Complete the following. Clearly mark your answers. YOU MUST SHOW YOUR WORK TO RECEIVE CREDIT.

Warm-up (2 points each).

1. The _thermal conductivity detector (TCD)__ utilizes a series of heated filaments in its detection mechanism.

2. In _solid phase microextraction (SPME)_ a small fiber is inserted into the sample container to allow analyte to adsorb to its surface. The fiber can then be introduced into a GC for desorption and analysis.

3. In APCI, nebulized LC eluent passes through a ___corona discharge________ to produce solvent ions that ultimately lead to analyte ions.

4. Capillary electrochromatography, aggregates of surfactants called _____micelles________ form a _pseudo_ stationary phase to allow partitioning of analytes.

5. The _partition coefficient (distribution coefficient)_ is an equilibrium constant that describes the tendency for a solute to exist in the stationary phase relative to the mobile phase during a chromatographic separation.

Complete six of the following. Be clear and concise. Clearly indicate which problem is not to be graded. (15 points each)

6. Selection of a detector for separations often involves a tradeoff between universality (or selectivity) and sensitivity. Briefly describe why this is so, using examples of specific gas chromatography detectors to illustrate your point.

   Often, detectors that are particularly sensitive are tuned to probe a particular property of the analyte. For example, FID detectors in GC are designed to monitor combustible analytes in a mixture, but are insensitive to anything that doesn’t burn in the flame (like CO₂). In order to expand the range of analytes that a detector sees, it is often necessary to probe a property of the analyte that is less unique to a particular class of compounds. A typical outcome of this choice is poorer sensitivity. Consider the TCD in GC. In order to be more universal, it responds to a change in thermal conductivity of the carrier gas, however, this response is less sensitive that that of a FID.
7. Briefly describe the mechanism of separation in capillary zone electrophoresis. What parameters can be changed to optimize separation conditions in CZE?

Separation in capillary zone electrophoresis results from two phenomena, electrophoretic mobility and electroosmotic mobility. Electrophoretic flow occurs as a result of movement of charges species in the electric field that exists when a high voltage is applied across the length of the capillary. As a result of this electric field, cations are attracted to the cathode at the negative end of the capillary, and anions are attracted to the anode at the positive end. The rate at which ions move depends on their charge (multiply charged ions move more quickly than singly charged ions) and their mass (small ions move more rapidly than large ions). Neutrals are unaffected by the electric field and do not move as a result of electrophoretic flow.

Electroosmotic flow results from the general flow of solvent (water) toward the cathode end of the capillary. Since cations are more highly solvated than anions, they tend to "drag" solvent along as they move toward the cathode. Since solvation is a dynamic process, bulk solvent flow results, carrying all species (anions, cations, and neutrals) toward the cathode. Elution order is: cations, neutrals, anions.

Separation conditions can be modified by changing the separation voltage, capillary length, and to some degree, running buffer composition.

8. Mass spectrometry and evaporative light scattering have emerged as powerful detection schemes for HPLC, yet UV detection is still the most common mode for LC. For either MS or ELSD, describe the benefits of employing the detection scheme and how these benefits are realized. Given these benefits, why is UV detection still the most popular?

Key considerations:

**MS:** The main benefit of MS detection is the potential to get both quantitative information and structural (identification) information. The main challenge is in interfacing the LC and MS. Techniques like electrospray and APCI allow this mating, but the hardware is much more complex and expensive than UV detection.

**ELSD:** The main benefit of ELSD is that it is a fairly universal detection scheme, like refractive index, but is more sensitive than RI detection (not more sensitive than UV). The main challenges is that the hardware is more complex and expensive than UV and mobile phase modifiers (like buffers) lead to a large background and problematic analysis.

The main reasons that UV is still the most popular are that it has good sensitivity for compounds that absorb UV light, it is an established method, and it is much less complex and costly than MS or ELSD.
9. What is the general elution problem? Describe one approach in gas chromatography and one approach in liquid chromatography aimed at addressing the general elution problem. From a physical chemistry perspective, how does each approach alleviate the problem?

The general elution problem states that conditions that result in a good separation for one pair of compounds, will likely not result in the same quality separation for other compounds in the mixture. Both GC and LC approach this issue by altering the separation conditions during the course of the separation.

In GC, temperature programming is typically used. In this approach, the temperature of the separation column is raised throughout the course of the separation. Increasing the temperature gives more kinetic energy to the gas-phase eluent, decreasing retention time.

In LC, the composition of the mobile phase is altered throughout the course of the separation. In gradient elution, the resulting change in polarity that comes with this composition change, alters the retention time of analytes in the mixture.

10. Consider the Van Deemter equation. Which term is likely to be the primary contributor to band broadening in GC? Justify your answer. Does the same argument hold true for LC and CE? Why or why not?

The Van Deemter equation describes three classes of phenomena that contribute to band broadening in chromatography. The multipath term (A) is independent of flow rate and corresponds to the large number of possible routes and analyte may take as it moves through a packed column. As particle size increases, the difference in length of these routes is magnified, leading to an increased contribution of the A-term. The B-term is inversely proportional to flow rate and deals with the contribution of diffusion of analyte in the mobile phase along the axis of the separation column (longitudinal diffusion). The C term is directly proportional to flow rate and corresponds to mass transport of material into and out of the stationary phase.

Since diffusion coefficients are very large in the gas-phase, the longitudinal diffusion term dominates band broadening in GC. Since packed columns in GC are typically one to several meters in length, contribution from the A-term get averaged out over the length of the column. Fast diffusion also helps to minimize the contribution of the C-term as well.

Since diffusion coefficients are much smaller in the liquid phase, the relative contribution of all three terms are much more comparable. In CE, the absence of stationary phase packing and partitioning causes the A and C terms to become negligible. Therefore only longitudinal diffusion plays a significant role.
For problems 11 and 12, consider the chromatogram below that was obtained for a reverse-phase HPLC separation on a 25 cm column, using UV absorbance detection. Unretained compounds elute in 0.15 minutes.

11. Complete the following.
   a. Calculate the number of theoretical plates for component B.
   b. Calculate the selectivity factor of compound D over compound C.
   c. Calculate the resolution of compounds C and B.
   d. Which compound is the most polar? Justify your choice.

   a: For compound B:
   \[ N = \frac{(4t_r)^2}{W^2} = \frac{(4 \times 5.32 \text{ min})^2}{(0.19 \text{ min})} = 12,500 \text{ plates} \]

   b: \[ \alpha = \frac{k'_D}{k'_C} = \frac{(t_r)_C - t_m}{(t_r)_B - t_m} = \frac{(6.02 - 0.15)\text{ min}}{(5.63 - 0.15)\text{ min}} = 1.07 \]

   c: \[ R_s = \frac{2\Delta Z}{W_C + W_B} = \frac{2(5.63-5.31)\text{ min}}{(0.22+0.19)\text{ min}} = 1.56 = 1.6 \]
   Or: \[ R_s = \frac{(N)^{1/2}(\alpha-1)k_B}{4\alpha(1+k_B)} = \frac{(12500)^{1/2}(1.06-1)(21.52)}{4\times1.06\times(1+21.52)} = 1.51 \]

   d: In reverse-phase separations, the stationary phase is less polar than the mobile phase, meaning nonpolar compounds will be more strongly retained, while more polar compounds will elute first. Soooooo, A is the most polar compound.

12. Your boss looks at the chromatogram and makes the following statement: “Well, it is clear to me that compound D is present at about 2 times the concentration of compound A and that compound B is methamphetamine since it elutes at 5.39 minutes under these conditions.” Discuss the validity of this statement.

While it is true that the height (and maybe the area) of peak D is about 2 times that of peak A, there is no guarantee that the response of the detector is the same for both components. Depending on the mode of detection, the response may be quite different. For example, if UV absorbance detection is used and compound D has a much larger molar absorptivity at the detection wavelength than compound A, it could be the case that the concentration of D is actually less than the concentration of A. The only way to be sure is to prepare calibration curves for each component.

While the retention time of a particular compound is essentially constant under constant conditions, there is no guarantee that another compound cannot have the same retention time. If you have a good understanding of the composition of your sample, this assertion is better, but the only way to be sure is to have a detection scheme that can provide the identity of the compound (such as MS).
Possibly Useful Information

A = \log\left(\frac{P_0}{P}\right) = \varepsilon \beta c

\pi = 3.14159

k_A' = \frac{V_S}{V_M} = \frac{t_R - t_M}{t_M}

\alpha = \frac{K_A}{K_B} = \frac{k_A'}{k_B'}

N = \frac{L}{H}

H = \frac{\sigma^2}{L} = \left(\frac{W}{4t_R}\right)^2

N = \left(\frac{2.35t_R}{W_1/2}\right)^2

H = \frac{A + B}{u} + Cu = \frac{B}{u} + (C_S + C_M)u

R_S = \frac{\Delta Z}{W_A / 2 + W_B / 2} = \frac{2\Delta Z}{W_A + W_B}

R_S = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k_B'}{1 + k_B}\right)

v = (\mu_e + \mu_{eo})E = (\mu_e + \mu_{eo})V/L

\frac{v}{2D}

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Numbers in parentheses are mass numbers of most stable or most common isotope.

Atomic weights corrected to conform to the 1961 values of the Conference on Atomic Weights.

The group designations used here are the former Chemical Abstracts Service numbers:

\* Lanthanide Series

† Actinide Series