CHEM 322 **Name**___________________________________
Exam 2 **Fall 2009**

Due at the beginning of class Monday, October 26
NO LATE PAPERS ACCEPTED!

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

Instructions

- This is **NOT** an open-book, open-note take exam. You MAY NOT consult any human or nonhuman resource besides Dr. Lamp as you complete the exam. This exam MUST be completed INDIVIDUALLY and in your own words. Group work or plagiarism will result in a zero for the exam.
- You will be allowed to ask Dr. Lamp a maximum of two (2) questions regarding the exam. Additional questions may be asked at a 3-point penalty per question. If you are working on the exam in the evening, you may try to reach Dr. Lamp on his cell phone at 660-341-0067 before 10:00 PM.
- Before opening the exam, prepare for it like you would for a traditional, in-class exam. Review concepts and examples from the text, as well as those discussed in class. This preparation will help to maximize your effort on the exam and allow you to complete it more efficiently.

Time Restriction

You may spend no more than two (2) hours working on this exam. This <u>must</u> be in one continuous block of time. You are on your honor to adhere to this restriction and record the time spent in the chart below.

Date	Time Began	Time Finished	Total Time
Total Time Spent on the Exam			

Pledge

I pledge on my honor that I have completed the exam in accordance with the above instructions and that I have not provided or received unethical assistance. I realize that failure to comply with these instructions will result in a score of zero on the exam.

____ __________________
Signature Date

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Warm-up (2 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.

Answer in a sentence or two, or with a calculation. Complete seven of the following. Clearly indicate which problem is not to be graded. (14 points each)

5. Why is the sample injection rate (or sample plug size) an important consideration in all separations?

   The rate at which sample is introduced onto the column (or capillary) determines the size of the "plug" of analyte at the beginning of the separation and determines the minimum width of a chromatographic band at the end of the separation. Since all separations are subject to band broadening due to diffusion, this initial band will be broadened as material moves through the column. The relative size of the sample plug compared to the column volume and detector sensitivity is also important. Too large of a plug could overload the column, leading to poor separation. Too small a plug could lead to difficulty detecting sample components.

6. Why is a thermal conductivity detector a much more universal GC detector than a flame ionization detector? If it 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 <u>any</u> 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.

7. Compare the operation of **two** of the following detectors in LC: UV absorbance, fluorescence, refractive index, electrochemical, ELSD. Consider the benefits and limitations of each detector, paying particular attention to selectivity and sensitivity.

   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, sensitve, (-) costly, can't handle salts in mobile phase.

8. In separations, we often refer to a theoretical plate as a representation of a single separation event. In general, the more events (plates) involved in the separation, the better the separation should be. Having said that, why don't we simply use very long columns to perform separations? These columns would provide large numbers of plates and good separation, right? In practice, how do we attempt to maximize the number of separation "events" that occur?

   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.

9. Describe the two general approaches to sample introduction for capillary electrophoresis. Include a description of how the sample is introduced to the capillary and any benefits or challenges of each approach.

   Electrokinetic Injection: The inlet end of the capillary and one of the electrodes (typically the anode) are placed in the sample solution, while the outlet end and the other electrode are placed in buffer. A high voltage is applied, resulting in movement of species toward the cathode end of the capillary through electroosmotic and electrophoretic flow. After a fixed amount of time, the potential is switched off and the electrode and inlet end of the capillary are removed from the sample solution and placed in buffer solution to begin the separation. The key benefit for this approach is its mechanical simplicity. No additional hardware is needed. A major drawback for electrokinetic injection is that species with high mobility are sampled more efficiently than those with low mobility.

   Pressure Injection: The inlet end of the capillary is placed in the sample solution. Pressure is applied to the surface of the sample solution, forcing sample into the inlet end of the capillary. After a fixed amount of time, the pressure is released and inlet end of the capillary is removed from the sample solution and placed in buffer solution to begin the separation.

The key benefit for this approach is that it does not discriminate on the basis of mobility. A more representative sample plug is injected. A major drawback for pressure injection is the necessity for additional hardware to supply the pressure needed to inject sample.

10. Sketch a van Deemter plot for an HPLC separation using a packed column. Describe the relative contribution of all three terms in the van Deemter relationship. How does the van Deemter plot change if we considered open tubular GC instead?

    I'd expect to see a plot similar to that below:

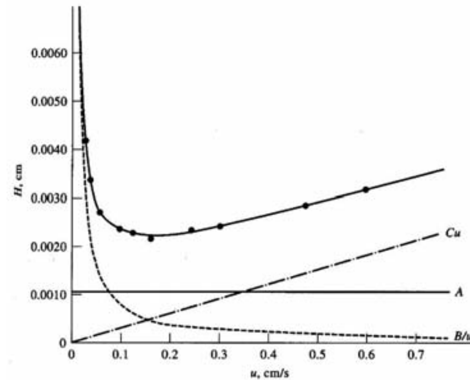


Figure 26-9 A van Deemter plot for a packed liquid chromatographic column. The points on the upper curve are experimental. The contributions of the various rate terms are shown by the lower curves: *A*, multipath effect; *B/u*, longitudinal diffusion; *Cu*, mass transfer for both phases. *(From E. Katz, K. L. Ogan, and R. P. W. Scott,* J. Chromatogr.*, 1983, 270, 51. With permission.)*

    Your discussion must demonstrate and understanding of the three components of the van Deemter equation as it applies to LC. Since LC use a packed column, the multipath term (A) will contribute to H. The longitudinal diffusion term also plays a significant role, given the relatively low flow rates in most LC experiments. Since the "thickness" of the stationary phase coating in LC is fairly small, contributions of the C term, while present, tend to be the least of the three.

    In an open tubular GC experiment, the A term disappears (no packing) and the C term holds greater weight because the thickness of the stationary phase coating is greater. The B term is also significant due to higher diffusion coefficients in the gas phase compared to the liquid phase

11. 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.

12. Clearly describe the mechanism of separation in capillary zone electrophoresis. What parameters can be changed to optimize separation conditions in CZE?

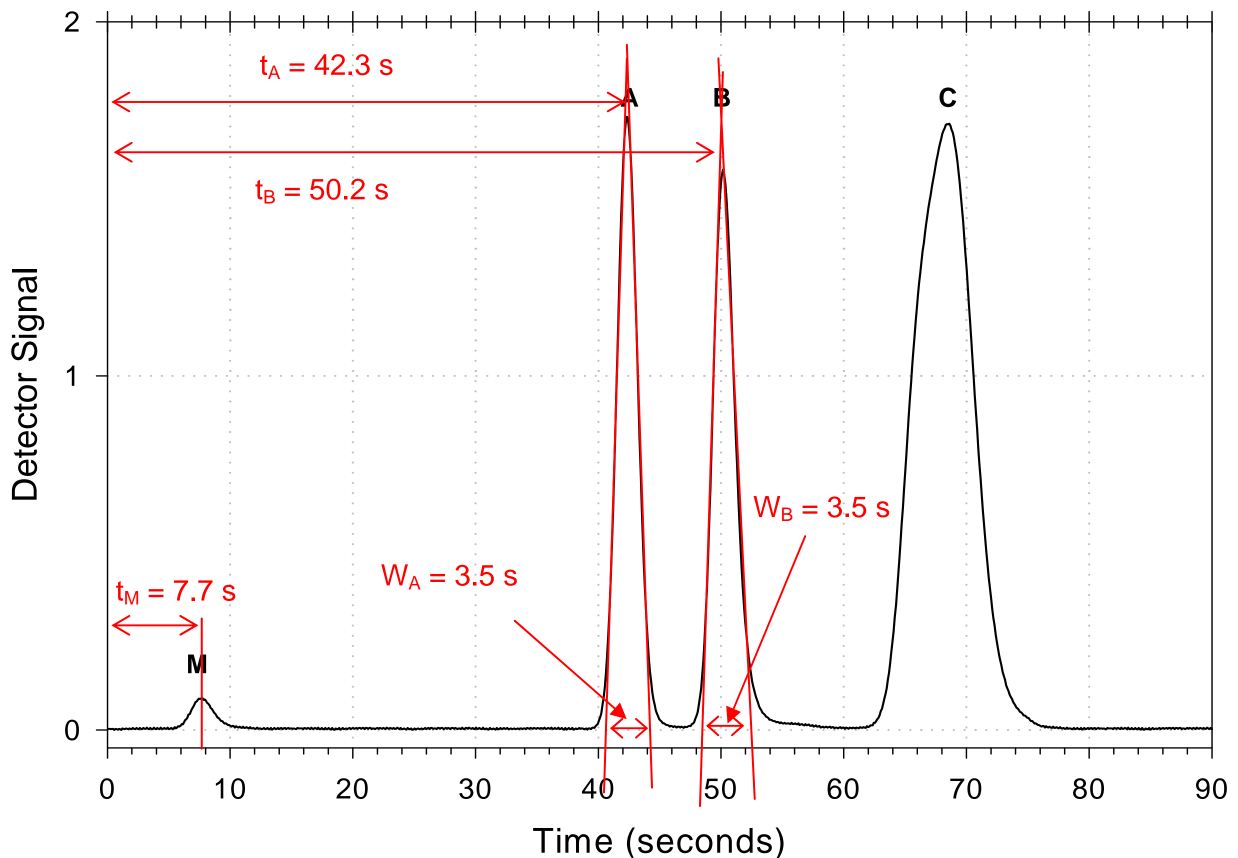
    Separation in capillary zone electrophoresis results from two phenomena, electrophoretic mobility and electroosmotic mobility. Electrophoretic flow occurs as a result of movement of charges species in the electric field that exists when a high voltage is applied across the

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 charge (multiply charged ions move more quickly than singly charged ions) and their mass (small ions move more rapidly than large 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. 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.

13. 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, TCD detector, column temperature = 100°C, injector temperature = 150°C, detector temperature = 150°C. Peak M corresponds to an unretained material.



a. Calculate the selectivity factor and resolution for peaks A and B.

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

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

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

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

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

b. Calculate the number of theoretical plates for peak B.

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

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

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?

Your discussion should describe how you would take advantage of <u>temperature programming</u> 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

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

PERIODIC CHART OF THE ELEMENTS

IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIII			IB	IIB	IIIA	IVA	VA	VIA	VIIA	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	59	60	61	62	63	64	65	66	67	68	69	70	71
Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
140.12	140.907	144.24	(147)	150.35	151.96	157.25	158.924	162.50	164.930	167.26	168.934	173.04	174.97

† Actinide Series

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