

LEARNING OBJECTIVES – SEPARATION SCIENCE – CHROMATOGRAPHY UNIT

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In-class Problem Set – Extraction

Problem #1

After completing this problem, the student will be able to:

1. Devise a scheme to be able to isolate organic acids, bases and neutrals through an extraction process.
2. Devise a scheme to be able to extract metal ions into an organic phase.
3. Predict the general effect of pH on the extraction efficiency of a metal ion into an organic phase.

Problem #2

After completing this problem, the student will be able to:

1. Devise a scheme to be able to solubilize an organic ion with a lipophilic component into an organic phase using ion pairing.

After the introductory lecture material on chromatography, the student will be able to:

1. Write the expression for the distribution coefficient
2. Write the expression for the partition coefficient
3. Describe how paper and thin-layer chromatography work
4. Write the definition of adsorption and partitioning
5. Describe the procedure for coating a liquid chromatographic phase
6. Write the expression for the selectivity factor
7. Write the expression for the retention or capacity factor
8. Describe the concepts of a theoretical plate and height equivalent of a theoretical plate
9. Write the expression for the resolution of two chromatographic peaks

Chromatography - In-class Problem Set #1

Problem #1

After completing this problem, the student will be able to:

1. Draw the idealized plot of the concentration of analyte in the stationary phase versus the concentration in the mobile phase as a function of increasing concentration of analyte injected into the chromatographic system.
2. Recognize that in a real situation, the stationary phase will become saturated and the plot will deviate from ideality and exhibit Langmuir behavior
3. Predict and justify what the chromatographic peaks will look like for Langmuir and anti-Langmuir behavior

Problem #2

After completing this problem, the student will be able to:

1. Realize that a molecule dissolved in a liquid stationary phase moves by the process of diffusion.

Problem #3

After completing this problem, the student will be able to:

1. Realize that a molecule dissolved in the mobile phase moves by two processes: (1) diffusion and (2) the physical motion of the flowing mobile phase.

Problem #4

After completing this problem, the student will be able to:

1. Realize that starch, silica gel and alumina have hydroxyl groups on their surfaces and are therefore highly polar materials.
2. Draw a plot of the distribution of enthalpies of adsorption for a molecule adsorbing onto silanol, disilanol and trisilanol groups.
3. Recognize that the overall enthalpy of adsorption is the sum of the individual plots.
4. Describe why the distribution of the enthalpy of adsorption is not symmetric.
5. Predict the shape of the peak that would result for such a distribution of the enthalpy of adsorption and explain why this is not a favorable situation for chromatographic efficiency.

Chromatography - In-class Problem Set #2

Longitudinal Diffusion

After completing this problem, the student will be able to:

1. Rationalize why a band of a compound in a chromatographic column will spread out in both directions due to normal diffusion processes.
2. Explain why longitudinal diffusion is more significant in gases than liquids
3. Predict and rationalize the effect that the mobile phase flow rate has on the contribution of longitudinal diffusion to chromatographic peak broadening.
4. Write an appropriate term for the van Deemter equation that includes the flow rate dependency of longitudinal diffusion.

Eddy Diffusion

After completing this problem, the student will be able to:

1. Rationalize why different molecules will have different paths with different path lengths through a packed bed of particles.
2. Explain why the difference between the shortest and longest path is a function of particle size and is smaller for smaller particles.

3. Explain the meaning of channeling in a chromatographic column and why channeling contributes significantly to broadening.
4. Explain why it is more difficult to efficiently pack small particles.
5. Explain why open tubular capillary columns do not exhibit eddy diffusion.
6. Rationalize why there is still debate about whether or not eddy diffusion has a flow rate dependency.

Stationary Phase Mass Transport Broadening

After completing this problem, the student will be able to:

1. Rationalize why molecules in the mobile phase will move ahead of molecules in the stationary phase in a chromatographic column and explain why this contributes to peak broadening in a chromatographic system.
2. Recognize that molecules must spend a finite amount of time in the stationary phase.
3. Justify why the contribution of stationary phase mass transport broadening to peak broadening depends on the flow rate and is more significant the higher the flow rate.
4. Write an appropriate term for the van Deemter equation that includes the flow rate dependency of stationary phase mass transport broadening.
5. Explain why the dependence on flow rate for stationary phase mass transport broadening represents a limitation to completing the chromatogram in a short period of time.
6. Rationalize why it is best to have the stationary phase be as thin as possible.
7. Rationalize why it is best to have a homogeneous coating of the stationary phase.
8. Explain the process for coating capillary gas chromatographic columns.
9. Rationalize why smaller particles lead to thinner coating and reduced stationary phase mass transport broadening when using coated phases for gas chromatographic separations that have the same weight percent loading.
10. Explain why stationary phase mass transport broadening is more significant in liquid chromatography than it is in gas chromatography.
11. Describe the process used to prepare bonded phase materials for use in liquid chromatography.
12. Explain the advantage of using bonded over coated stationary phases in liquid chromatography.

Mobile Phase Mass Transport Broadening

After completing this problem, the student will be able to:

1. Rationalize why it is best for molecules in the mobile phase to be able to encounter the stationary phase as quickly as possible and explain why the finite time it takes for a molecule to encounter the stationary phase contributes to peak broadening in a chromatographic system.
2. Recognize that molecules must spend a finite amount of time in the mobile phase before encountering the stationary phase.
3. Justify why the contribution of mobile phase mass transport broadening to peak broadening depends on the flow rate and is more significant the higher the flow rate.
4. Write an appropriate term for the van Deemter equation that includes the flow rate dependency of mobile phase mass transport broadening.

5. Explain why the dependence on flow rate for mobile phase mass transport broadening represents a limitation to completing the chromatogram in a short period of time.
6. Rationalize why a capillary column with a narrower internal diameter will have less mobile phase mass transport broadening.
7. Justify why capillary liquid chromatography is usually not a practical method to use widely in the laboratory.
8. Rationalize why packed columns with smaller particles will have less mobile phase mass transport broadening than those with larger particles.
9. Explain why mobile phase mass transport broadening is much more significant in liquid chromatography than it is in gas chromatography.
10. Justify why the particles used in liquid chromatographic columns are much smaller than those in gas chromatographic columns.
11. Explain why, when using bonded liquid chromatographic phases, shorter columns can be used if smaller particles are used.

After completing the development of the van Deemter equation, the student will be able to:

1. Draw a plot of h versus v for a typical gas and liquid chromatographic packed column.
2. Rationalize the general form of the plot in (1).
3. Explain why most analyses are not performed at the flow rate that optimizes the efficiency of the column.
4. Explain for each of the following (separately for gas and liquid chromatography), referring back to specific terms and processes incorporated into the van Deemter equation, its expected impact of or relationship to column efficiency:
 - a. Particle size
 - b. Flow rate
 - c. Using a gas versus a liquid as the mobile phase
 - d. Switching from a packed to a capillary column
 - e. Coating a thicker stationary phase
 - f. The presence of channels in the packing material
 - g. Coating a more homogeneous mobile phase
 - h. Carry out a more extensive end-capping of silanol groups on bonded liquid chromatographic phases.
5. For the same items listed in number (4), explain what other positive or negative effects it has on the chromatographic process. This can include aspects such as analysis time or experimental variables or constraints that it places on the analyst.

Chromatography - In-class Problem Set #3

Problem #1 – Fundamental Resolution Equation

After completing this problem, the student will be able to:

1. Describe each of the different ways in which the number of plates on a chromatographic column can be increased.
2. Explain and rationalize whether there any tradeoffs associated with these changes?
3. Describe ways in which the separation factor can be increased.

4. Explain why there are limits to the effect that increasing the separation factor has on chromatographic resolution.
5. Describe ways in which the retention factor can be increased in gas and liquid chromatography
6. Explain and rationalize whether there any tradeoffs associated with changes designed to increase the retention factor.
7. Explain why there are limits to the effect that increasing the retention factor has on chromatographic resolution.
8. Examine a chromatogram with unacceptable levels of resolution and determine which term of the three in the fundamental resolution equation is the best one to alter and the best way to first go about altering it.

Problem #2 – Fundamental Resolution Equation

After completing this problem, the student will be able to:

1. Recognize peaks in a chromatogram that have either too small or too large of a retention factor.
2. Describe a way in gas chromatography to optimize the retention factor for each component of a multicomponent mixture.
3. Describe a way in liquid chromatography to optimize the retention factor for each component of a multicomponent mixture.

After lecture material on the basics of liquid chromatography, the student will be able to:

1. Draw and describe the basic setup of a liquid chromatograph.
2. Draw and describe the operation of a reciprocating small-volume pump with inlet and outlet check valves.
3. Describe the operation of and flow paths through a liquid chromatographic injection valve.

After lecture material on steric exclusion chromatography, the student will be able to:

1. Describe the influence of the pore size on the separation
2. Rationalize why larger molecules elute earlier in steric exclusion chromatography
3. Rationalize why steric exclusion separations occur in one column volume
4. Describe the procedure through which steric exclusion chromatography can be used to determine the approximate molecular weight of a substance
5. Explain why steric exclusion chromatography is actually a size-based rather than weight-based method

In-class Problem Set #4 – Ion-Exchange Chromatography

Problem #1

After completing this problem, the student will be able to:

1. Describe a scheme using ion exchange chromatography that would enable you to deionize water.
2. Explain why high capacity ion exchange resins are desirable for deionizing water.

Problem #2

After completing this problem, the student will be able to:

1. Explain why high capacity ion exchange resins are not desirable for analytical (trace analysis) separations.

Problem #3

After completing this problem, the student will be able to:

1. Justify the order of retention for Li(I), Na(I), and K(I) on a cation exchange column based on their attractiveness toward the resin.
3. Justify the order of retention for Li(I), Na(I), and K(I) on a cation exchange column based on their attractiveness toward the mobile phase.
4. Relate which of the two situations (attractiveness toward the resin or mobile phase) is more significant in affecting retention order for ions of the same charge but different size.
5. Justify the order of retention on a cation exchange column for two ions that have the same size but a charge of +1 or +2 based on their attractiveness toward the resin.
6. Justify the order of retention on a cation exchange column for two ions that have the same size but a charge of +1 or +2 based on their attractiveness toward the mobile phase.
7. Relate which of the two situations (attractiveness toward the resin or mobile phase) is more significant in affecting retention order for two ions of the same size but different charge.

Problem #4

After completing this problem, the student will be able to:

1. Describe and rationalize a detection scheme for ion chromatography that would allow for the measurement of the conductivity of the analyte ions while selectively removing the conductivity of the eluent ions.
2. Describe and rationalize a detection scheme that would allow you to detect ions separated in ion chromatography using indirect spectrophotometric methods.

After lecture material on bonded phase liquid chromatography, the student will be able to:

1. Explain the difference between a normal and reversed-phase separation
2. Predict the retention order for compounds separated on a normal or reversed phase liquid chromatographic column.
3. Determine the effect of pH on the elution time and retention order of compounds separated by reversed-phase liquid chromatography.
4. Describe how liquid chromatography can generally be used to separate pairs of enantiomers.
5. Describe the technique of ion-pairing chromatography.

After lecture material on gas chromatography, the student will be able to:

1. Draw and describe the basic setup of a gas chromatograph
2. Describe the difference between a split and splitless injection and justify when and why you would want to use each
3. Describe the different injection technique for gas chromatography. This includes the use of liquid injection and adsorbent traps.
4. Describe the purge and trap method

5. Predict the retention order for a series of compounds separated by gas chromatography.
6. Describe the following detection devices for a gas chromatograph, including their advantages
 - a. Flame ionization
 - b. Thermal conductivity
 - c. Electron capture
 - d. Mass spectrometry