CHEMISTRY 447 **Separations—Problem Set**

1. Please answer the following questions True or False (3 points each):

a) Gradient elution liquid chromatography refers to systems where the flow rate of the mobile phase is changed (either as gradual increase or in steps) during the course of the separation.

b) In chromatography, column efficiency always increases when the length of the column is increased.

c) In packed liquid chromatographic columns, H.E.T.P. values will always increase as the diameter of the column increases for a given volumetric flow rate of mobile phase and column length.

d) When used in HPLC, an electrochemical detector is generally more sensitive and selective than either a refractive index or UV-Vis absorbance detector.

e) To perform sensitive anion exchange chromatography using conductivity detection, the suppressor or stripper component of such a system would normally be made with a high capacity anion exchange packing or membrane.

f) The primary advantage of performing electrophoresis within a narrow bore capillary (i.e., CZE) is that a very small volume of sample is required.

2). Given the following chromatogram for the separation of two solutes on a 25 cm long HPLC column, determine:

a) the H.E.T.P achieved under the operating conditions of the column using solute A values for calculations;

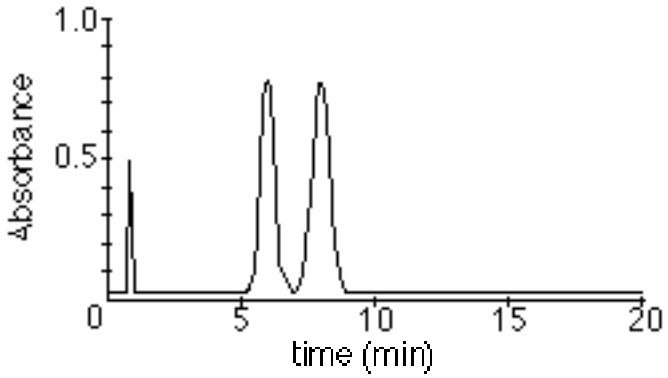
b) the retention volumes (V_R) for solutes A and B;

c) the resolution achieved for solutes A and B;

d) the capacity factors for solutes A and B;

e) the linear velocity of the mobile phase in the column.

Assume that the exact retention time for the compound A ($t_{R(A)}$) is 6.0 min and $t_{R(B)}$ = 7.8 min and that the width of the eluting peak at the baseline for compound A is 1.5 min and for compound B is 1.9 min. Also, the column is 4.2 mm in diameter and has a void volume of 1.2 ml yielding a t_m = 0.9 min. (25 points)



3. A technician at a local waste disposal company was trying to develop a separation of substituted aromatic compounds present in industrial sewage by normal phase adsorption chromatography. He first tried separating chlorobenzene, nitrobenzene, toluene, and xylene on a Dupont Zorbax® silica column using dichloromethane as the mobile phase and a fixed wavelength UV detector set at 254 nm. While chlorobenzene and nitrobenzene were adequately resolved on the silica column, toluene and xylene were found to coelute. Being an expert at liquid chromatography, he realized a less polar mobile phase solvent would improve the separation of toluene and xylene. When carbon tetrachloride was used as the mobile phase eluent, none of the solutes injected were detected by the UV detector. Puzzled, he analyzed the effluent from the hplc system by gas chromatography and discovered that the four solutes had in fact eluted from the column. After closely reading the labels of the two solvent bottles (see below), the technician realized his mistake. What error of mobile phase selection did he make?
(5 points) ?

Carbon Tetrachloride		Dichloromethane	
Mol. Wt.	153.82	Mol. Wt.	84.93
Boiling Point	77 ° C	Boiling Point	40 ° C
Refract. Index	1.457	Refract. Index	1.421
UV cutoff	265 nm	UV cutoff	233 nm
Viscosity	0.90 cP	Viscosity	0.41 cP
Dielectric Const.	2.24	Dielectric Const.	8.9

4. On the same graph, sketch the relative H.E.T.P (H) vs. linear velocity flow rate curves you would expect to observe for the following three GC capillary columns: (10 points))

a) a 10 meter column with 0.1 mm inner diameter

b) a 10 meter column with 0.2 mm inner diameter

c) a 20 meter column with 0.2 mm inner diameter

5. Briefly explain, via an appropriate diagram and words, how a flame ionization detector functions as a very sensitive GC detector. (10 points)

6. If you were paying attention during your Chem 447 lectures, you learned that modern ion-exchange chromatography systems set-up for the determination of inorganic ions generally utilize suppressor columns or membrane systems in conjunction with conductivity detection for the most sensitive measurement of the separated ions. Interestingly, a few years ago, your beloved (perhaps this is not the best word to use as this stage of this exam!!) 447 Professor introduced a slightly different way to detect the eluted ions in such suppressed ion chromatography systems. Indeed, a paper with the following title was published in the journal Analytical Chemistry (M. Trojanowicz and M. E. Meyerhoff, Anal. Chem. , 61, 787 (1989):

"Potentiometric pH Detection in Suppressed Ion Chromatography"

Quite surprisingly, this detection scheme yielded sensitivities equal to or better than the usual conductivity mode of detection. Using the determination of cations via ion-exchange as an example, explain via appropriate chemical reactions, how in the world you could use a simple pH electrode in place of the conductance cell to detect the eluted cations. (i.e., why does the pH of the effluent change as the cations are eluted?). Will the pH increase or decrease? (10 points)