

OUT OF CLASS PROBLEMS – SEPARATION SCIENCE CHROMATOGRAPHY UNIT

Homework 1

1. What would be the effect on a gas chromatographic peak of introducing the sample at too slow a rate (i.e., making a very slow injection into a gas chromatograph from a syringe)?
2. Describe the different contributions to peak broadening in a gas or liquid chromatographic system.
3. Consider the following changes in a chromatographic system. Other than the specified change, everything else about the system is kept constant.
 - a) Increasing the weight of stationary phase on the inner walls of a capillary gas chromatographic column.
 - b) Increasing the flow rate of a packed liquid chromatographic column.
 - c) Reducing the particle size of the packing in a liquid chromatographic column.
 - d) Decreasing the column temperature of a capillary gas chromatographic column.

What would be the effect of each of the above on the plate height? Explain each answer by referring specifically to terms in the equation that describes chromatographic peak broadening.

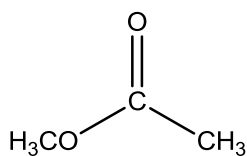
What would be the effect of each of the above on the resolution of two chromatographic peaks? Explain each answer by referring specifically to terms in the fundamental resolution equation.

What would be the effect of each of the above on the analysis time? Justify each answer.

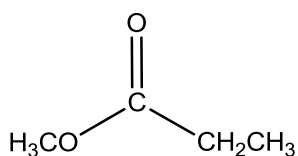
4. Describe three general terms that can be adjusted to improve resolution in chromatographic separations and explain specific experimental changes that can be made to adjust these parameters.

Homework 2

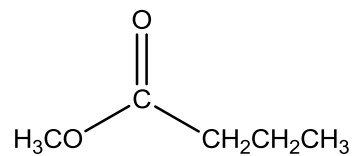
1. Consider the gas chromatographic separation of the esters methyl acetate, methyl propionate, and methyl *n*-butyrate on a column containing a stationary phase of intermediate polarity.
 - (a) What would be the retention order on this column?
 - (b) Would this retention order change if a non-polar stationary phase had been used instead? Explain.
 - (c) Would the retention times change on the non-polar column? Explain.



Methyl acetate

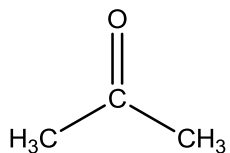


Methyl propionate

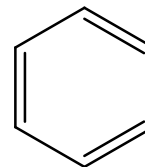


Methyl butyrate

2. In preparing a benzene/acetone gradient for a silica gel liquid chromatographic column (note, this is silica gel and not a bonded-phase material), is it desirable to increase or decrease the proportion of benzene as the column is eluted? Explain your answer.

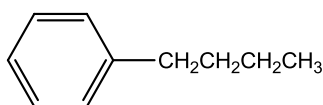


Acetone

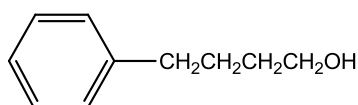


Benzene

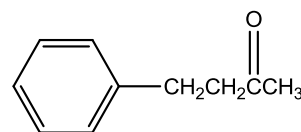
3. a) For a reversed-phase liquid chromatographic separation on a C-18 column, predict the elution order of 4-phenylbutane, 4-phenyl-1-butanol, and 4-phenyl-2-butanone. Explain your answer.
b) Suppose the liquid chromatogram of these three compounds came out as shown below. What is the problem with this chromatogram (there is a specific chromatographic term to describe it) and explain specifically what you would do experimentally to improve the chromatography?



4-phenylbutane



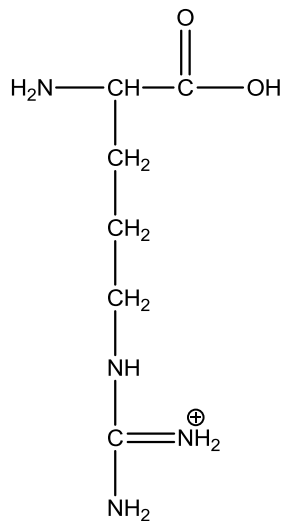
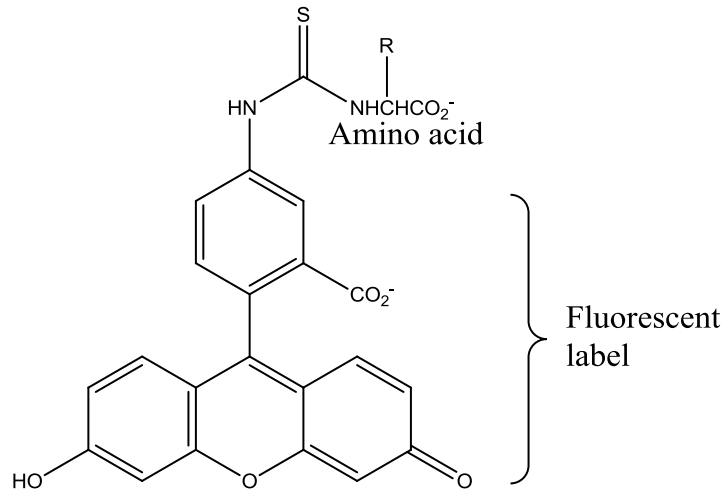
4-phenyl-1-butanol



4-phenyl-2-butanone

Capillary Electrophoresis – Out-of-class Question

Fluorescent derivatives of amino acids were prepared and separated by capillary electrophoresis at pH = 9.3. Predict the elution order of the four amino acids. Explain your reasoning.



pKa = 12.5

(protonated form of arginine side chain is shown)

