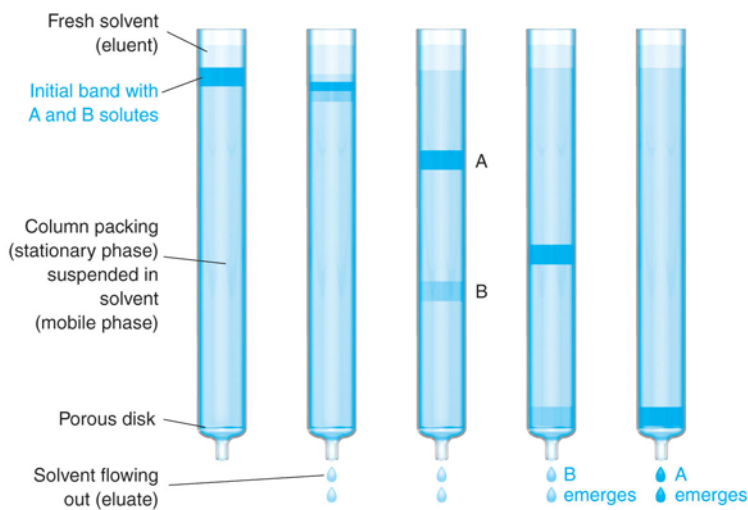


Chem 321 Lecture 21 - Chromatography

11/12/13

Student Learning Objectives

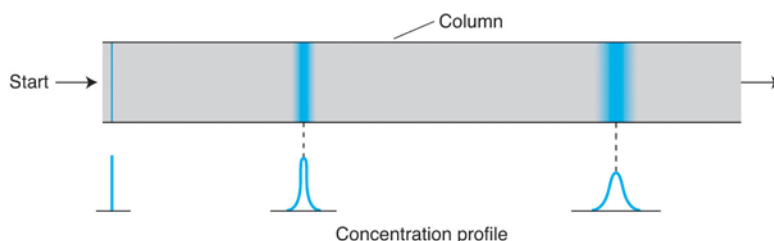
The last three lab experiments that you perform require that the analyte(s) be separated before a quantitative determination is made. Various forms of column chromatography are used to achieve the necessary separations. In each case, a sample mixture is injected onto a chromatography column consisting of a **stationary phase** and supporting material. The sample is then carried through the column by a flowing **mobile phase**. Each sample component interacts with the stationary phase to a different extent, hence the components migrate through the column at different rates and elute from the column separated. This situation is illustrated for liquid column chromatography in Figure 14.1. Your three experiments differ primarily in the nature of the stationary and mobile phases employed to effect this separation.



© 2011 W. H. Freeman and Company

Figure 14.1 Separation of analytes A and B using liquid column chromatography

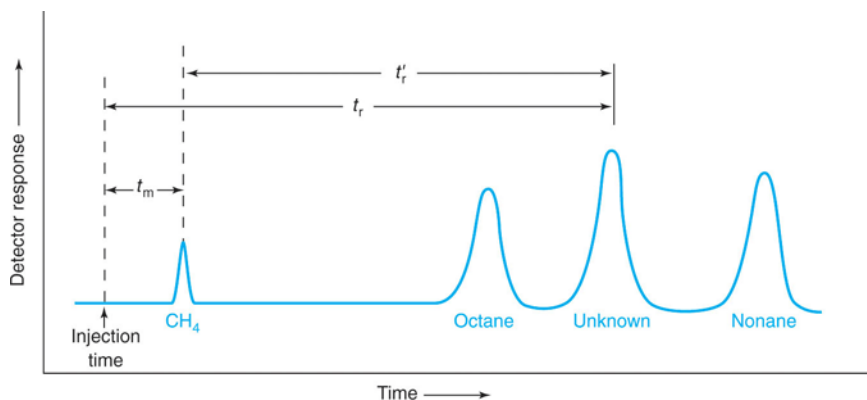
As each component moves through the column, it spreads out in a band that is most concentrated in the center (Fig. 14.2). A successful separation is achieved if this band broadening is small compared to the separation of the different component bands.



© 2011 W. H. Freeman and Company

Figure 14.2 Broadening of band of analyte as it moves through a chromatography column

If the components are detected continuously as they elute from the column, a **chromatogram** similar to that shown in Figure 14.3 for a gas chromatography separation is obtained.



Harris, *Quantitative Chemical Analysis*, 8e
© 2011 W. H. Freeman

Figure 14.3 Typical chromatogram of a multi-component mixture

The time it takes from when the sample is injected onto the column until a particular component elutes is the retention time (t_R) for that component. If a component is not retained by the column and flows through at the same rate as the mobile phase, its retention time is symbolized by t_m . The approximately Gaussian shape to the chromatography peaks reflects the concentration profile of the analyte band.

The best measure of column efficiency is **plate height (H)**. Plate height is defined as

$$H = \frac{\sigma^2}{L}$$

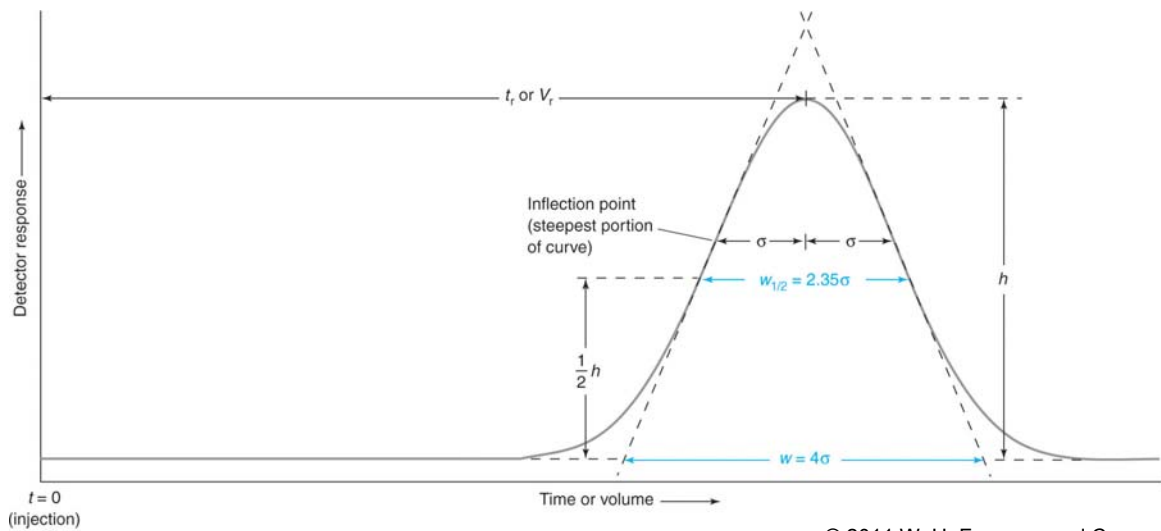
where σ is the standard deviation, in units of length along the column, of the analyte concentration profile in its band, and L is the length of the column. Thus, H has units of length. The smaller the value of H , the less the analyte band broadens and the more efficient the column. Each component has a plate height that depends on the stationary phase and mobile phase conditions. The number of plates (N) associated with a column is related to H by $N = L / H$. This permits a ready determination of H ,

since N can be calculated from experimental parameters by

$$N = 16 \left(\frac{t_R}{w} \right)^2 = 5.54 \left(\frac{t_R}{w_{1/2}} \right)^2$$

where t_R is the component retention time;
 w is the chromatography peak width at the baseline;
 $w_{1/2}$ is the peak width at half height.

Figure 14.4 illustrates how w and $w_{1/2}$ are measured. It is generally more accurate to measure the width at half height so this method is preferred for calculating N. In calculating N, the retention time and the peak width must have the same units, either time units or distance (length units) along the chromatogram. The larger the number of plates for a particular component, the more efficient the column is for separating this component.



© 2011 W. H. Freeman and Company

Figure 14.4 Experimental parameters used to calculate the number of plates

The degree of separation of two components is assessed by the resolution (R_s) which is calculated as

$$R_s = \frac{(t_R)_B - (t_R)_A}{(w_A + w_B)/2}$$

where $(t_R)_A$ and $(t_R)_B$ are the retention times for components A and B, respectively (A elutes before B);

w_A and w_B are the baseline widths for peaks A and B, respectively.

Figure 14.5 illustrates how the value of R_s correlates with peak separation. A resolution of $R_s = 1.5$ or greater is generally needed in order for the detector response to return to the baseline between the peaks.

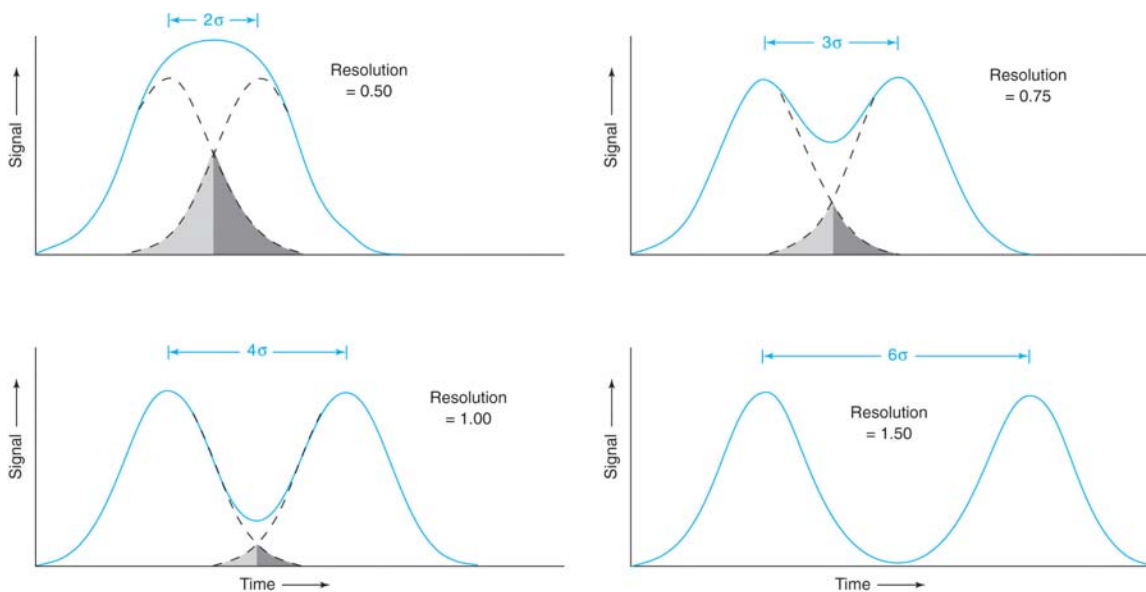


Figure 14.5 Separation of component peaks at various resolutions

Another parameter that you will use to characterize your separations is the retention factor, or capacity factor, (k'). For component A, the retention factor is defined as

$$k'_A = \frac{t_R - t_m}{t_m}$$

For our liquid chromatography experiment, the retention time for an unretained component (t_m) is the solvent peak retention time. For gas chromatography the retention time of methane is often used for t_m . The retention factor increases with increasing component retention time.

For a given set of analysis conditions, the area of a chromatogram peak is proportional to the amount of component present in a sample. The peak area is manually calculated using chromatogram data by

$$AREA = h \times w_{1/2}$$

where h is the peak height and $w_{1/2}$ is the width at half height. Unless the chromatogram peak is sufficiently broad, measurement of $w_{1/2}$ is not very accurate. Your gas chromatograph and liquid chromatograph are interfaced to a computer for automatic data collection. The software will also determine the location (t_R) of each peak in the chromatogram and the peak area. It is important to verify that the program has made a reasonable choice of where each peak starts and ends, and whether it has selected an appropriate baseline for calculating the peak area. The software permits you to manually alter these parameters if you decide it is necessary.

Check for Understanding 14.1

Solution

1. If a peak has a height of 123.4 mm and a half height width of 26.5 mm, what is the approximate ppth error in the peak area? Assume the peak dimensions can be measured to ± 0.3 mm.