

CHEM 322 Homework

Chapter 13: LC problems 2, 4, 6, 10, 12, 16. CE problems: 9, 5, 17a, 23

- 2 a. Advantage: May bind water to serve as a polar stationary phase (as in normal phase chromatography)
Disadvantage: May degrade separation by serving as active sites in a reverse phase separation.
- b. Suppress interaction by endcapping.
- c. Enhance interaction with water by first rinsing with strong base to ionize silanol groups.
4. HPLC support particles are typically smaller and spherical to allow efficient packing, while maintaining flow. The particles are also porous to allow for large surface areas. The stationary phase is typically a monomolecular layer bonded to the particles in HPLC, compared to a multilayer film coated on the particles in GC.

6.

	Separation type	Detector
a	Size Exclusion	UV (polymer should absorb)
b	RP-HPLC	ELSD
c	RP-HPLC	UV (aromatic)
d	Ion Chromatography	Conductivity or indirect UV
e	RP-HPLC	MS (gives molar mass information)
f	Chiral Separation using cyclodextrin columns	UV (phenols should absorb), or ELSD, or MS
g	Affinity Chromatography	UV or MS
h	RP-HPLC	MS or RI (May be too volatile to use ELSD)
i	Size Exclusion	RI or MS
j	RP-HPLC	ELSD
k	RP-HPLC	RI (no need for sensitivity)

10. Guard columns are attached prior to the separation column and are packed with the same type of stationary phase as the analytical column. Their purpose is to preserve the useful life of the analytical column by “catching” anything that might irreversibly adsorb on the stationary phase. Suppressor columns are attached after the separation column in an ion chromatography separation. They are packed with a material that is different than that in the analytical column. Their purpose is to decrease the overall conductivity of the mobile phase by introducing ions that form molecular species with mobile phase ions. Considering porous vs pellicular packing, it is desirable for the guard or suppressor columns to have minimal impact on the quality of the separation, therefore pellicular particles allow faster flow and minimal band broadening.+

12. a. High pressure is required because particle size is small and the stationary phase is well packed in the column.
 b. Stationary phases are bonded to prevent dissolution of the stationary phase material.
 c. Supports are endcapped to prevent interaction of the analyte with unreacted silanol groups, leading to peak broadening.
 d. At extreme pH, Si-O-Si bonds can be hydrolyzed, releasing stationary phase from the support.
16. a. If the stationary phase is a weak acid, pH can be used to change the number of ionized groups, therefore changing exchange capacity.
 b. The exchange group on the suppressor column has the opposite charge as the ion to be suppressed so that it can undergo a reaction to form a neutral compound, thus decreasing conductivity.
 c. The separator column is regenerated by flowing a concentrated solution containing the original, low affinity ions
 d. The suppressor column is regenerated buy backflowing an appropriate strong acid or base through the column, followed by a water rinse.
 e. The suppressor can be regenerated continuously by using an electrochemical cell to continuously produce the appropriate ions to restore the suppressor ion.

9.

Detector	Sensitivity	Selectivity	Gradient Compat.	Buffer/pH Compat.	Structural Info	Mass/Conc.
RI	Poor	Universal	No	OK	No	Conc.
ELSD	V. Good	Universal	Yes	No	No	Mass.
UV-Vis	Variable	Variable	Yes	Yes	Minimal	Conc.
Fluoresc.	V. Good	Very	Yes	Yes	Minimal	Conc.
EChem.	Variable	Very	Poor	OK	Minimal	Depends
Conduct.	OK	Universal	No	No	No	Conc.
MS	Good	Universal	Yes	OK	Yes	Mass

15.

- a. Increasing pH will promote the ionization of surface silanol groups on the capillary, increasing the charge on the surface and increasing EOF.
 b. Increasing buffer concentration should increase the charge density of the solution and increase EOF.
 c. Since EOF is proportional to potential gradient, increasing V for a given length increases EOF.
 d. Increasing length while maintaining voltage produces a smaller potential gradient, therefore lower EOF.
 Encapping silanol groups prevents the formation of an electrical double layer and inhibits EOF.
- 17 a. i) The choice of column depends on the pH range you plan on working in. If the pH is lower than the isoelectric point, the amino acids will be protonated (cationic)

- and a cation exchange column could be used. If the pH is basic, the amino acids will be anionic and an anion exchange column could be used. The analytical column could be coupled with a suppressor and conductivity detection used, or electrochemical detection is also a possibility.
- ii) React amino acids with derivatizing agent (like orthophthalaldehyde). Separate on C18 column and used fluorescence (or UV) detection.
 - iii) See discussion on page 862
23. See discussion beginning on p 863. MEKC separations rely on partitioning between the solvent (mobile phase) and micelles suspended in the mobile phase. This partitioning is much like that in chromatography. Since everything is moving as a result of EOF, the dynamic micellar phase is termed a pseudostationary phase.