Homework 3

1. (a) Why is high pressure needed in HPLC?
   (b) What is a bonded phase in LC?
   (c) Why does the efficiency (plate height) of liquid chromatography in creases as the stationary-phase particle size is reduced?

2. (a) What is ion suppression chromatography?
   (b) What is ion pair chromatography?
   (c) What is Hydrophobic interaction Chromatography?
   (d) What is Ion exchange chromatography?
   (e) What is the size exclusive chromatography?
   (f) What is affinity chromatography?
   (g) What is gradient polymer elution chromatography?
   (h) What is absorption chromatography?

3. Compare Gel permeation chromatography (GPC) and Gel filtration chromatography (GFC)?

4. In preparing a benzene/acetone gradient for an alumina HPLC column, is it desirable to increase or decrease the proportion of benzene as the column is eluted?

5. Compare the role of the mobile phase in GC, LC and SFC.

6. What is gradient elution? What is the most useful gradient elution in (a) GC, (b) LC, or (c) SFC, respectively?

7. How can the separation factor be manipulated in (a) GC, (b) LC, and (c) SFC?

8. (a) Nonpolar aromatic compounds were separated by HPLC on a bonded phase containing octadecyl group covalently attached to silica particles. The elution was 65% methanol in water. How would the retention time be affected if 90% methanol were used in stead?

(b) Octanoic acid and 1-aminooctane were passed through the same column described in (a), using an eluent of 20% methanol in water adjusted to pH 3.0 with HCl. State which compound is expected to be eluted first and why.

9. Suppose the HPLC column produces ideal Gaussian profiles for which

$$\text{area of peak} = (1.19)*h*w_{1/2}$$

Where h is peak height and is the width at half of the maximum peak height. The detector measures absorbance at 254 nm. A sample containing equal moles of compounds A and B was injected into the column. It is found on the record

paper that compound A (ε_{254}=2.26 x 10^4 $M^{-1}cm^{-1}$) has h = 128 mm and $w_{1/2}$ = 10.1 mm, and compound B (ε_{254}=1.68 x 10^4 $M^{-1}cm^{-1}$) has $w_{1/2}$ = 7.6 mm. What is the height of peak B in millimeters?

10. A series of protein and polymer MW standards were injected onto a 15 mm ID x 30 cm size exclusion column. The following elution volumes were at a flow-rate of 0.7 mL/min:

Standard	MW (g/mol)	V_R(mL)
Cyrochrome C	13,370	36.1
Bovine Serum Albumin	65,400	35.9
Aldolase	158,000	35.4
Catalase	210,000	32.5
Ferritin	440,000	28.4
Thyroglobulin	669,000	25.0
Blue Dextran 2000	2,000,000	24.8

a. Over what range can this column can be used in the determination of unknown molecular weights?
b. A compound with unknown NW was injected under the same conditions and eluted at 31.2 mL. What is the MW of this compound?

11. An affinity column containing an immobilized antibody which binds to insulin is to be used in quantitation of recombinant insulin produced by E. coli. A 4.1 mm ID x 5 cm column (V_m = 0.53 mL) is used which contains approximately 50 nmol of antibody. The equilibrium constant for the binding of this anitbody to insulin under physiological condition (pH 7.4) is 2×10^8 M^{-1}.

a. Assuming that two moles of insulin can bind per mole of antibody and that all of the antiboday on the column is active, what would be the value of k for insulin on this column at pH 7.4? What would be the retention time (t_R) for insulin at a flow of 1 mL/min?

b. The equilibrium constant for this system is 1×10^4 M^{-1} at pH 3.0. What would be the value of k and t_R for insulin under these condition?

12. Consider a protein with a net negative charge tightly adsorbed on an anion-exchange gel at pH 8.

a. Will a gradient of elution pH (from pH 8 to pH 4) be useful for eluting the protein? Assume that the ionic strength of the elution is kept constant.

b. Will be a gradient of ionic strength (at constant pH) would be useful for eluting the protein?

13. Compare liquid chromatography and thin-layer chromatography.

14. What is retardation factor (R_f) in TLC? and what is the relationship between R_f and capacity factor (k)?

15. Explain why the separation resolution is increased when using circular development instead of linear development in thin-layer chromatography?

16. a. What is supercritical fluid? b. list the advantages of supercritical fluid extraction over liquid extraction. c. What are the advantages of supercritical fluid chromatography?

17. What is electrophoresis and electroosmosis?

18. Explain how neutral molecules can be separated by micellar Electrokinetic capillary chromatography.

19. Compare HPLC and Capillary electrochromatography.

20. Compare capillary gel electrophoresis and gel electrophoresis.

21. The observed behavior of benzyl alcohol ($C_6H_5CH_2OH$) in capillary electrophoresis is given below. Explain what happens as voltage is increased.

Electric field (V/m)	Number of plates
6400	38000
12700	78000
19000	96000
25500	124000
31700	124000
38000	96000