

## IN-CLASS PROBLEMS – SEPARATION SCIENCE CROMATOGRAPHY UNIT

Thomas Wenzel, Bates College

### Extraction

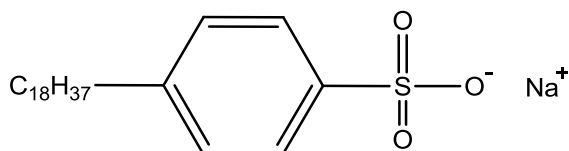
1. Devise a way to separate the materials in the following sample by performing an extraction.

The sample consists of water with a complex mixture of trace levels of organic compounds. The compounds can be grouped into broad categories of organic acids, organic bases and neutral organics. The desire is to have three solutions at the end, each in methylene chloride, one of which contains only the organic acids, the second contains only the organic bases, the third contains only the neutrals.

Remember - Ions are more soluble in water than in organic solvents.

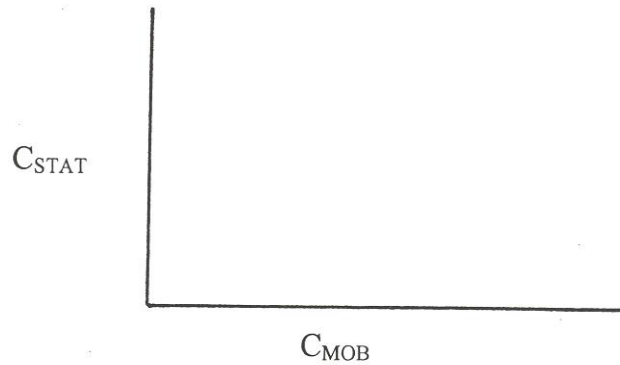
- Neutrals are more soluble in organic solvents than in water.

2. Devise a way to solubilize the organic anion shown below in the organic solvent of a two-phase system in which the second phase is water. As a first step to this problem, show what might happen to this compound when added to such a two-phase system.

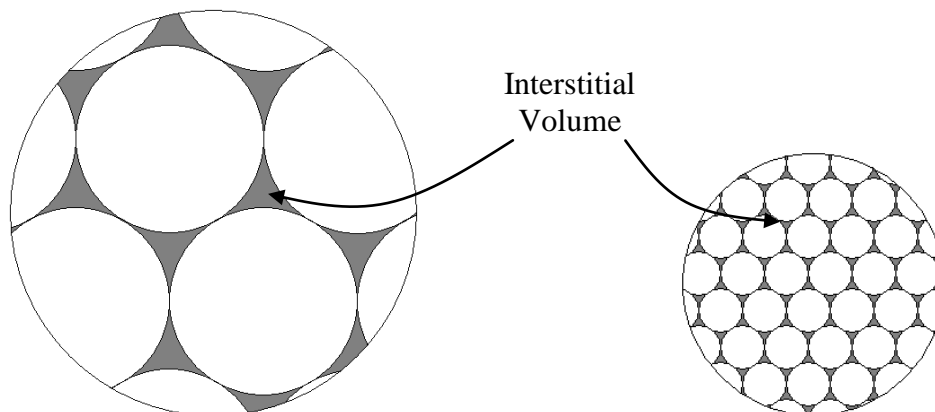


## General Chromatographic Concepts

1. Consider a plot that has the concentration of analyte in the stationary phase on the Y-axis and the concentration of analyte in the mobile phase on the X-axis.



- a) Draw an idealized plot as greater concentrations of analyte are injected into the chromatographic column.
  - b) Draw what you suspect would really happen.
  - c) What might the peaks look like in the real versus ideal situations?
2. What term would we use to describe the movement of a molecule in a liquid stationary phase?
  3. What processes would account for the movement of a molecule through a region of interstitial volume in a mobile phase?

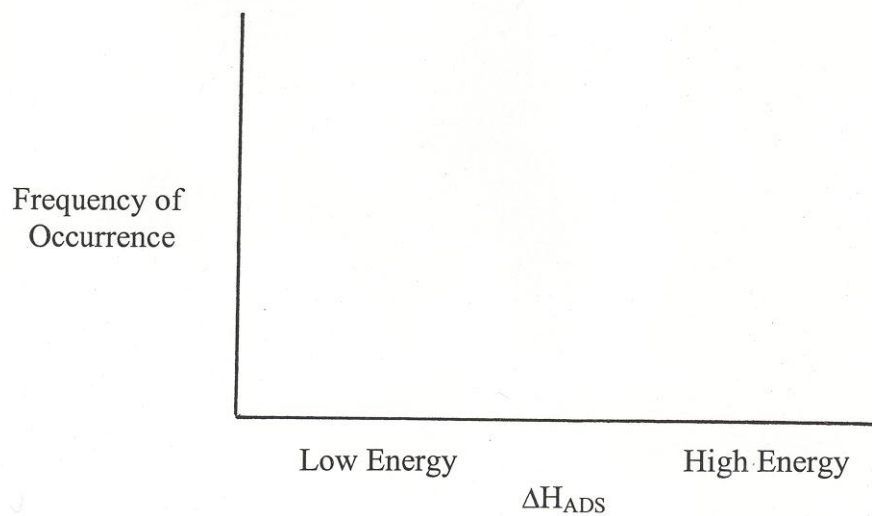


4. Tswett used starch as his stationary phase.

a) What is the dominant surface functionality of starch?

b) The two other common solid stationary phases are silica gel ( $\text{SiO}_2$ ) and alumina ( $\text{Al}_2\text{O}_3$ ). What do you think are the surface functionalities of these materials?

c) Draw a plot of the distribution of enthalpies of adsorption for a molecule on the surface of starch, silica gel, or alumina.



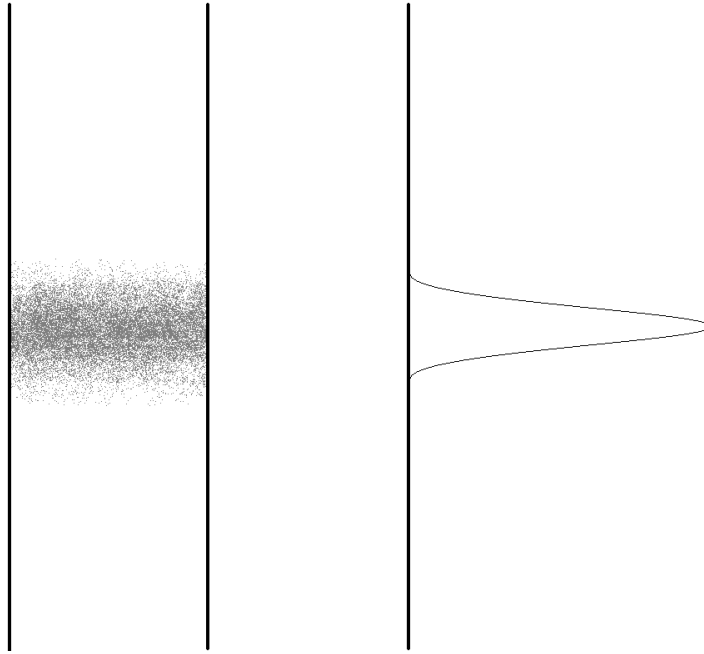
d) What would a chromatographic peak look like on such a phase with such a plot of adsorption enthalpies?

e) What is the problem with your peak?

## Peak Broadening

Development of **Longitudinal Diffusion Broadening** in Chromatography

Consider a “band” of a compound in a chromatographic column. The band has the following concentration profile.



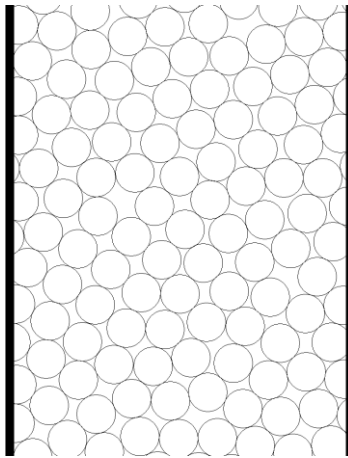
a) What would happen to this profile if the flow of the column were stopped and the column was allowed to sit?

b) Would this phenomena be more significant (i.e., happen faster) in a gas or a liquid?

c) Does this phenomena contribute more to band broadening at higher or lower flow rates?

## Development of **Eddy Diffusion Broadening** in Chromatography

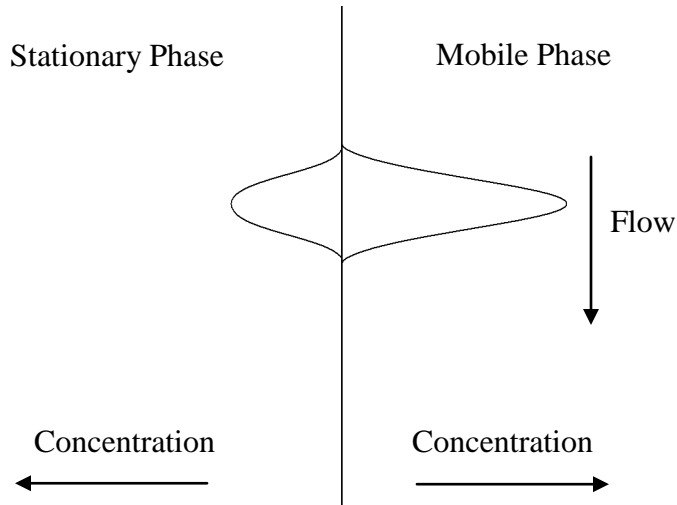
Consider molecules flowing through a packed bed of particles.



- a) Would different molecules have different path lengths as they passed through the bed?
  
- b) Is the difference in length between the shortest and longest path dependent at all on the diameter of the particles? If so, which particles (smaller or larger) would lead to a greater difference?
  
- c) Some packed columns exhibit channeling. What do you think is meant by this term?
  
- d) Would channeling be more likely to occur with smaller or larger particles? In other words, which is more difficult to pack efficiently, larger or smaller particles? A column is packed efficiently when the particles are in a uniform bed with the minimum amount of voids.
  
- e) Do open tubular capillary columns exhibit eddy diffusion?
  
- f) Does this phenomena exhibit any dependence on the flow rate?

## Development of **Stationary Phase Mass Transport Broadening** in Chromatography

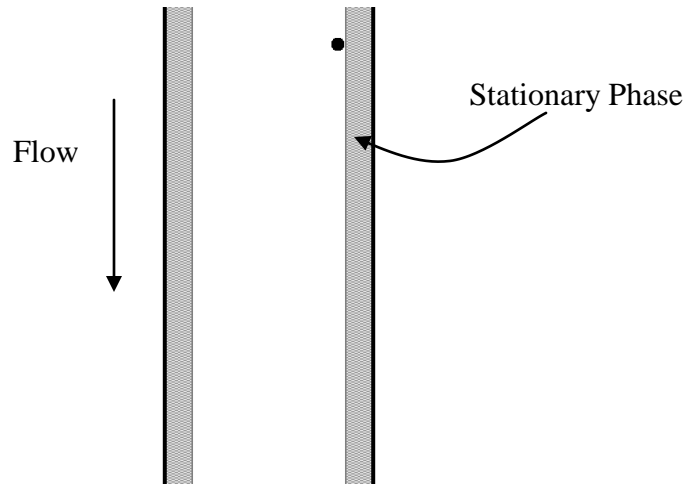
Consider a compound that has distributed between the mobile and stationary phase within a plate in a chromatographic column. The following might represent the concentration distribution profiles in the two phases (note that the compound as depicted has a slight preference for the mobile phase).



- What would happen to these concentration profiles a brief instant of time later?
- Will what happens in (a) contribute to band broadening? Explain.
- Does the contribution of this phenomenon to band broadening exhibit a dependence on flow rate? If so, are there any troublesome aspects to this dependence?
- What happens to this effect as the stationary phase coating is made thicker?
- Capillary GC columns have very thin coatings. Describe one advantage of these columns.
- Compare the effect of this phenomenon on a uniform versus non-uniform stationary phase coating.
- Is this effect of more concern in gas or liquid chromatography?

## Development of **Mobile Phase Mass Transport Broadening** in Chromatography

Consider a capillary column as shown below.



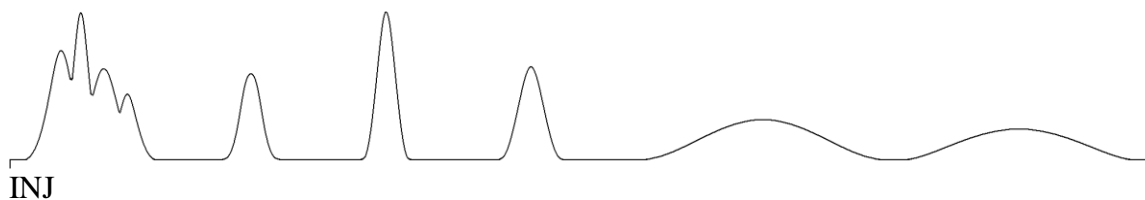
The dot represents a molecule that has just left the stationary phase and is about to diffuse across the mobile phase and re-encounter the stationary phase on the other side of the column.

- Draw a line representing the path of the molecule.
- What would this path look like if the flow rate were doubled?
- Is it important for the molecule to encounter the stationary phase? Think about a situation in which the flow was so fast that the molecule never re-encountered the stationary phase.
- If using capillary columns, what does this suggest about the desirable diameter for such a column?
- Is this phenomenon worse in gas or liquid chromatography?
- How does the contribution to band broadening depend on flow rate?
- Would this effect be observed in a packed column? If so, how?
- How could you reduce this effect in a packed column?
- Say something about the length of column needed if you undertake what you have suggested in (h) assuming that you have maintained a constant thickness of stationary phase.

## Fundamental Resolution Equation

1. Describe ways in which the number of plates on a chromatographic column can be increased. Are there any tradeoffs associated with these changes?
2. Describe ways in which the separation factor can be increased. Are there limits to the effect that increasing the separation factor has on chromatographic resolution?
3. Describe ways in which the retention or capacity factor can be increased. Are there any tradeoffs associated with these changes? Are there limits to the effect that increasing the retention factor has on chromatographic resolution?

4. Consider the following chromatogram.



The early eluting peaks and later eluting peaks exhibit a problem.

- a) Describe the chromatographic nature (there is a particular term that describes each) of the problem.
- b) Propose a way in gas chromatography to eliminate both problems.
- c) Propose a way in liquid chromatography to eliminate both problems.



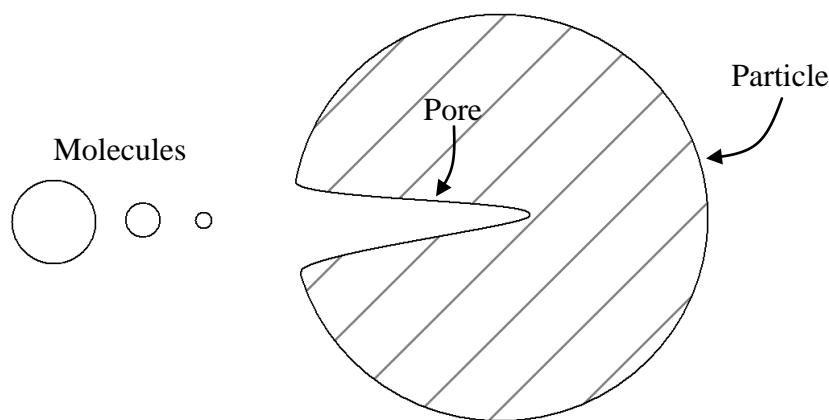
## Steric Exclusion Chromatography

Steric exclusion chromatography is a technique that separates compounds solely on the basis of size. In order for the results of steric exclusion separations to be meaningful, there can be no directed forces (no attractive forces whatsoever) between the compounds being separated and the surface of the particles used as the stationary phase. Instead, the particles are prepared with well-characterized pore sizes.

Steric exclusion chromatography requires large compounds, and is not generally effective on things with molecular weights of less than 1000.

The figure below shows a particle with one pore in it. It also shows representations for several molecules of different size.

Note: the pore size and molecule size are not representative of the scale that would exist in real steric exclusion phases – the pore is bigger than would actually occur and the molecules would never have sizes that big relative to the size of the particle.



1. Draw a chromatogram showing the elution order of the three molecules and label the peaks. Justify your retention order.
2. Suppose we had a molecule that was even larger than the biggest one in the picture above. Where would this elute in your chromatogram?
3. Suppose we had a molecule that is smaller than the smallest pictured above. Where would this elute on your chromatogram?
4. How could you separate the two large molecules?
5. How could you separate the two small molecules?
6. What conditions would you use for the mobile and stationary phase to eliminate any attractive forces between the molecules being separated and the stationary phase?

7. There are four important volume terms to consider for the column.

$V_T$ : The total volume of the column.

$V_I$ : The interstitial volume between the particles.

$V_P$ : The volume of all of the pores.

The volume occupied by the material that makes up the particles.

8. What amount of the total column volume do you think is interstitial volume?

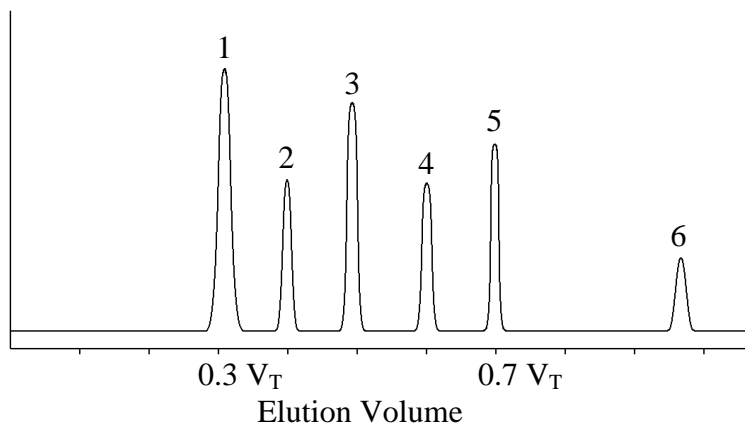
$$V_I = (??) \times V_T$$

9. What amount of the total column volume do you think is pore volume?

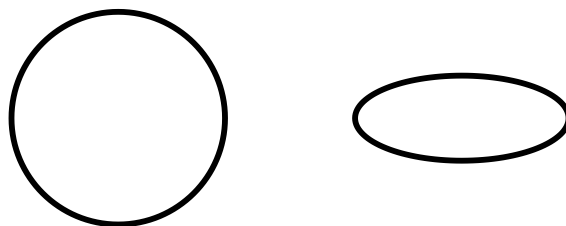
$$V_P = (??) V_T$$

10. Remembering that there are no directed forces in a steric exclusion column between the molecules and the stationary phase, label the elution volume on the chromatogram that you drew for question (1).

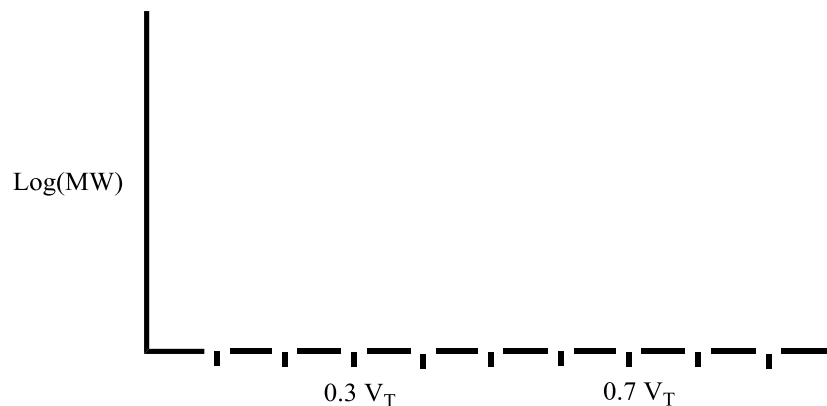
Suppose you obtained the following chromatogram for a series of compounds being separated on a steric exclusion.



11. What concern may you have about the peaks labeled 1 and 5?
12. How would you explain the elution volume of the peak labeled 6?
13. The picture below shows representations for spherical and rod-shaped molecules. Which of the two molecules would elute earlier in a steric exclusion separation?



14. Assuming the molecular weights of a set of molecules correlates with their size (i.e., the larger the molecule, the higher the molecular weight). Draw a general plot that would be obtained for the log of the molecular weight versus the elution volume.

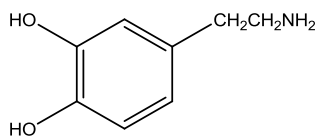


## **Ion-Exchange Chromatography**

1. Describe a scheme using ion exchange chromatography that would enable you to deionize water. Say something about the capacity of the ion exchange resins you would use for this purpose.
2. Would ion exchange resins that are useful for deionizing water be useful for analytical separations?
3. What would be the order of retention for the ions Li(I), Na(I), and K(I) on a cation exchange resin? Justify your answer.
4. Consider the case of separating the alkali ions in (3) on a polystyrene resin using a fairly dilute solution of hydrochloric acid as the mobile phase.
  - a) What is the bound ion and mobile counter ion?
  - b) One problem is how to detect these ions. They do not absorb ultraviolet or visible light in the accessible portion of the spectrum. They do not absorb infrared light. Conductivity might work except that the hydrochloric acid in the mobile phase produces too high a background signal. Devise a way to remove the conductivity of the eluent ions (HCl) but retain the conductivity of the alkali ions you wish to detect.

## Bonded-phase Liquid Chromatography

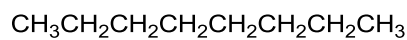
1.  $C_{18}$  silica gel phases are stable over the range of 2 to 8 (although 3 to 7 really represents a safer window).
2. Predict the relative retention times for a carboxylic acid on a  $C_{18}$  column using a mobile phase where the aqueous portion has a pH 3 versus pH 7.
3. Predict the relative retention times for a primary amine on a  $C_{18}$  column using a mobile phase where the aqueous portion has a pH 3 versus pH 7.
4. Predict the retention order of pyridine and *tert*-butylpyridine on a reversed-phase  $C_{18}$  column column.
5. A number of neurotransmitters such as dopamine have aromatic and amine functionalities. Suppose these amines are protonated in the mobile phase being used on a  $C_{18}$  column. Can you think of a way to lengthen the retention time that does not involve adjustment of the pH?



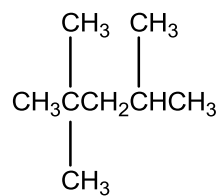
Dopamine

## Gas Chromatography

1. What factors influence the retention order in gas chromatography?
2. Which compound do you think has a higher boiling point, pentane or 1-pentanol?
3. Which compound do you think has a higher boiling point, 1-pentanol or 1-hexanol?
4. Which compound do you think has a higher boiling point, *n*-octane or *iso*-octane.

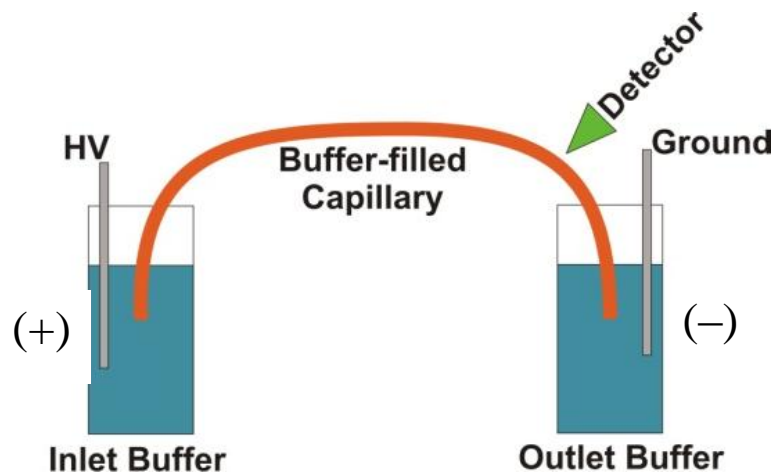


*n*-octane



*iso*-octane

## Capillary Electrophoresis

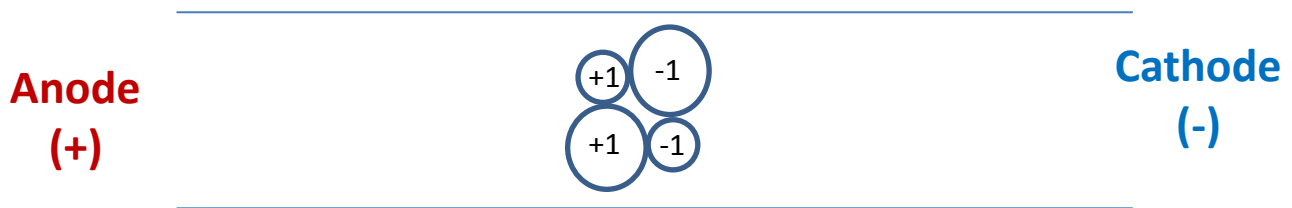


1. What is the functional group on the surface of a fused silica capillary column? Fused silica is a silicon dioxide ( $\text{SiO}_2$ ) polymeric material.
2. Would the nature of the interior surface of the capillary be different if the background electrolyte was at pH 2 versus pH 9?

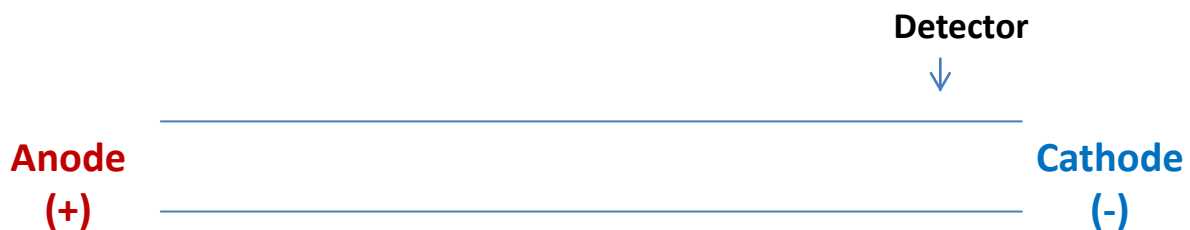
3. Assuming the silanol groups are deprotonated to anionic silanate groups, what do you think will happen in the portion of the solution in immediate contact with the surface of the fused silica?
4. What happens in the diffuse layer in the presence of this applied electric field?
5. Which flow profile, hydrodynamic or electroosmotic, results in greater peak broadening?
6. As previously discussed, broadening in chromatographic columns results from eddy diffusion, longitudinal diffusion, and mass transport broadening in the stationary and mobile phases. Which of these processes contribute to peak broadening in capillary electrophoresis?
7. What would happen to the electroosmotic flow rate at pH 2.5 versus pH 10?
8. Suppose the compounds being analyzed have a charge. Is there any other process that will result in analyte ions moving in the capillary column?
9. What variables influence the electrophoretic migration rate of a charged analyte?



10. Imagine that four ions start in the middle of the capillary as shown below. Assuming that the ions only move as a result of electrophoresis, draw the position of the ions after 30 kV has been applied for a short time.



11. Now consider the separation of a sample that has anionic, cationic and neutral analytes and has both electroosmotic and electrophoretic flow. The detector is placed on the end of the capillary near the cathode as shown below. Anions, cations and neutrals are all detected. Explain.

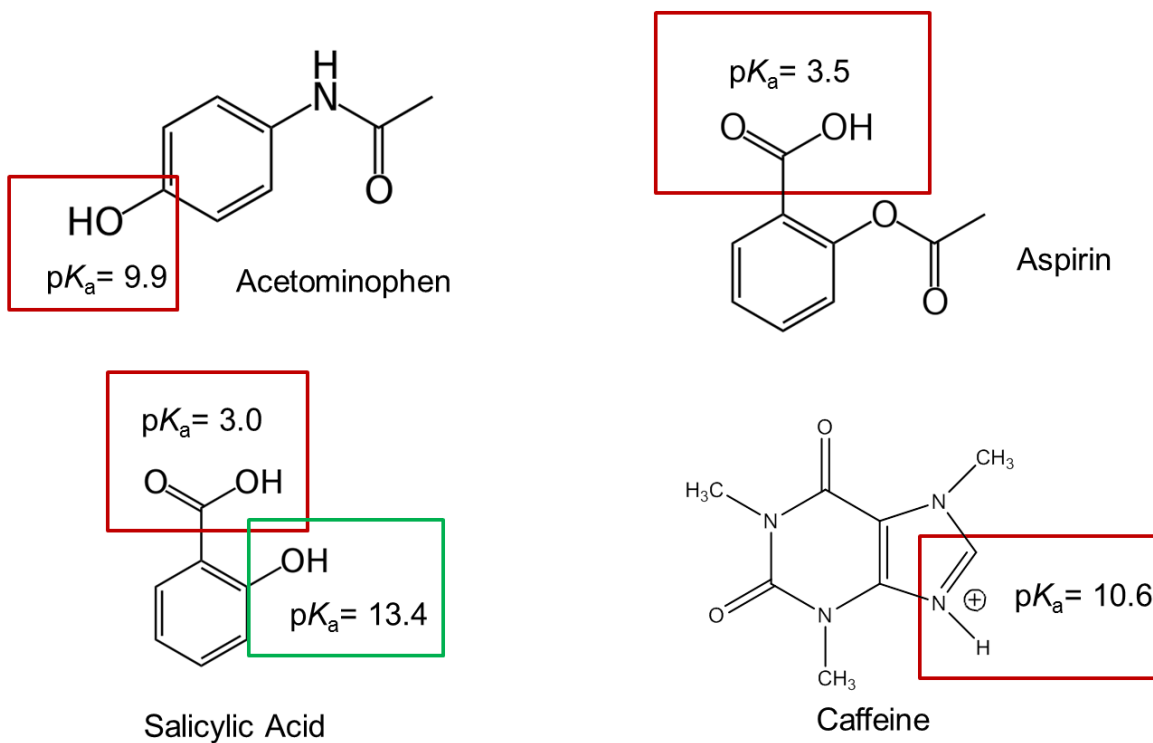


12. What is the predicted elution order of the following five substances? In addition, draw an electropherogram of the results and explain your reasoning.

- Substance A: neutral
- Substance B: -1 charge
- Substance C: +1 charge
- Substance D: neutral
- Substance E: -2 charge

Substances A, B, C, and E are all the same size. Substance D is twice as large as the others.

13. The structural formulas and relevant  $pK_a$  values for the four components of an Exedrin tablet are shown below. (The protonated form of the boxed group is shown.) Predict the elution order in CE with a 20 mM borate buffer at  $pH = 9.3$ .



14. What could you do experimentally to confirm your predicted elution order?