

Chem 434
Instrumental Analysis
Hour Exam III

In class portion. Do any 7 of the following 8 essay questions (10 points apiece).

1. Describe the major components of a scanning electron microscope (excluding an x-ray spectrometer) and the function of each component in image formation.

A. Column

Electron gun - Tungsten filament produces e^- for primary beam

Wehnelt cylinder - used to focus electrons at crossover point and to 'push' electrons down the column

Accelerating voltage - is the difference between the - filament and the + anode

Electromagnetic lenses - Condenser Lenses

Condenser apertures

Objective lens aperture

Objective Lenses

Stigmator coils

Scan coils

B. Detector system

Screen - I^- charged to attract II^+ electrons

Scintillator - Phosphor - transfers e^- to photons

Light pipe - carries photons

Photocathode/photomultiplier tube - converts photons to e^-

Amplifier

BSE detector

C. Vacuum System

roughing pump ~ 100 l/min to 10^{-2} torr

oil diffusion pump ~ 420 l/sec but only 10^{-2} torr or lower

2. Compare and contrast Electron-Impact and Chemical Ionization sources in molecular mass spectrometers. How are these sources the same or different in physical design, how they ionize molecules, and the kinds of ions they release for a given compound?

In an electron impact ionization source - molecular gas is exposed to an electron beam with about a 70V potential. Near collisions of electrons and molecules tend to make a high energy, positively charged ion (but the process is not very efficient, only about 1 in 1,000,000 molecules actually ionized). Ions then attracted through a series of slits and plates that focus and accelerate ions into the mass detector. Throughout this process the high energy ions collapsing, colliding and rearranging themselves in a variety of ways, so the final product is a unique spectrum of fragments, rather than a simple molecular ion.

In a chemical ionization source is designed almost identically, but a small slit is placed at the end of the ionization chamber so the chamber can be flooded with 10^3 to 10^4 more reagent gas molecules than target molecules. Here it is the reagent gases, simple compounds like methane or ammonia that are ionized, and the molecule ion is formed when these ionized reagent gases collide with the target molecule. This is a much lower energy process so the molecular ion tends to stay intact, and very little fragmentation is seen.

3. Describe how a quadrupole mass spectrometer works.

In a quadrupole mass spec the ions are ejected into a vacuum chamber that contains 4 parallel metal rods. One pair of rods, located opposite each other are positively charged, while the other pair is negatively charged. On top of the static positive and negative charges of the sets of rod, an additional +/- oscillation is also imposed on the rods. The combination of oscillating positive and negative fields makes the ions in the vacuum chamber move in a spiral pattern. At a given oscillation frequency, ions of either very large or very small mass spiral from the center and collide with the rods rather than reaching the mass detector at the end of the chamber. Thus only ions of a particular mass can be observed at a given oscillation frequency. By changing the oscillation frequency other ions of other masses are allowed to hit the detector. Thus a scan of all frequencies gives a scan of all ions in the sample.

4. List the variables that lead to zone broadening.

Linear velocity of the mobile phase

Diffusion of solute in the mobile phase

Diffusion of solute in the stationary phase

Retention factor of solute

Diameter of packing material

Thickness of coating of stationary phase on packing material

5. Name two different detectors used in gas chromatography and tell how they work. (A mass Spectrometer doesn't count)

FID - Flame Ionization Detector - Burn the column effluent in a H_2 flame. As organic compounds burn various ions are formed. These ions create an electrical current that can be detected by holding a potential of a few hundred volts between the burner tip and a second electrode located above the flame. The signal is directly proportional to the number of reduced carbons in the column effluent.

TCD - Thermal Conductivity Detector. The detector works by having two resistors in identical heated chambers, one of which is surrounded by the column effluent gas, the other is surrounded by the identical gas with an identical flow, but it has not passed through the column. When the column effluent contains other molecules, the conducts heat slightly differently so the resistor is at a slightly different temperature. The electronics are set up to detect the slightly different resistance in the effluent and non-effluent resistors, and this difference in resistance is your signal that tells when some foreign substance is coming out of the column.

6. What is a guard column and why is it used in HPLC?

It is a very small, short column containing packing material identical to the packing material used in the main HPLC column. It is placed in the liquid flow before the main column where it is used as a cheap sacrifice to protect the main column from anything that might permanently bind and clog it. As the samples are injected into the HPLC system, they come through this column first and bind to it instead of the main column. This guard column can then be removed and replaced for \$20-50 instead of replacing the entire main column for ~\$500

7. What are the Normal Phase, Reverse Phase, Ion Exchange and Size Exclusion Chromatography. What are the physical principles behind these different methods, and what properties of the molecule do they use to separate different compounds from each other.

Normal phase refers to a chromatographic system in which the stationary phase is polar and the mobile phase is non-polar. Reverse phase has a non-polar stationary phase and a polar mobile phase. Both of these methods use the relative polarity of the solute to separate it from other compounds.

In ion exchange chromatography the stationary phase has either cations or anions built into it, and then ions of the opposite charge are attracted to this matrix and retained on it as the solvent is washed by. The retained ion can be removed by the column either by manipulating the ionic strength of the medium or by changing the pH of the medium. So this method separates by ionic charge.

In Size exclusion chromatography the stationary phase is a matrix that contains pores of a set size. Molecules smaller than these pores enter into the matrix and elute slower than molecules that are larger than the pores, so they remain outside the matrix and elute faster. This method elutes by molecular weight.

8. What do the following letters mean?

EDX - Electron Dispersive X-ray

BSE - Back Scattered Electron (detector)

CI Chemical Ionization

MALDI Matrix Assisted Laser Desorption Ionization

H (in general Chromatography) Plate Height

t_R Retention Time

t_m Dead time

SCOT Support Coated Open Tubular

FID Flame Ionization Detector

TCD Thermal Conductivity Detector

Take Home questions

1. (20 points) The following data came from an HPLC separation

Length of packing: 10 cm

Flow rate: 1 ml/minute

<u>Material</u>	<u>Retention Time(min)</u>	<u>Width of Peak Base (min)</u>
non-retained	1.45	-
A	3.0	.25
B	3.5	.29
C	5.0	.42
D	6.0	.48

Calculate the number of plates for each peak?

$$\begin{aligned}N &= 16 \left(\frac{t_r}{W} \right)^2 \\&= 16 \left(\frac{3.0}{.25} \right)^2 = 2304 \\&= 16 \left(\frac{3.5}{.29} \right)^2 = 2331 \\&= 16 \left(\frac{5.0}{.42} \right)^2 = 2268 \\&= 16 \left(\frac{6.0}{.48} \right)^2 = 2500\end{aligned}$$

What is the average and standard deviation for N?

Average = 2351, standard deviation = 103
so call it 2400 +/- 100

What is the plate height for the column?

$H = L/N = 10\text{cm}/2300 = .0043\text{cm/plate} = 43\mu\text{m/plate}$

Which peaks are better resolved, A&B or C&D?

$$R_s = \frac{2[(t_r)_B - (t_r)_A]}{W_A + W_B}$$

For A&B

$$= \frac{2(3.5 - 3.0)}{.25 + .29} = 1.85$$

For C&D

$$= \frac{2(6.0 - 5.0)}{.42 + .48} = 2.22$$

For resolution the bigger number is better, so C and D are better resolved.

What is the selectivity factor for B relative to A?

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} = \frac{3.5 - 1.45}{3.0 - 1.45} = 1.32$$

2. (5 points) Calculate the Retention Index for cyclohexane based on the following GC information.

Retention time for air = .6 min.

For n-pentane = 5.6 min.

For n-hexane = 6.1 min

For Cyclohexane = 5.9 min.

Here the log of the adjusted retention time is linear with the number of carbons, and we use a system where n-pentane = 500, and n-hexane = 600

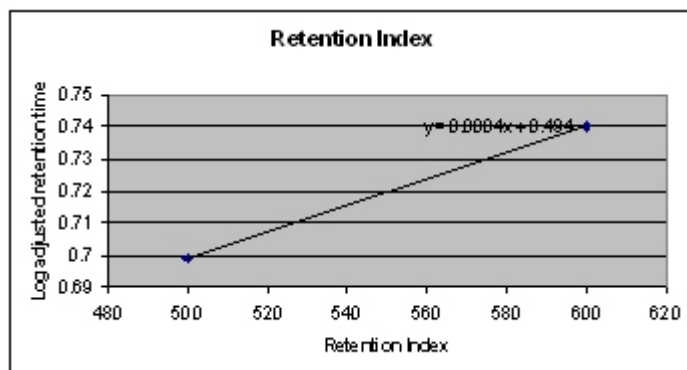
The log of the adjusted retention times are:

n-pentane = $\log(5.6-.6)=.699$

n-hexane = $\log(6.1-.6) = .740$

cyclohexane = $\log(5.9-.6)=.724$

You can plot this out to see it better



For cyclohexane $.724 = .0004X + .494$

$(.724-.494)/.0004 = 575$

Or you can simply do a linear extrapolation

$$\frac{.724 - .699}{.740 - .699} = \frac{X - 500}{600 - 500}$$

$$\frac{.025}{.041} = \frac{X - 500}{100}$$

$$.061(100) = X - 500$$

$$61 = X - 500$$

$$X = 561$$

You should have the same number with both methods, I assume the number I got from the plot is off a bit because the computer rounded the slope to a single significant figure.

3. (5 points) In the lab we used a mixture of 40% methanol and 60% water to elute caffeine. Suggest two other solvents I could have used instead of methanol, and what % of solvent and water should I mix together to get an elution at roughly the same time on the HPLC

$$k' = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right) \left(\frac{V_m}{V_m + V_s} \right) = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right)^2$$

$$k' = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right)^2$$

$$A + B = 1$$

$$A \cdot X + B \cdot Y = 1$$

$$W \cdot X + (1 - W) \cdot Y = 1$$

$$A = \frac{V_m}{V_m + V_s} \cdot X$$

$$B = \frac{V_m}{V_m + V_s} \cdot Y$$

$$k' = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right)^2$$

$$A \cdot X + B \cdot Y = 1$$

$$A \cdot X + (1 - A) \cdot Y = 1$$

$$Y = \frac{1 - A \cdot X}{1 - A}$$

$$k' = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right)^2$$

Substituting into the equation for k'

$$k' = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right)^2 \left(\frac{1 - A \cdot X}{1 - A} \right)^2$$

$$k' = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right)^2 \left(\frac{1 - A \cdot X}{1 - A} \right)^2$$

$$k' = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right)^2 \left(\frac{1 - A \cdot X}{1 - A} \right)^2$$

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$$k' = \frac{K \cdot V_s}{V_m} \left(\frac{V_m}{V_m + V_s} \right)^2 \left(\frac{1 - A \cdot X}{1 - A} \right)^2$$

Therefore, the solvent composition that would give the same retention time as 40% methanol and 60% water is 40% of the other solvent and 60% water.