Worksheet 16: Chromatography

Objectives

1. Relate the separation process to features of the chromatogram, such as retention time and resolution
2. Explain how the chemical interactions between a solute and both the stationary phase and the mobile phase impact retention
3. Predict the elution order for a set of compounds given the mobile and stationary phase composition

Key Questions

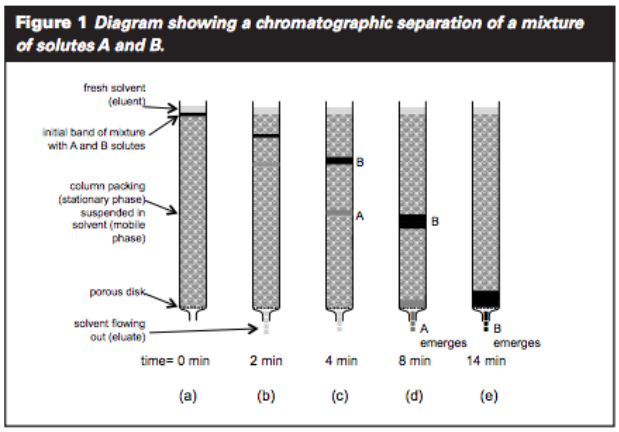
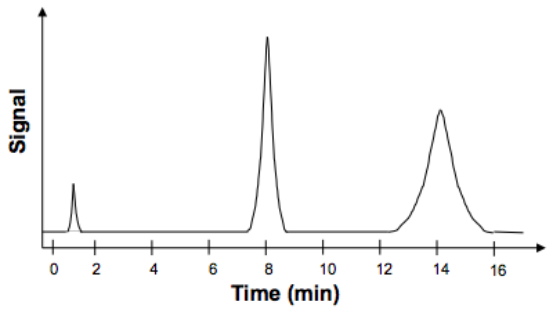


Figure 1 *Diagram showing a chromatographic separation of a mixture of solutes A and B.*

1. In Figure 1a, solutes A and B are mixed together in the initial band. List differences you observe as time progresses from (a) to (d).

2. The retention time for a solute (t_r) is the time needed for the compound to emerge from the column. The retention factor (k') is the ratio of the time a solute spends in the stationary phase to the time spent in the mobile phase. Retention time for an unretained solute is called the void time (t_v or t_m). Using the figure above, what are the retention times for analytes A and B?

Figure 2 *A chromatogram.*



The plot of the signal detected at the outflow of the column in Figure 1 during the elution of the solute mixture A and B. The first peak represents the time it takes for an unretained substance to pass through the column; it has no interaction with the stationary phase.

3. Which peak on the chromatogram corresponds to analyte A and which corresponds to analyte B? How do you know?

4. Determination of the retention time should be consistent from person to person. Which part of the peaks in Figure 2 makes this easiest?

5. Based on Figure 2, what is the void time for this column?

6. How is the void time related to the time the solutes spend in the mobile phase?

7. How is the void time related to the time the solutes spend in the stationary phase?

8. Write an equation for the calculating the retention factor from retention time and void time data. Use this equation to calculate the retention factors for analytes A and B.

9. What is the relationship between the broadness of the peaks in Figure 2 and the width of the bands in Figure 1?

10. Do you notice any relationship between the retention factor and peak broadness?

11. In chromatography, the term resolution (R_s) is used to express the quality of the separation between two peaks. How would changes in retention time and peak broadness affect the resolution of Figure 2?

12. Resolution can be computed by dividing the difference in retention times between the two peaks by their average width along the baseline:

$$\frac{t_{r1} - t_{r2}}{(w_1 + w_2)/2} \tag{1}$$

A value of $R_s = 1.50$ represents what is called *baseline resolution* between peaks. Use Figure 2 to estimate the resolution of the peaks corresponding to analytes A and B. Are the peaks baseline resolved?

13. What retention time for analyte B would be required to achieve baseline resolution? Assume that the width of both peaks and the retention time of A is unchanged.

14. How could a chemist change the resolution of two peaks?