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

1. [**Related to CE topic not in Exam 4**] In capillary electrophoresis, hydrodynamic injection means:
a) using an injection valve before the capillary
b) using pressure to force the sample into the capillary
c) using a voltage to migrate sample into the capillary [that is electroosmotic injection]
d) using a change in height to force the sample into the capillary [that is hydrostatic injection]

2. [**Related to CE topic not in Exam 4**] The main reason narrow capillaries are used in capillary electrophoresis is:
a) to limit dispersion when analytes partition to and from the capillary walls
b) thick walls are needed to avoid current leaks through the walls
c) to limit "joule" heating in the capillaries at high voltages
d) to keep the multiple flow term of the van Deemter equation small

3. [**Related to CE topic not in Exam 4**] Which term of the van Deemter equation contributes the most to band broadening in capillary zone electrophoresis?
a) the A term **b) the B term** c) the C term d) the u term

4. [**Related to CE topic not in Exam 4 and MEKC**] List a capillary electrophresis method that can be used for uncharged molecules.
Method = __*MEKC, capillary electrochromatography*__

5. [**Related to MEKC**] Micellar electrokinetic chromatography (MEKC) is performed with sodium dodecyl sulfate (SDS). The SDS concentration must be greater than the critical micelle concentration or:
a) No micelles form b) inverted micelles (hydrophobic exteriors) form
c) A reversal of polarity occurs on the fused silica surface (from negative to positive charge)
d) There are insufficient charge carriers for electrophoretic mobility

6. [**Related to SMB Chromatography**] How is the moving bed simulated in simulated moving bed chromatography?
a) by computer data reduction methods
b) by switching valves going to, between, or from an array of connected columns
c) by moving the column walls instead of the bed
d) by using a high potential to stimulate migration of the bed toward the cathode

7. [**Related to SMB Chromatography**] Why would SMB not be useful for isolation of natural products from plant extracts [in a single batch]? *SMB is only useful for binary separations. In the isolation of natural products from plant extracts, there will almost always be several compounds present. Normally, there will be impurities with greater or lesser retention than the*

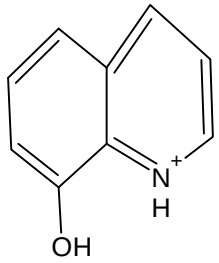
compound of interest. Isolation would require multiple passes through an SMB system while a single pass in column chromatography could possibly lead to isolation.

8. [**Related to SPME-HPLC**] For what type of compounds can SPME-HPLC be used but not SPME-GC? *Non-volatile or thermally labile compounds cannot be analyzed well by GC.*

9. [**Related to SPME-HPLC**] List two factors that influence the time it takes for analytes to diffuse off of an SPME fiber in an SPME type injection. factor 1 = _the analyte's size or molecular weight (affects diffusion constants) factor 2 = _the thickness of the coating (thinner is faster)___

Longer Answer Questions:

1. [**Related to CE topic not in Exam 4**] Given hydroxyquinoline's pK_a values (pK_{a1} = 4.94 for NH^+ proton and pK_{a1} = 9.8 for OH proton – see structure below) can be separated from neutral molecules by capillary electrophoresis using a silica capillary. Separations are attempted at pH = 4, 7.5, and 11. Indicate whether hydroxyquinoline will be well separated from neutral compounds at those pH values and the relative migration speed (fastest to slowest).



At pH < pK_{a1}, the predominant form is as shown (positively charged), and can be written as H_2L^+ and will migrate faster than neutrals. This will occur at pH = 4. At pK_{a1} < pH < pK_{a2}, the predominant form is HL, which is neutral (not even zwitterionic in this case). At that pH, the EOF will be somewhat slower, due to fewer $–O^-$ groups on the silica. At pH = 7, it will migrate nearly with the neutrals. Finally, at pH > pK_{a2}, the predominant form will be L^-, which will migrate slower than the neutrals.

2. [**Related to MEKC**] Discuss how the answers to question 1) would be changed if the separations were performed at the same pH but using MEKC.
The main effect here would be for neutral compounds. At pH = 7, the migration rate will decrease relative to hydrophilic neutrals because HL would exist part of the time within the negatively charged micelles which migrate at a rate less than the neutral compounds.

3. [**Related to SMB Chromatography**] A large-scale synthesis produces two diastereomers and also has left over excess reagents.
a) If the diastereomer of interest is less polar than the other diastereomer and the other reagents, could SMB (simulated moving bed) chromatography be used to collect at good purity the diastereomer of interest in a single run? Explain your answer.
Yes. The separation is a binary separation, so it is only possible to isolate two fractions. However, if the compound of interest is the least polar, it can be retained less (assuming normal phase) with all of the other compounds in the other fraction.
b) Could the other diastereomer also be collected in good purity?
No, at least not in one separation. That is because the other diastereomer will likely have compounds more polar (other reagents) and less polar (the diastereomer of interest). It could be collected in good purity, but it would require at least one other separation.

4. [**Related to ion-pairing HPLC**] Alkyl amines are bases with conjugate acid pK_a of around 10. Why can these be separated easily using C18 columns and ion-pairing HPLC while it is very difficult to do so by reversed-phase HPLC [without ion-pairing?]
The problem with standard reversed-phase HPLC is that the bases must be in their non-ionized form, and that only occurs at high pH. Unfortunately, most C18 columns are not stable at pH > 8 where the separation could occur. At pH under 8, these bases will be in their cation form. Those form ion-pairs with anionic species which will separate well.

5. [**Related to ion-pairing HPLC**] Glycine, $NH_3^+CH_2CO_2^-$, shown in its neutral pH zwitterionic form, has a pK_{a1} for $-CO_2H$ acid of 2.35 and a pK_{a2} of 9.78 for its $-NH_3^+$ acid. At what pH and with what type of counter ion could glycine be retained through ion-pairing HPLC on a reversed phase (C18) column? Can it be separated at any pH using standard reversed phase HPLC?
Glycine can exist in three forms: H_2L^+ at pH < pK_{a1}, HL (as a zwitterion) at pH between 2.35 and 9.78, and as L^- at pH > pK_{a2} of 9.78.

Separation of glycine in the anionic form is not possible on a standard C18 column because C18 columns are not stable at those pH values.

At low pH (e.g. 2), the main form will be cationic. At this pH, one can use an anionic counter ion (e.g. heptyl sulfate) and ion-pairing.

It may also be possible to use ion-pairing for the zwitterion, but this is complicated by the dual charges. Assuming the ion-pair species theory, any ion pair would still have a charge (e.g. alkyl sulfonate + glycine would have an extra negative charge from the $-CO_2^-$ group and shouldn't be retained. Assuming the alkyl sulfate is bound to the stationary phase and then interacts with ions, it would be possible for an ion-exchange mechanism of retention. Since at neutral pH glycine is a zwitterion, one could use either an anionic or a cationic ion-pairing reagent.