Che380
Fall 2019
Stoll

Offline Homework #1 – Due Friday October 19th in class

2. In class we will discuss the concept and importance of resolution at length. Many simple treatments of resolution are only concerned with the situation where two adjacent peaks are of equal size; in practice, however, this rarely the case. Given the following initial conditions, what resolution is required for it to be very clear that there are two components present, and not just one (this is a qualitative assessment)? What resolution is required if we want to be able to accurately quantify how much of each component is present (this is a quantitative assessment)? In your calculation, you should assume that analyte B is more retained than analyte A. The following table may help organize your results.

   You should build a model of a two-component chromatogram in Excel which allows you to systematically vary the peak height ratio by varying the number of moles of each analyte injected and the retention of both peaks, observe the effect on the chromatogram, and report as an output the resolution of the peak pair. Assume the detector response is the same for both molecules. You are encouraged to work on this in groups of two. Please submit via email the spreadsheet you put together to answer this question. As part of your model, include the following input parameters:

   - Column length
   - Column diameter
   - Flow rate
   - Particle size
   - Van Deemter A, B, and C parameters
   - Mobile phase composition (i.e., %ACN…this will be a model for RP LC)
   - ln kw and S values for each analyte

   As part of this exercise, you should measure retention factors for ethylbenzene and butyrophenone under the following conditions, and use those data to get the ln kw and S values you need to use the model. Use the following conditions for these measurements:

   - Column: Zorbax SB C18, 50 mm x 4.6 mm i.d., 3.5 or 5.0 micron
   - Mobile phase: ACN and water
   - Temperature: 40 C
   - Vary the mobile phase composition to get at least five k' values between 1 and 30.
   - A good starting point would be to inject 1 uL of the analyte at 100 ug/mL

For the specific exercise outlined in Table 1, use either ethylbenzene or butyrophenone as analyte A, and then put in some arbitrary ln kw and S values for the other compound so that you can adjust the retention as needed.

Table 1. Initial Separation Conditions and Required Resolution for Qualitative and Quantitative Analysis as a Function of Peak Height Ratio

Initial Conditions				Required Resolution	
	Analyte A		Mole Ratio (A/B)	Qualitative	Quantitative
k'	5.6		1		
t_m (min.)	1.00		5		
N	5000		10		
			100		
			1000		
			10000		