FID while attempting to light the FID assists in the ignition of this detector.)

You may also test for ignition by holding a cold, shiny surface (e.g. a chrome-plated wrench) over the detector exit. Steady condensation indicates the flame is lit.

The red RUN LED:
On indicates a run is actively in progress
Off indicates no run is currently in progress
Blinking indicates it will be ready soon

The green NOT READY LED
On indicates one or more parts of the system are not ready. Press “clear” to display what is not ready
Off indicates the instrument is ready for a run
Blinking indicates hardware fault. Press “clear” to display fault messages.

2. Software

a. Put in run sample info and filename before each run. Or new data will overwrite a previously file.
b. During a GC run, in the DataAnalysis|Specify report menu, check the box for Report to Screen
c. After the run, to print, uncheck the box for Report to Screen in the DataAnalysis|Specify report menu. Then check the box for combined Chromatogram and Report. The select Print Report.
d. If some peaks do not get integrated, modify the settings of integration|integration events for initial area reject (to a lower value), initial peak width (narrower) and initial threshold (lower).

4. Shutdown procedure

Press: "oven temp" and then "off" to switch off the oven heater.
Close the flow of H_2, Air and He to the FID. Again, please do not force the delicate needle valves.
Close the main cylinder valves on the H_2 and Air tanks. Do not shut off the flow of He (Why?). Reduce the flow to about 15 psi to save He.

Liquid Chromatography Laboratory

Prelab Preparations

1. Find the chemical structures for the chemical compounds that we use in this lab. Find their dipole moments or predict their order from the chemical structures.
2. Find the polarity index of water, acetonitrile and methanol and the formula to evaluate the polarity index of a binary solvent mixture in reversed phase HPLC.
3. Review the standard addition and internal standard procedures and learn how to combine them.
4. Determine how to prepare a test mixture (100 mL) containing 25 ppm of each compound
5. Do necessary calculations and prepare a table for the preparation of the various solutions for performing standard additions.

Overview

This experiment is comprised of 2 sections as follows:
1. To study the effect of mobile phase composition on isocratic separation and determine a gradient elution program for the best performance
2. To perform quantitative analysis of an unknown using internal standard and standard addition method

Note that in HPLC separations, the first solvent mixture attempted should be acetonitrile and water. Acetonitrile has a low viscosity that allows a relatively low operating pressure, and it permits UV detection down to 190 nm. Methanol is the second choice for organic solvent because it has a higher viscosity and longer UV wavelength cutoff.

Materials
- HPLC-grade acetonitrile and methanol.
- An acidic aqueous buffer (contains K_2HPO_4 adjusted by H_3PO_4 to pH 3.10)
- solutions of 100-ppm of each of the 4 compounds (phthalic acid, salicylic acid benzoic acid and p-nitrophenyl acetic acid)
- seven 50-mL volumetric flasks
- four 10-mL graduated pipette

References

Review chapters 26 & 28 of Skoog/Holler/Nieman, Principles of Instrumental Anlaysis, 5th edition, 1998, Saunders College Publishing, Florida for theory of chromatography and HPLC.
Review Chapter 5 of Harris, Qualitative Chemical Analysis, 5th Ed., Freeman, 1999 for the standard addition procedure.

A discussion of columns suitable for high backing pressure is provided by Halasz, I. Columns for Reversed Phase Liquid Chromatography, Anal. Chem., 1980, 52 (13), 1393A.

A detailed report on column optmization is provided by Glajch, J. L.; Kirkland, J. J. Optimization of Selectivity in Liquid Chromatography, Anal. Chem., 1983, 55(2), 319A-336A.

Procedures

(1) Effect of mobile phase composition on the separation using a water/acetonitrile mobile phase (one 50-mL flask)

Prepare a test mixture (50 mL) containing 25 ppm of each compound.

(a) Isocratic separation:

Run the test mixture at 1.5 mL/min using mobile phase: water/acetonitrile in the ratios of 60:40, 70:30, 80:20, and 90:10. Identify the elution order using individual standard solutions.

Q. Calculate the retention factor, k', for all compounds.
Q: Calculate the resolution, R_s, of the separation of two closely eluted compounds in each chromatographic run.
Q: Determine the water/acetonitrile blend that yields the best compromise between retention time and resolution.
Q. Has the elution order of the solutes changed when the mobile phase composition changes? Explain in terms of the change in mobile phase polarity.
Q. Calculate the polarity index of this optimized solvent blend.
Q: If methanol would be used instead of acetonitrile, calculate the solvent composition of water/methanol required to achieve the same polarity index as calculated above.

(b) Gradient elution

With the knowledge of the isocratic separations using various water/acetonitrile compositions, perform a gradient elution program to achieve the best separation resolution of the solutes in the shortest time.

Q. Discuss, with calculations, the resolution obtained with isocratic versus gradient elution for the separation of the solutes in test mixture.

(2) Quantitative analysis of an "unknown" sample by standard addition method (six 50-mL flasks)

In any chemical analysis, there are possibly some changes in analytical signal due to the presence of interferences or the matrix. This is called interference or, in general, the matrix effect. Standard addition method is well suited to minimize any errors caused by the matrix effect (as you have learnt in atomic spectroscopy).
In the case of two unresolved or partially resolved peaks in chromatography, one analyte can be considered as an interferent to the other if we take the combined peak area as the analytical signal. Therefore, from your previous experiments in (1), a 60:40 composition does not result in two peaks completely resolved from each other. Imagine that you could not improve the resolution, then use this separation conditions to illustrate the concept of matrix effect and the use of standard addition to minimize errors.

Prepare an "unknown" sample (50 mL) that contains the analyte of your choice (say, p-nitrophenylacetic acid at 25 ppm) and the partially resolved substance (at 25 ppm). Any one of the other two substances (at 25 ppm) can be used as the internal standard.

Use 25 mL of your test mixture as the unknown sample, perform the standard addition method to establish the concentrations of the added analyte to be 0, 10, 20, 30 and 40 ppm, using the 100-ppm stock solution of the analyte. Remember to make up the final volume to 50 mL using distilled water.

Q: Calculate the volumes of 100-ppm analyte to be added in each of the 5 samples to produce 0, 10, 20, 30 and 40 ppm of added analyte in each case.

Then perform an HPLC run on each of these 5 samples.

Q: Select an internal standard, and calculate the peak area ratios for the 5 samples using the combined peak areas of the two overlapping peaks in each case. (Note that you will use the internal standard to minimize errors due to sample loss and the standard addition procedure to minimize errors due to matrix effect)
Q: Perform linear regression analysis and determine the amount of the analyte in the "unknown" sample. Estimate the associated uncertainty too. Set up the spreadsheet while you are waiting for the results.
Q: Comment on the advantages and disadvantages of the standard addition method.

Optional question:
Comment on the degree of tailing associated with each of the solute peaks in test mixture.

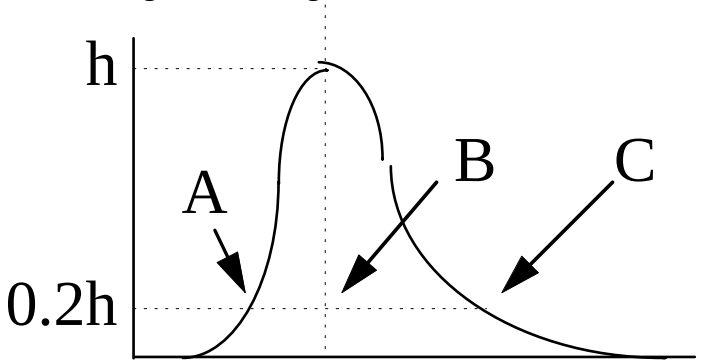

Fig. 1 Peak tailing can be defined as the length of the line segments AB relative to BC. The line segment AC is drawn parallel to the x-axis at a height equal to 0.2 x the peak maximum. Alternatively, peak tailing is defined as the width of the peak W (where W = AB + BC) divided by 2 AB.

Notes on the use of the HPLC Equipment (Series 1050)

6. Overview

PURGE GAS SUPPLY

Helium is used to purge the solvents for 15 min. Open the main valve and needle valve on the gas cylinder. Switch on purge valve control. Adjust purge rates using the knobs A, B, C and D.

SOLVENT RESERVOIRS

Bottle A contains water, bottle B contains acetonitrile, bottle C contains methanol, and bottle D contains water buffered at pH 3.1

SAMPLE INJECTOR

The injector is a 6-port rotary valve consisting of a 20-μL sample loop.

Good sample injection procedures are:

1. Fill the 0.025-mL syringe (note that a HPLC syringe needle is blunted) with sample and empty it to the waste about 3 times to clean the syringe.
2. Immerse the needle into the sample, and if necessary, pump the syringe by slowly aspirating and rapidly expelling the solvent to remove any air bubbles.
3. Fill the syringe to more than 20 μL
4. Switch to the "Load" position. Insert the needle (insert fully, you should feel some resistance) into the injection port, depress the plunger quickly.
5. Do not bend a needle or a plunger!
6. Make sure the "not ready" LED is off. Switch to the "Inject" position.

COLUMN

Spherisorb ODS 2, 125 x 4 mm, 5 μm, 80 Å pore diameter, 0.5 mL/g pore volume,
220 m^2/g surface area, 12.0% w/w C loading, pH range 2-8, maximum pressure 6000 psi

DETECTOR

An UV-absorbance detector is used. Set the wavelength at 254 nm.

2. HPLC START-UP PROCEDURE:

Turn on the switches on the detector and the pump.

SLOWLY open the main cylinder valves for He for purging.

The HPLC METHOD for this experiment is stored in the directory on the hard drive of the control computer (chem316.m).

Create your own subdirectory in the directory for storage of your data, giving each datafile a name unique to you and your partner.

3.Software control

- In RunControl}Setup Sample Info, put in filename and comments.
- In Instrument|Setup Pump flow, input flow rate and solvent composition. For isocratic separation, make sure the bottom gradient elution table is empty.
- In Instrument|Setup VWD Signal, set up wavelength to be 254 nm.
- When you are ready to load the sample, do RunControl|Run method, and watch for the "waiting for injection" message.
- Then load your sample, and inject it.