CHEM 230
Fall, 2014
Special Topics Additional Problems
For Your Own Benefit – not to turn in

1. [**Related to FFF**] List one type of Field Flow Fractionation and the separation field used in that type:
Flow FFF – cross flow field
Electrical FFF – electrostatic force field
Thermal FFF – thermophoretic mobility
(other examples)

2. [**Related to FFF**] Relative to the direction of the flow in FFF, in which direction is the separation field pointed
a) with the flow
b) against the flow
c) at a right angle to the flow
d) it changes depending on movement

3. [**Related to FFF**] In FFF, analytes that are pushed more strongly toward a wall elute _*elute later (due to lower velocities at the wall)*_ (earlier or later).

4. [**Related to SFE**] A scientist testing extraction of a polar compound from tree bark through Soxhlet extraction using water notices that longer extraction periods gives lower yields. He suspects that the cause is compound degradation.
a) Is supercritical fluid extraction with CO_2 likely to decrease thermal losses? *Yes. SFE only needs a T > 30°C which will be lower than needed for water based Soxhlet (around 100°C).*
b) Is supercritical fluid extraction with CO_2 likely to be efficient? *No. Polar compounds are not extracted well by non-polar carbon dioxide.*

5. [**Related to SFE**] What properties of supercritical fluids make extractions of solids more time efficient than standard methods?
Greater diffusivity and lower viscosity allows better solvent penetration into solids. Greater diffusivity allows faster removal of analytes from solid (vs. conventional liquid extraction). Greater solvating power is an improvement over thermal desorption methods.

6. [**Related to SFE**] In the analysis of natural products in solid plant parts, it is desired to remove compounds of interest and then to obtain them at relatively high concentrations. Which step can be eliminated or minimized by switching from conventional extractions to SFE?
Solvent reduction steps (such as rotovapping) should not be needed because carbon dioxide will completely transfer to the gas phase leaving either no solvent (for pure carbon dioxide) or minimal solvent (for polar additives).

7. [**Related to Chiral Separations**] List one advantage and one disadvantage for separating enantiomers through derivation with chiral reagents and achiral (regular type) chromatography.
Advantage = _*cheaper, frequently effective*_ Disadvantage = __*more labor intensive*__

8. [**Related to Zirconia in HPLC**] Running a packed HPLC column at higher temperatures, while not common, generally results in a lower H value. In considering sources of broadening from the van Deemter equation, the main reason for a decrease in H is:
a) shrinking of particle size resulting in a smaller A term
b) an increase in diffusion resulting in a smaller B term
c) an increase in molecular diffusion resulting in faster mass transport and a smaller C term
d) all of the above

9. [**Related to Zirconia in HPLC**] What type of zirconia based HPLC column would be a good replacement of silica based C18 columns (e.g. for the analysis of bases at high pH)?
PBD-coated zirconia also shows similar retention as for silica based non-polar stationary phases.

10. [**Related to 2D HPLC**] In what way does the effectiveness of 2D HPLC depend on both the two methods of separation (done in the two columns) and on the types of samples?
The two methods should be orthogonal, there should not be transfer broadening (e.g. undersampling), and the sample should have solute that will fill the full two dimensional chromatographic space.

11. [**Related to 2D HPLC**] 2D HPLC is a fairly popular method for separating proteins in biological samples. List two possible orthogonal separation methods that could be used for proteins.
Combinations of reversed-phase, ion exchange (most commonly cation exchange) and size exclusion are common.

12. [**Related to Zwitterion stationary phase**] Why can conductivity detection be used with zwitterionic stationary phase (and no suppressor) with better success than with ion exchange (also without a suppressor)?
Compared to standard ion-exchange columns, more moderate reagents are needed to elute compounds. This means lower ionic strength eluents (with lower conductivity) can be used.

Longer Answer Questions:
1. [**Related to Chiral Separations**] A researcher is carrying out an asymmetric synthesis to predominantly synthesize one enatiomer. She then is analyzing the products (both enantiomers) to determine how successful the synthesis is using GC with a chiral column. If two product peaks are observed to elute, describe how she can determine the enatiomeric ratio. Are quantitative or qualitative standards needed?
The enantiomeric ratio generally can be given just by the peak area ratio. This is because enantiomers should give the same response (as their physical properties – other than light rotation – should be the same). So no quantitative standards are needed.
However, it is not possible to know a priori which enatiomer elutes first. So, qualitative standards are needed.

2. [**Related to Zwitterion stationary phase, ion-pairing and CE topics**]. List two special topic methods that allow analysis of neutrals, cations, and anions in a single run? In this method, what determines the elution order?
A) MEKC – elution order is determined by 1) charge of tubing wall (e.g. usually – in silica), 2) the charge of the analytes (cations generally elute fastest, then neutrals, and finally anions), and 3) incorporation into micilles (hydrophobic incorporate most and will elute faster than other molecules in charge group)
B) Ion-Pairing HPLC – elution order is determined by charge of ion-pairing reagent with analytes eluting fastest if same charge of ion-pairing ion (can't pair and typically not retained on C18 column), and slowest if opposite charge of ion-pairing reagent. Beyond that more hydrophobic molecules and ions elute later.
C) Zwitterionic Stationary phase – elution order depends on which charge is within the hydrophobic region. Typically, there is more retention for analyzes of charge opposite to the hydrophobic region charge.

3. [**Related to 2D HPLC**] A set of peptides is separated by 2D HPLC using a SEC first column and a RP second column. Assuming the peptides can vary by 1) fragment length and 2) % of non-polar amino acids (e.g. phenylalanine, leucine and valine). Indicate where long, polar peptides and short, non-polar peptides would come out in a 2D chromatogram.