

Complete these problems on separate paper and staple it to this sheet when you are finished. Please initial each sheet as well. Clearly mark your answers. YOU MUST SHOW YOUR WORK TO RECEIVE CREDIT.

Warm-up (3 points each)

1. In size exclusion chromatography, species are separated based on their ability to move in and out of the pores in the stationary phase packing material.
2. An electron capture detector, is the detector of choice for GC separations of halogenated compounds.
3. A guard column, is attached to the inlet end of an HPLC column to extend its useful life.
4. In a CE experiment, electroosmosis, results in the general movement of all species toward the cathode.

You must complete problem 5.

5. Much of the development in LC recently has been focused on methods to decrease particle size from the 5 μm diameter particles that had become the industry standard to particles of 2 μm or smaller. Why has there been such a focus on decreasing particle size? Be sure to reference the van Deemter equation in your discussion. What challenges accompany the implementation of LC columns with smaller particles? (15 points)

Considering the van Deemter relationship, two of the three terms are dependent on the size of the stationary phase packing. The multipath term (the "A" term) contributes less as particle size decreases because there is less of a variation in the multiple paths that molecules take through the column. This ultimately translates to narrower bands and narrower peaks and therefore better separation efficiency.

The mass transport term (C) also is strongly dependent on particle size. As particles decrease in size, the depth of the pores in the particle surface also decreases, meaning that molecules cannot diffuse as far into the center of the pores, and as a result, are more well able to keep up with the flow of mobile phase by having their partitioning interactions take place closer to the exterior of the particle as opposed to being "buried" inside the particle's pores. The end result is that the C-term flattens out above some threshold mobile phase velocity, meaning that separations using small particles can occur at higher flow rates without a loss in separation efficiency.

Benefits: Faster, more efficient separations, smaller sample volumes, smaller volume flow rates, decreased solvent consumption.

Challenges: Higher back pressures due to efficient packing of small particles leads to more stringent demands on pumping system. The necessity for small, homogenous particles leads to more stringent stationary phase preparation requirements.

Complete 5 of the following. Be concise in your answers and show work for problems involving calculations. Clearly indicate which problems are not to be graded. (15 pts ea)

6. For years, mating LC and MS had posed a significant challenge. Why was this the case? Describe two approaches for interfacing LC with mass spec.

The key challenge in interfacing LC and MS 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, 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.

One of major developments in the coupling of MS to LC is the electrospray ionization source. In electrospray, the sample solution eluting from the LC flows through a needle which is subject to a large electric field. As solution leaves the needle, it obtains a charge. Electrostatic repulsion causes the charged stream to break into smaller charged droplets, which continue to "explode" until solvent is essentially evaporated and ionized analyte remains.

Atmospheric pressure chemical ionization (APCI) has also become a popular ionization source for LCMS. With this method, column eluent is nebulized and the spray of droplets is directed over a corona discharge needle, which is held at high voltage. As mobile phase and analyte pass through the corona discharge, ions may be formed. Since there is much more solvent than analyte, solvent molecules are ionized preferentially. These solvent ions can then produce analyte ions through a chemical ionization pathway.

7. Compare the operation of a UV absorbance detector with one of the following detectors in LC: fluorescence, refractive index, electrochemical, ELSD. Include a diagram of the components and discuss the benefits and limitations of each detector, paying particular attention to selectivity and sensitivity.

See your text for examples of diagrams.

You should discuss the basic operation and the following benefits and challenges.

UV: (+) sensitive, (-) analytes must absorb in UV (no good for aliphatics)

Fluorescence: (+) can be very sensitive, (-) most analytes don't fluoresce.

Electrochemical: (+) inexpensive, (-) not very universal, susceptible to fouling.

Refractive Index: (+) fairly universal, inexpensive, (-) not very sensitive

ELSD: (+) universal, sensitive, (-) costly, can't handle salts in mobile phase.

8. Compare and contrast the role of the mobile phase in GC with that in LC. Include a description of the important properties of the mobile phase in each separation and its impact on the quality of a separation.

Your discussion should focus on the fact that intermolecular interactions between analyte and the mobile phase are much more significant (and critical) to the separation in LC than in GC. In GC the primary role of the mobile phase is to provide inertia for gas phase species to move through the column. Intermolecular interactions between the mobile phase and analyte are minimal (if nonexistent) as the mobile phase serves to kick the gas phase analytes along through the column. In LC, however, the mobile phase must also provide a thermodynamic reason for the analytes to leave the column. This must be done by

solubilizing (“dissolving”) the analyte away from the stationary phase so that it can be carried to the detector end of the column.

9. One method for evaluating the efficiency of a separation is to calculate the number of theoretical plates (N) for the separation, with larger numbers of theoretical plates generally leading to better separations. Given that $N = H/L$, where H is the “size” of the theoretical plate and L is the length of the column, we can increase N by decreasing H or increasing L. Given this relationship, why don't we simply use very long columns to perform separations? In an HPLC experiment, how might you work to decrease H in order to increase N?

In terms of overall separation quality, the length of the column required to perform a separation event is a critical factor. While using infinitely long columns would allow more separation events (plates) to occur, they would also lead to unacceptably long separations. Longer separations are also more susceptible to broadening as a result of longitudinal diffusion. As a result, overall separation quality is diminished. In practice, we try to optimize the length (height) of a theoretical plate to allow us to package a larger number of separation events in a shorter column.

There are several ways to decrease H:

- Use smaller packing material: This diminishes band broadening due to multipaths
- Decrease the thickness of the stationary phase coating this diminished band broadening due to mass transfer terms.
- Use gradient elution: by altering separation conditions during the experiment, you can tune conditions for each analyte and improve separation efficiency.

10. You intend to perform a separation of a mixture of the five components below using capillary electrophoresis with pressure injection and absorbance detection at 200 nm at the cathode end of the capillary. The table below describes the properties of each of the components under the conditions of the separation. Sketch an electropherogram you would expect for two experiments:

- (1) capillary zone electrophoresis in a fused silica capillary **and**
- (2) capillary zone electrophoresis in a capillary whose surface has been reacted with trimethylchlorosilane.

Identify each peak in your electropherograms and describe why you chose to draw them as you did.

species	concentration (ppm)	molar mass (g/mol)	molar absorptivity @ 200 nm ($M^{-1}cm^{-2}$)	charge
A	50.0	101.3	1000	+1
B	100.0	100.9	500	-1
C	50.0	100.2	2000	+2
D	100.0	99.9	1000	0
E	50.0	100.5	1000	-2

Your electropherograms should illustrate both the elution order and relative size of the peaks for each compound. Since we have concentration and molar absorptivity information, we can calculate a “response” for each. Since $A = abc$, the “response” will be proportional to the product of molar absorptivity and concentration, as shown above. As a result, peaks for C and D should be twice as large as those for A, B, and E (if they appear).

In terms of electrophoretic mobility, since all species have roughly the same mass, the main indicator will be charge. Therefore electrophoretic mobility toward the cathode will correspond to the following order $+2 > +1 > 0 > -1 > -2$ or $C > A > D > B > E$.

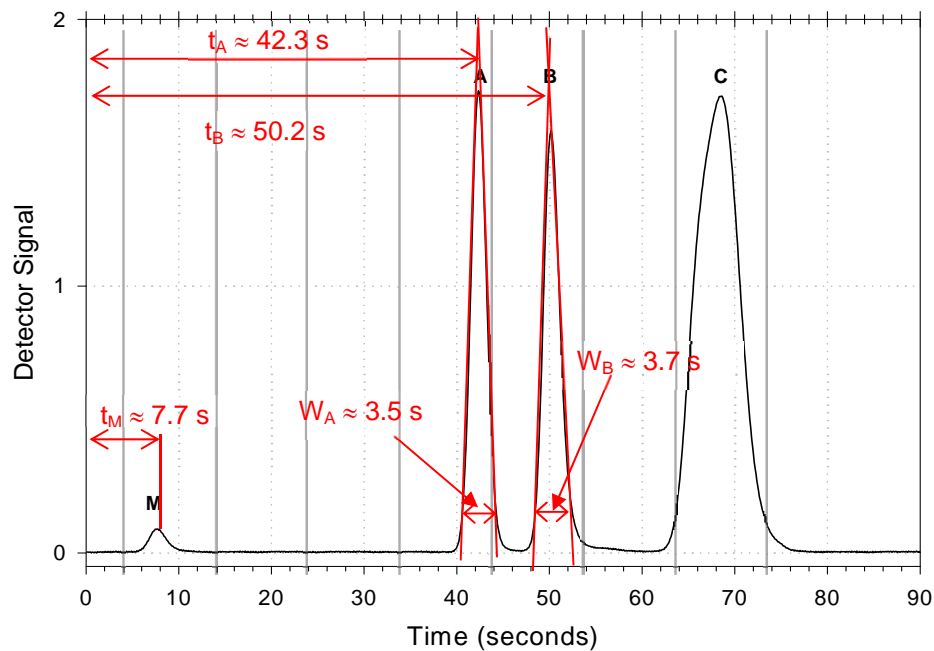
For separation (1), both electrophoresis and electroosmosis will be in play and all species will move toward the cathode in the order listed above. Therefore your electropherograms should show peaks for all 5 species with C appearing first, followed by A, D, B, and E. The peaks for C and D should be similar in size but twice as large as those for A, B, and E (which are similar in size to one another).

For separation (2), electroosmotic flow will be suppressed, leaving electrophoresis as the only mode of transport. Therefore, only cationic analytes will be detected at the cathode end, with C moving more rapidly than A. So, your electropherogram should have two peaks, with C appearing first and being roughly twice as large as A.

11. Why is a thermal conductivity detector a much more universal GC detector than a flame ionization detector? If the TCD is so much more universal, why use an FID at all?

The thermal conductivity detector works by monitoring the heat-transfer characteristics of the column effluent. Typically, the mobile phase in GC (helium, hydrogen) has a very high thermal conductivity compared to other compounds, therefore, when an analyte elutes from the column, there is a large decrease in thermal conductivity of the effluent. This decrease is observed whenever any species other than H_2 or He elutes from a column, a change in signal is observed. With an FID, only combustible species are detectable. BUT, the FID has a built in degree of gain, because the signal is related to the combustibility (# of carbon atoms) of the sample. So, FID tends to be significantly more sensitive than TCD.

12. Answer the following questions related to the gas chromatogram below. Experimental conditions: Packed column (4 mm diameter x 2 m long), Carbowax stationary phase, 40 mL/min helium carrier gas flow rate, FID detector, column temperature = 100°C, injector temperature = 150°C, detector temperature = 150°C. Peak M corresponds to an unretained compound.



Depending on how you determined your peak width and retention times, your numbers will probably vary a little from mine. As long as your approach to doing the calculations was sound, and your values are correct for the w and t_r you determined, things were fine.

- a. Calculate the selectivity factor and resolution for peaks A and B. (4 points)

Before we can calculate α , we calculate k' for each peak:

$$k_A' = \frac{(t_R)_A - t_M}{t_M} = \frac{(42.3 - 7.7) \text{ s}}{7.7 \text{ s}} = 4.49$$

$$k_B' = \frac{(t_R)_B - t_M}{t_M} = \frac{(50.2 - 7.7) \text{ s}}{7.7 \text{ s}} = 5.52$$

$$\alpha = \frac{k_B'}{k_A'} = \frac{5.52}{4.49} = 1.23$$

$$R_s = \frac{2\Delta Z}{W_A + W_B} = \frac{2(50.2 - 42.3)}{3.5 + 3.7} = 2.20$$

- b. Calculate the number of theoretical plates for peak B. (3 points)

$$N = \frac{16(t_R)_B^2}{W_B^2} = \frac{16 \times 50.2^2}{3.7^2} = 2950 \text{ Plates}$$

- c. Based on the size of the peaks, what can you say about the relative concentrations of components A and B? (4 points)

Even though the sizes of the peaks are similar, we cannot say anything quantitative about the relative concentrations unless we know something about the relative sensitivities of the detector to each component. Therefore we would need a calibration curve for each peak to say with confidence anything about concentration.

- d. It appears that peak C is the result of co-elution of two compounds. How would you change experimental conditions to resolve these two peaks? What effect are these changes likely to have on the separation of components A and B? (4 points)

Your discussion should describe how you would take advantage of temperature programming to improve the separation. One approach would be to ramp the temperature after the two well-resolved peaks elute. In your discussion you should mention that increasing the temperature before peaks A and B elute would result in a decrease in resolution. Lowering the column temperature too much will likely result in band broadening and may decrease the quality of the separation.

Possibly Useful Information

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

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
--------------------	---------------------	--------------------	-------------------	--------------------	--------------------	--------------------	---------------------	--------------------	---------------------	--------------------	---------------------	--------------------	--------------------

† 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)
---------------------	-------------------	-------------------	-------------------	-------------------	-------------------	-------------------	-------------------	-------------------	-------------------	--------------------	--------------------	--------------------	--------------------