CHEM 322 **Name**_____
Exam 2 **Spring 2010**

Due by 3:00 PM Friday, February 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 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 must 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. The _____ utilizes a series of heated filaments in its detection mechanism.

2. In _____ a small fiber is inserted into the sample container to allow analyte to adsorb to its surface. The fiber can then be introduced into a GC for desorption and analysis.

3. Capillary electrochromatography, aggregates of surfactants called _____ form a *pseudo* stationary phase to allow partitioning of analytes.

4. The _____ 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.

Complete <u>seven</u> of the following. Be clear and concise. Clearly indicate which problem is not to be graded. (14 points each)

5. Describe each of the following, indicating why each is utilized in a separations experiment.
   a. A guard column in HPLC.

   A guard column is used to trap material that would irreversibly adsorb to the analytical column, diminishing its effectiveness. The packing in the guard column is the same as that in the analytical column, but the guard column is sacrificial.

   b. Split injection in GC.

   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.

   c. A buffer in CE.

   In CE, we need a conductive solution to facilitate charge migration in the electric field. Having this solution buffered prevents dramatic changes in pH, and therefore proton concentrations during the run. Changing proton concentrations would impact electroosmosis and therefore separation conditions.

6. 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 gas chromatography detectors to illustrate your point.

   Often, detectors that are particularly sensitive are tuned to probe a particular property of the analyte. For example, FID detectors in GC are designed to monitor combustible analytes in a mixture, but are insensitve to anything that doesn't burn in the flame (like CO_2). 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 class of compounds. A typical outcome of this choice is poorer sensitivity. Consider the TCD in GC. In order to be more universal, it responds to a change in thermal conductivity of the carrier gas, however, this response is less sensitive that that of a FID.

7. Briefly 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 thy 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.

8. Briefly describe how an ELSD works and why it is more universal than UV-Vis detection.

   In an ELSD, effluent from the LC column is passed through a nebulizer (much like in AA) to break the solution into a stream of small droplets. This stream then passes through a heated drift tube, where solvent is evaporated, leaving (if a solute is present), an aerosol of solid particles. This aerosol passes through a laser beam. The presence of particles results in the scatter of laser light. This scatter is detected as the analytical signal.

   With UV-Vis detection, the analyte must absorb UV or Visible radiation (at the detection wavelength), or else it will not be "seen" at the detector. For ELSD, the only requirement is that the solute have a higher boiling point than the mobile phase, so that it is left as an aerosol after the solvent evaporates. There are no requirements that the analyte absorb the laser radiation.

9. What is the general elution problem? Sketch a generic chromatogram to illustrate the problem. Describe one approach in gas chromatography and one approach in liquid chromatography aimed at addressing the general elution problem. From a physical chemistry perspective, how does each approach hope to alleviate the problem?

   The general elution problem states that conditions that result in a good separation for one pair of compounds, will likely not result in the same quality separation for other compounds in the mixture. Both GC and LC approach this issue by altering the separation conditions during the course of the separation.

   In GC, temperature programming is typically used. In this approach, the temperature of the separation column is raised throughout the course of the separation. Increasing the temperature gives more kinetic energy to the gas-phase eluent, decreasing retention time.

   In LC, the composition of the mobile phase is altered throughout the course of the separation. In gradient elution, the resulting change in polarity that comes with this composition change, alters the retention time of analytes in the mixture.

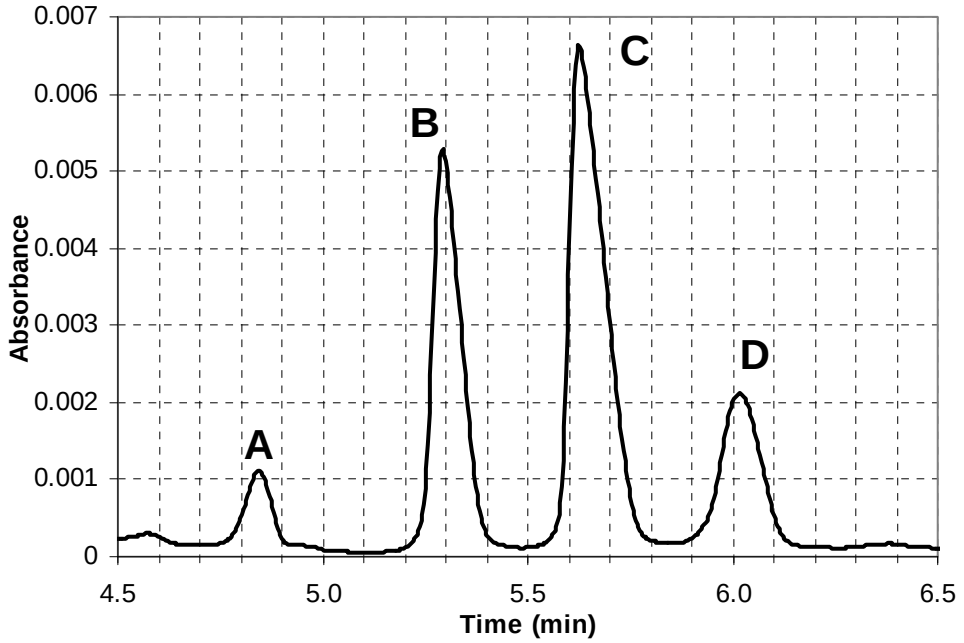
10. Consider the Van Deemter equation. Which term is likely to be the primary contributor to band broadening in GC? Justify your answer. Does the same argument hold true for LC and CE? Why or why not?

    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 magnifies, 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. Since packed columns in GC are typically one to several meters in length, contribution form the A-term get averaged out over the length of the column. 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 0.15 minutes. NOTE: Problems 11 and 12 are independent of one another!



11. Complete the following.
    a. Calculate the average plate height for the separation. Based on this average, calculate the number of theoretical plates for the separation.

For compound A:

$$H = \frac{LW^2}{(4t_r)^2} = \frac{25\text{ cm}(0.16\text{ min})^2}{(4 \times 4.84\text{ min})^2} = 0.0017\text{ cm}$$

For compound B, W = 0.19 min, t_r = 5.31 min, H = 0.0020 cm
For compound C, W = 0.22 min, t_r = 5.63 min, H = 0.0024 cm
For compound D, W = 0.21 min, t_r = 6.02 min, H = 0.0019 cm
Average H = 0.0020 cm

Since N = L/H, $N_{average}$ = 25 cm/0.0020 cm = 12,500 plates.

    b. Calculate the selectivity factor of compound D over compound C.

$$\alpha = \frac{k'_D}{k'_C} = \frac{(t_r)_c - t_m}{(t_r)_B - t_m} = \frac{(6.02 - 0.15)\text{min}}{(5.63 - 0.15)\text{min}} = \mathbf{1.07}$$

    c. Calculate the resolution of compounds C and B.

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

Or:

$$R_s = \frac{(N)^{1/2}(\alpha\text{-}1)k'_B}{4\,\alpha(1+k'_B)} = \frac{(12500)^{1/2}(1.06\text{-}1)(21.52)}{4*1.06*(1+21.52)} = \mathbf{1.51}$$

d. Which compound is the most polar? Justify your choice.

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.

12. Your boss looks at the chromatogram and makes the following statement: "Well, it is clear to me that compound D is present at about 2 times the concentration of compound A and that compound B is methamphetamine since it elutes at 5.39 minutes under these conditions." Discuss the validity of this statement.

While it is true that the height (and maybe the area) of peak D is about 2 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 D has a much larger molar absorptivity at the detection wavelength than compound A, it could be the case that the concentration of D 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. We have seen examples in our GC and LC experiments of compounds having similar retention times (or coeluting in a mixture). 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

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

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 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)