

Complete the following. Clearly mark your answers. YOU MUST SHOW YOUR WORK TO RECEIVE CREDIT.

Warm-up (3 points each).

1. The **_thermal conductivity detector (TCD)_** utilizes a series of heated filaments in its detection mechanism.
2. The **_Kovats retention index_** scales the retention characteristics of a compound to those of aliphatic hydrocarbons and provides a means of comparing chromatographic results between labs.
3. **_Electrophoresis_** describes the movement of charged species in the electric field during an CE experiment.
4. The **_partition coefficient (distribution coefficient)_** is an equilibrium constant that describes the tendency for a solute to exist in the stationary phase relative to the mobile phase during a chromatographic separation.

You must complete problem 5. (15 points)

5. Perhaps the single most important advance in separations science in the past few decades has been the mating of mass spectrometric detection with HPLC. Why has this been such a significant development? What two key challenges must have been overcome to make LC-MS a reality?

The coupling of HPLC with MS allows the user to take advantage of the ability to HPLC to separate mixtures of a variety of classes of compounds (many more than can be separated using GC) with the powerful quantitative and qualitative analysis capabilities of the MS. While GCMS had been a workhorse technique for small molecules, LCMS opens the door to separations of large molecules, biomolecules, and ions.

The two key challenges in coupling the LC and MS focus on the interface between the two components. One challenge is that the material that exits the LC must first be converted to gas phase ions for the MS to be useful and most forms of LC are built around the separation of neutrals dissolved in solution. A second challenge is the very different conditions at which each instrument operates. In traditional HPLC, eluent exits the column at mL/min flow rates, resulting in a large amount of material exiting the LC in a short time. If all of this material were introduced into the MS, it would be impossible to maintain the high vacuum conditions required to provide a large mean free path for the ions produced in the MS.

In most LC-MS interfaces (such as electrospray and APCI), the ions are generated at or around atmospheric pressure and only a fraction of the ions produced are allowed to pass through the aperture of a sampler cone into a portion of the vacuum chamber that is held at an intermediate pressure. A fraction of the ions that remain are passed through a skimmer cone into the main MS vacuum chamber.

Complete five of the following. Be clear and concise. Clearly indicate which problem is not to be graded. (15 points each)

6. Briefly describe the mechanism of separation of a mixture of cations, anions, and neutrals in capillary zone electrophoresis. What parameters can be changed to optimize separation conditions in CZE?

Separation in capillary zone electrophoresis results from two phenomena, electrophoretic mobility and electroosmotic mobility. Electrophoretic flow occurs as a result of movement of charges species in the electric field that exists when a high voltage is applied across the length of the capillary. As a result of this electric field, cations are attracted to the cathode at the negative end of the capillary, and anions are attracted to the anode at the positive end. The rate at which ions move depends on their mass to charge ratio (small multiply charged ions move more quickly than large singly charged ions). Neutrals are unaffected by the electric field and do not move as a result of electrophoretic flow.

Electroosmotic flow results from the general flow of solvent (water) toward the cathode end of the capillary due to the presence of deprotonated silanol groups on the capillary walls and their attraction for solvated protons in solution. Since cations are more highly solvated than anions, they tend to "drag" solvent along as they move toward the cathode. Since solvation is a dynamic process, bulk solvent flow results, carrying all species (anions, cations, and neutrals) toward the cathode. Elution order is: cations, neutrals, anions.

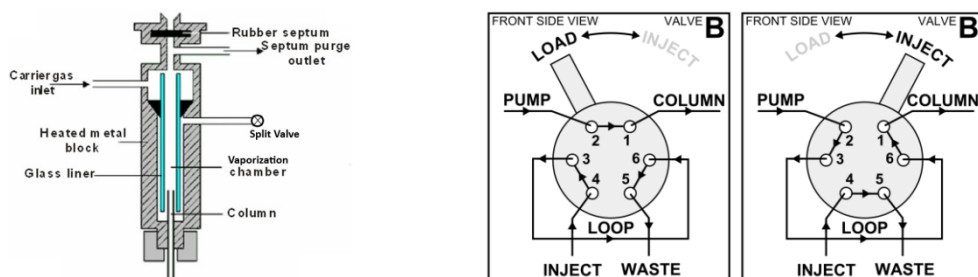
Separation conditions can be modified by changing the separation voltage, capillary length, and to some degree, running buffer composition.

7. Selection of a detector for separations often involves a tradeoff between universality (or selectivity) and sensitivity. Briefly describe why this is so, using examples of specific liquid chromatography detectors to illustrate your point.

Often, detectors that are particularly sensitive are tuned to probe a particular property of the analyte. For example, fluorescence detectors in LC offer wonderful sensitivity, but are limited to analytes that fluoresce. In order to expand the range of analytes that a detector sees, it is often necessary to probe a property of the analyte that is less unique to a particular compound. A typical outcome of this choice is poorer sensitivity. Consider the refractive index detection in LC. In order to be more universal, it responds to a change in the refractive index of the eluting solution, however, this response is much less sensitive than that of a fluorescence detector. Even new universal detectors, like the ELSD cannot rival the sensitivity of fluorescence. Really the only detection scheme which approaches both the optimum in sensitivity and selectivity is mass spectrometric detection, which comes with its own set of challenges (see problem 5!).

8. Clearly describe one of the two injection methods for chromatography listed below. Include a diagram and a discussion of the key characteristics of the method in your description.
- Split/Splitless injection for GC
 - An injection loop and injection valve for HPLC.

- Split/Splitless injection allows the user to determine whether “all” or only a fraction of a sample that is injected into the GC makes it onto the capillary. Split injection is primarily used to reduce the effective size of the sample introduced into a GC capillary. Since the dimensions of the capillary are so small, direct injection of neat liquids can overload the column and lead to poor separations. Split injections help minimize this by carrying a portion of the sample away prior to it reaching the capillary. Splitless injection allows most of the sample to enter the capillary and is useful for more dilute solutions.
- You should indicate that the purpose for an injection loop is to improve reproducibility (precision) of the volume of sample injected in to the HPLC. Your description should include the operation of “load” mode, where the injection loop is filled by the user while effluent from the pump bypasses the loop and “inject” mode where the mobile phase is allowed to flow through the loop and carry the sample on to the column.



9. Consider the Van Deemter equation. Which term is likely to be the primary contributor to band broadening in capillary GC? Justify your answer. Does the same argument hold true for LC and CE? Why or why not?

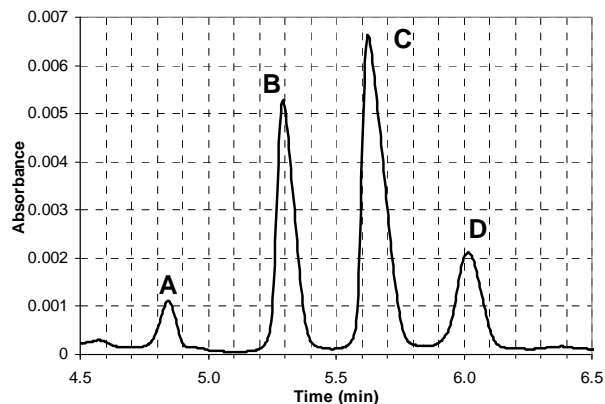
The Van Deemter equation describes three classes of phenomena that contribute to band broadening in chromatography. The multipath term (A) is independent of flow rate and corresponds to the large number of possible routes and analyte may take as it moves through a packed column. As particle size increases, the difference in length of these routes is magnified, leading to an increased contribution of the A-term. The B-term is inversely proportional to flow rate and deals with the contribution of diffusion of analyte in the mobile phase along the axis of the separation column (longitudinal diffusion). The C term is directly proportional to flow rate and corresponds to mass transport of material into and out of the stationary phase.

Since diffusion coefficients are very large in the gas-phase, the longitudinal diffusion term dominates band broadening in GC, regardless of whether it is in a packed column or capillary. For capillary GC, the absence of packing eliminates the A term. Fast diffusion also helps to minimize the contribution of the C-term as well.

Since diffusion coefficients are much smaller in the liquid phase, the relative contribution of all three terms are much more comparable. In CE, the absence of stationary phase packing and partitioning causes the A and C terms to become negligible. Therefore only longitudinal diffusion plays a significant role.

For problems 11 and 12, consider the chromatogram below that was obtained for a reverse-phase HPLC separation on a 25 cm column, using UV absorbance detection. Unretained compounds elute in 0.85 minutes.

10. Complete the following.
- Calculate the number of theoretical plates for component C.
 - Calculate the selectivity factor of compound D over compound C.
 - Calculate the resolution of compounds C and B.
 - Which compound is the most polar? Justify your choice.



a: For compound C:

$$N = \frac{(4t_r)^2}{W^2} = \frac{(4 \times 5.63 \text{ min})^2}{(0.22 \text{ min})^2} = \mathbf{10,500 \text{ plates}}$$

b:

$$\alpha = \frac{k'_D}{k'_C} = \frac{(t_r)_D - t_m}{(t_r)_C - t_m} = \frac{(6.02 - 0.85) \text{ min}}{(5.63 - 0.85) \text{ min}} = \mathbf{1.08}$$

c:

$$R_s = \frac{2\Delta Z}{W_C + W_B} = \frac{2(5.63 - 5.31) \text{ min}}{(0.22 + 0.19) \text{ min}} = \mathbf{1.56 = 1.6}$$

Or:

$$R_s = \frac{(N)^{1/2}(\alpha - 1)k'_B}{4\alpha(1 + k'_B)} = \frac{(10500)^{1/2}(1.08 - 1)(5.62)}{4 \times 1.08 \times (1 + 5.62)} = \mathbf{1.61}$$

d: In reverse-phase separations, the stationary phase is less polar than the mobile phase, meaning nonpolar compounds will be more strongly retained, while more polar compounds will elute first. Soooooo, A is the most polar compound.

11. Your boss looks at the chromatogram and makes the following statement: "Well, it is clear to me that compound B is present at about 5 times the concentration of compound A and that compound C is caffeine since it elutes at 5.62 minutes under these conditions." Discuss the validity of this statement.

While it is true that the height (and maybe the area) of peak B is about 5 times that of peak A, there is no guarantee that the response of the detector is the same for both components. Depending on the mode of detection, the response may be quite different. For example, if UV absorbance detection is used and compound B has a much larger molar absorptivity at the detection wavelength than compound A, it could be the case that the concentration of B is actually less than the concentration of A. The only way to be sure is to prepare calibration curves for each component.

While the retention time of a particular compound is essentially constant under constant conditions, there is no guarantee that another compound cannot have the same retention time. If you have a good understanding of the composition of your sample, this assertion is better, but the only way to be sure is to have a detection scheme that can provide the identity of the compound (such as MS).

Possibly Useful Information

$A = \log(P_0/P) = \epsilon bc$	$\pi = 3.14159$
$k'_A = K_A \frac{V_S}{V_M} = \frac{t_R - t_M}{t_M}$	$\alpha = \frac{K_A}{K_B} = \frac{k'_A}{k'_B}$
$N = L/H$	$H = \frac{\sigma^2}{L} = L \left(\frac{W}{4t_R} \right)^2$
$N = \left(\frac{4t_R}{W} \right)^2 = \left(\frac{2.35t_R}{W_{1/2}} \right)^2$	$H = A + \frac{B}{u} + Cu = A + \frac{B}{u} + (C_s + C_m)u$
$R_s = \frac{\Delta Z}{W_A/2 + W_B/2} = \frac{2\Delta Z}{W_A + W_B}$	$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k'_B}{1 + k'_B} \right)$
$v = (\mu_e + \mu_{e0})E = (\mu_e + \mu_{e0})V/L$	$N = \frac{(\mu_e + \mu_{e0})V}{2D}$

PERIODIC CHART OF THE ELEMENTS

IA	IIA	IIIB	IVB	VB	VIB	VII B	VIII	IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA	INERT GASES	
1 H 1.00797														1 H 1.00797	2 He 4.0026		
3 Li 6.939	4 Be 9.0122										5 B 10.811	6 C 12.0112	7 N 14.0067	8 O 15.9994	9 F 18.9984	10 Ne 20.183	
11 Na 22.9898	12 Mg 24.312										13 Al 26.9815	14 Si 28.086	15 P 30.9738	16 S 32.064	17 Cl 35.453	18 Ar 39.948	
19 K 39.102	20 Ca 40.08	21 Sc 44.956	22 Ti 47.90	23 V 50.942	24 Cr 51.996	25 Mn 54.9380	26 Fe 55.847	27 Co 58.9332	28 Ni 58.71	29 Cu 63.54	30 Zn 65.37	31 Ga 69.72	32 Ge 72.59	33 As 74.9216	34 Se 78.96	35 Br 79.909	36 Kr 83.80
37 Rb 85.47	38 Sr 87.62	39 Y 88.905	40 Zr 91.22	41 Nb 92.906	42 Mo 95.94	43 Tc (99)	44 Ru 101.07	45 Rh 102.905	46 Pd 106.4	47 Ag 107.870	48 Cd 112.40	49 In 114.82	50 Sn 118.69	51 Sb 121.75	52 Te 127.60	53 I 126.904	54 Xe 131.30
55 Cs 132.905	56 Ba 137.34	*57 La 138.91	72 Hf 178.49	73 Ta 180.948	74 W 183.85	75 Re 186.2	76 Os 190.2	77 Ir 192.2	78 Pt 195.09	79 Au 196.967	80 Hg 200.59	81 Tl 204.37	82 Pb 207.19	83 Bi 208.980	84 Po (210)	85 At (210)	86 Rn (222)
87 Fr (223)	88 Ra (226)	†89 Ac (227)	104 Rf (261)	105 Db (262)	106 Sg (266)	107 Bh (262)	108 Hs (265)	109 Mt (266)	110 ? (271)	111 ? (272)	112 ? (277)						

Numbers in parenthesis are mass numbers of most stable or most common isotope.

Atomic weights corrected to conform to the 1963 values of the Commission on Atomic Weights.

The group designations used here are the former Chemical Abstract Service numbers.

* Lanthanide Series

58 Ce 140.12	59 Pr 140.907	60 Nd 144.24	61 Pm (147)	62 Sm 150.35	63 Eu 151.96	64 Gd 157.25	65 Tb 158.924	66 Dy 162.50	67 Ho 164.930	68 Er 167.26	69 Tm 168.934	70 Yb 173.04	71 Lu 174.97
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† Actinide Series

90 Th 232.038	91 Pa (231)	92 U 238.03	93 Np (237)	94 Pu (242)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (249)	99 Es (254)	100 Fm (253)	101 Md (256)	102 No (256)	103 Lr (257)
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