CHEM 322   **Name**_____

Exam 2   **Fall 2012**

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.

Complete 6 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 points each)

5. Why is the sample injection volume (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 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 <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 a UV absorbance detector with **one** of the following detectors in LC: 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. 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	"response"
A	50.0	101.3	1000	+1	50k
B	100.0	100.9	500	-1	50k
C	50.0	100.2	2000	+2	100k
D	100.0	99.9	1000	0	100k
E	50.0	100.5	1000	-2	50k

Your electopherograms 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 electropherorgram should have two peaks, with C appearing first and being roughly twice as large as A.

9. 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 a chromatography experiment?

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. 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.

11. 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.
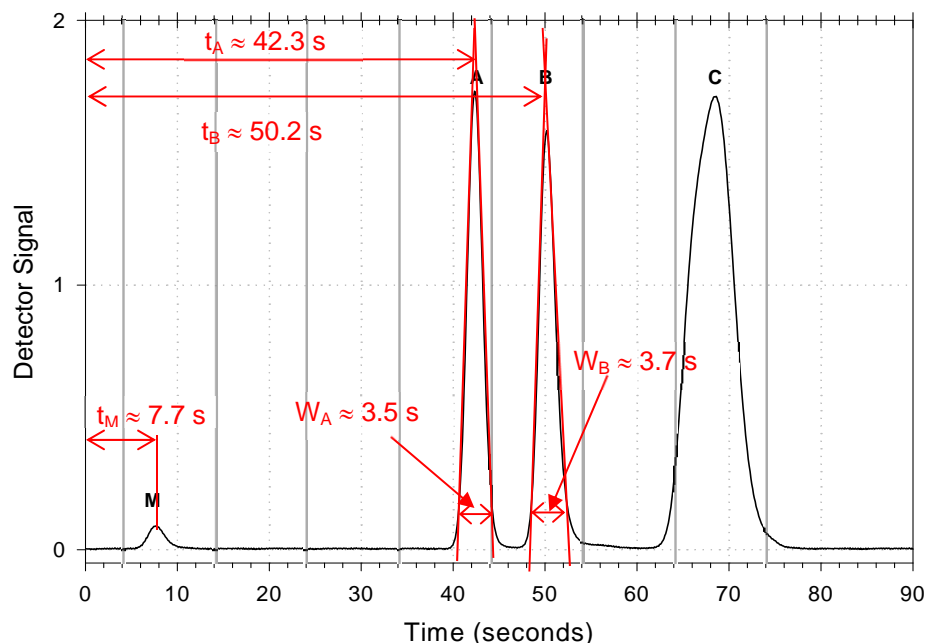
a. Calculate the selectivity factor and resolution for peaks A and B.
Before we can calculate α, we calculate k' for each peak:

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

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

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

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

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

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

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 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

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

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