Problem Set 9

Introduction to Chromatographic Separations

26-1 Define
(a) elution
(b) mobile phase
(c) stationary phase
(d) distribution constant
(e) retention time
(f) retention factor
(g) selectivity factor
(h) plate height
(i) longitudinal diffusion
(j) eddy diffusion
(k) column resolution
(l) eluent

26-1.

a. Elution: the separation process through which solutes are washed through a column by addition of a suitable solvent.

b. Mobile phase: a solvent of specific composition that moves through a column containing a stationary phase.

c. Stationary phase: a solid or liquid phase through which a mobile phase passes where solutes in the mobile phase interact or partition differently with the stationary phase.

d. Distribution constant: the ratio of the analyte concentrations in both stationary and mobile phases at equilibrium.

e. Retention time: the time interval between analyte injection at one end of the column and the appearance of its maximum peak at the other end.

f. Retention factor: the ratio of the number of moles of solute in stationary and mobile phases and is also defined as the distribution constant multiplied by the volume ratio of stationary and mobile phases.

g. Selectivity factor: the ratio of the retention factors of two species is called their selectivity factor where the value of the selectivity factor is equal or greater than unity.

h. Plate height: the ratio of the column length to the number of theoretical plates and is defined as the length of column which contains 34% of the solute as solute leaves from the column.
i. Longitudinal diffusion: diffusion of concentrated solute zones into more diluted zones where longitudinal diffusion is a source of band broadening, especially in gas chromatography.

j. Eddy diffusion: a mechanism through which molecules travel through a column in different speeds due to following different paths. Band broadening is the result.

k. Column resolution: how well two peaks are resolved from each other is called resolution. This can be defined as the ratio of the separation distance (in time units) between two peaks to have their total peak widths at the baseline.

f. Eluent: the mobile phase in chromatography is referred to as the eluent.

26-2 Describe the general elution problem.

The general elution problem is the separation case where solutes of very different distribution constants are to be separated. When conditions are optimized for the separation of late eluting peaks, bad resolution is obtained for fast eluted peaks. On the other hand, when conditions are optimized for the separation of early eluting peaks, very long retention of late eluting peaks is realized, including severe band broadening. The general elution problem can be overcome using gradient elution in liquid chromatography or temperature programming in gas chromatography.

26-3. List the variables that lead to zone broadening in chromatography.

Zone broadening is affected by the following factors:

a. Particle diameter where as $d_p$ is decreased $H$ decreases.

b. Flow rate of mobile phase where an optimum flow rate must be used. The flow rate is usually low in LC but high in GC.

c. Thickness of stationary phase where generally small thicknesses are necessary.

d. Diffusion of solute in mobile and stationary phases where in LC a low viscosity mobile phase is used to increase $D_M$ while the opposite is used in GC.

e. Temperature is especially important in GC where high temperatures are usually used.

26-4 What are the major differences between gas-liquid and liquid-liquid chromatography?

In gas liquid chromatography the mobile phase is a gas while the mobile phase is a liquid in liquid liquid chromatography.
26-5. What are the differences between liquid-liquid and liquid-solid chromatography?

The stationary phase is a solid in liquid solid chromatography while a liquid stationary phase is used in liquid chromatography.

26-6. What variables are likely to affect the selectivity factor \( a \) for a pair of analytes?

The selectivity factor for a pair of analytes is affected by:

a. Mobile phase composition.


c. Column temperature.

d. Use of special chemical effects like complexing agents, surfactants, ion pairing reagents, etc.

26-7. Describe how the retention factor for a solute can be manipulated.

The retention factor can be manipulated by the following:

I. In LC:

a. Mobile phase composition.


c. Use of special chemical effects like complexing agents, surfactants, ion pairing reagents, etc.

   d. Change column length.

II. In GC:

a. Change column length.

c. Column temperature.

26-8. Describe a method for determining the number of plates in a column.

The number of plates in a chromatographic column can be calculated by finding the retention time of analyte as well as the width of the peak at half height then applying the equation:

\[ N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2 \]

26-9. How does temperature affect separations in liquid chromatography?

Usually, temperature has little effects on separations in liquid chromatography. However, shorter retention times are realized as temperature is increased in gas chromatography.

26-10. Why does the minimum in a plot of plate height versus flow rate occur at lower flow rates with liquid chromatography than with gas chromatography?

Longitudinal diffusion terms are the reason for the minima obtained in van Deemter equation. This term is very important in gas chromatography where \( D_M \) must be decreased in gas chromatography to decrease \( H_L \). Therefore, high flow rates must be used. However, the longitudinal term in LC is marginal and increasing the flow rate will significantly increase band broadening. Thus the minimum in van Deemter equation occurs at much lower flow rates in LC than GC.

26-11. What is gradient elution?

Gradient elution is a process used in LC whereby the composition of the mobile phase, or its flow rate, is changed during the separation process to improve separation characteristics.

26-12. The following data apply to a liquid chromatographic column:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column length</td>
<td>25.7</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.313 mL/min</td>
</tr>
</tbody>
</table>
A chromatogram of a mixture of A, B, C, and D resulted in the following data:

<table>
<thead>
<tr>
<th></th>
<th>Retention time</th>
<th>Width of peak base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonretained</td>
<td>3.1</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>5.4</td>
<td>0.41</td>
</tr>
<tr>
<td>B</td>
<td>13.3</td>
<td>1.07</td>
</tr>
<tr>
<td>C</td>
<td>14.1</td>
<td>1.16</td>
</tr>
<tr>
<td>D</td>
<td>21.6</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Calculate
(a) the number of plates from each peak.
(b) the plate height for the column.

\[ N = 16 \left( \frac{t_R}{W} \right)^2 \]

a. \[ N_A = 16 \left( \frac{5.4}{0.41} \right)^2 = 2775.49 \]
b. \[ N_B = 16 \left( \frac{13.3}{1.07} \right)^2 = 2472.04 \]
c. \[ N_C = 16 \left( \frac{14.1}{1.16} \right)^2 = 2363.97 \]
d. \[ N_D = 16 \left( \frac{21.6}{1.72} \right)^2 = 2523.31 \]

b. \[ H = \frac{L}{N} \]

\[ H = \frac{25.7}{2534} = 0.010 \text{ cm} \]

26-13. From the data in the previous problem, calculate for each peak:

a. the retention factor

b. the distribution constant

\[ k' = \frac{(t_R - t_M)}{t_M} \]

a. \[ k'_A = \frac{(5.4 - 3.1)}{3.1} = 0.74 \]

\[ k'_B = \frac{(13.3 - 3.1)}{3.1} = 3.3 \]

\[ k'_C = \frac{(14.1 - 3.1)}{3.1} = 3.5 \]

\[ k'_D = \frac{(21.6 - 3.1)}{3.1} = 6.0 \]

b. the distribution constant, \[ k = k' V_M/V_S \]
$K_A = 0.74 \times 1.37/0.164 = 6.2$

$K_B = 3.3 \times 1.37/0.164 = 26$

$K_C = 3.5 \times 1.37/0.164 = 29$

$K_D = 6.0 \times 1.37/0.164 = 50$

26-14. From the data in the previous problem, for species B and C, calculate:

a. Resolution

The resolution is given by:

$$R = \frac{2(t_{RC} - t_{RB})}{W_C + W_B}$$

$$\alpha = \frac{k_C}{k_B}$$

b. The selectivity factor, $\alpha = k_C'/k_B$

$$\alpha = \frac{3.5}{3.3} = 1.06$$

c. Column length

$$R_1/R_2 = (L_1/L_2)^{1/2}$$

$$0.72/1.5 = (25.7/L_2)^{1/2}$$

$$L_2 = 112 \text{ cm}$$

d. The time required to separate B from C in a 25.7 cm column is 14.1 min. When the column length is increased from 25.7 to 112 cm the retention time increases in the same proportion:

$$t_{R1}/t_{R2} = L_1/L_2$$

$$14.1/t_{R2} = \frac{25.7}{112.5}$$
t_{R2} = 61 \text{ min}

26-15. From the data in the previous problem, for species D and C, calculate:

a. Resolution

b. The length of the column necessary to give a resolution of 1.5

a. Resolution = \frac{2(t_{RD} - t_{RC})(W_C + W_D)}{}

R = \frac{2(21.6 - 14.1)}{(1.16 + 1.72)} = 5.21

b. The length of the column necessary to give a resolution of 1.5

\frac{R_1}{R_2} = \left(\frac{L_1}{L_2}\right)^{1/2}

\frac{5.21}{1.5} = \left(\frac{25.7}{L_2}\right)^{1/2}

L_2 = 2.1 \text{ cm}

26-16. The following data were obtained by gas-liquid chromatography on a 40-cm packed column:

<table>
<thead>
<tr>
<th>Compound</th>
<th>t_R, min</th>
<th>W_{1/2}, \text{ min}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>1.9</td>
<td>-</td>
</tr>
<tr>
<td>Methylcyclohexane</td>
<td>10.0</td>
<td>0.76</td>
</tr>
<tr>
<td>Methylcyclohexene</td>
<td>10.9</td>
<td>0.82</td>
</tr>
<tr>
<td>Toluene</td>
<td>11.4</td>
<td>1.06</td>
</tr>
</tbody>
</table>

Calculate
(a) an average number of plates from the data.
(b) the standard deviation for the average in (a).
(c) an average plate height for the column.

a. N = 5.54 \left(\frac{t_R}{W_{1/2}}\right)^2

a. N_{\text{MCHexane}} = 5.54(10.0/0.76)^2 = 959

b. N_{\text{MCHexene}} = 5.54(10.9/0.82)^2 = 979

c. N_{\text{Toluene}} = 5.54(13.4/1.06)^2 = 885
Mean = (959 + 979 + 885)/3 = 941

N = 941 plates

b.

<table>
<thead>
<tr>
<th>x_i</th>
<th>x_i - \bar{x}</th>
<th>(x_i - \bar{x})^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>959</td>
<td>18</td>
<td>324</td>
</tr>
<tr>
<td>979</td>
<td>38</td>
<td>1444</td>
</tr>
<tr>
<td>885</td>
<td>56</td>
<td>3136</td>
</tr>
<tr>
<td>\bar{x} = 941</td>
<td></td>
<td>\Sigma (x_i - \bar{x})^2 = 4904</td>
</tr>
</tbody>
</table>

σ = \sqrt{(4904)/2} = \pm 50

c. H = L/N

H = 40/941 = 0.043 cm

26-17. Referring to previous problem, calculate the resolution for
(a) methylcyclohexene and methylcyclohexane.
(b) methylcyclohexene and toluene.
(c) methylcyclohexane and toluene.

N = 5.54 \left\{\frac{t_R}{W_{1/2}}\right\}^2 and N = 16 \left\{\frac{t_R}{W}\right\}^2

5.54 \left\{\frac{t_R}{W_{1/2}}\right\}^2 = 16 \left\{\frac{t_R}{W}\right\}^2

2.354 \frac{t_R}{W_{1/2}} = 4 \frac{t_R}{W}

W = 1.70 W_{1/2}

a. Resolution = \frac{2(t_{R,MCHexene} - t_{R,MCHexane})}{1.7(W_{1/2, MCHexene} + W_{1/2, MCHexane})}

R = \frac{2(10.9 - 10.0)}{1.7(0.82 + 0.76)} = 0.67

b. Resolution = \frac{2(t_{R,Toluene} - t_{R,MCHexene})}{1.7(W_{1/2, Toluene} + W_{1/2, MCHexene})}
\[ R = 2(13.4 - 10.9)/1.7(0.82+1.06) = 1.56 \]

c. Resolution = \[ 2(t_{R, \text{Toluene}} - t_{R, \text{MCHexane}})/1.7(W_{1/2, \text{Toluene}} + W_{1/2, \text{MCHexane}}) \]
\[ R = 2(13.4 - 10.0)/1.7(0.76 + 1.06) = 2.20 \]

26-18. List the variables in chromatography that lead to zone separation.

Zone broadening is affected by the following factors:

a. Particle diameter where as \( d_p \) is decreased \( H \) decreases.

b. Flow rate of mobile phase where an optimum flow rate must be used. The flow rate is usually low in LC but high in GC.

c. Thickness of stationary phase where generally small thicknesses are necessary.

d. Diffusion of solute in mobile and stationary phases where in LC a low viscosity mobile phase is used to increase \( D_M \) while the opposite is used in GC.

e. Temperature is especially important in GC where high temperatures are usually used.

26-19. Which is better slow or fast sample injection?

Slow sample introduction (injection) leads to serious band broadening.

26-20 Why does the minimum in a plot of plate height versus flow rate occur at lower flow rates with LC than with GC?

The most important term in va Deemter equation in GC is the B term where \( H_L \) is inversely proportional to flow rate. Therefore, high flow rates should be used to lower H.

26-21. Given the following chromatogram for the separation of two solutes on a 25 cm long HPLC column, determine:

a) the H.E.T.P achieved under the operating conditions of the column using solute A values for calculations;

b) the resolution achieved for solutes A and B;

c) the capacity factors for solutes A and B;

d) the linear velocity of the mobile phase in the column. Assume that the exact retention time for the compound A (\( t_{R(A)} \)) is 6.0 min and \( t_{R(B)} = 7.8 \) min and that the width of the eluting peak at the baseline for compound A is 1.5 min and for compound B is 1.9 min. Also, the column yielding a \( t_m = 0.9 \) min.
a. \[ N_A = 16\left(\frac{r_{RA}}{W_A}\right)^2 = 16\left(\frac{6.0}{1.5}\right)^2 = 64 \]

\[ H = \frac{L}{N} = \frac{250 \text{ mm}}{64} = 3.9 \text{ mm} \]

b. \[ R = \left\{ \frac{2(7.8-6.0)}{(1.5+1.9)} \right\} = 1.06 \]

c. \[ K'_A = \frac{6.0 - 0.9}{0.9} = 5.67 \]
\[ K'_B = \frac{7.8 - 0.9}{0.9} = 7.67 \]

d. the linear velocity of the mobile phase = \[ \frac{L}{t_m} = \frac{25.0}{0.9} = 27.78 \text{ cm/min} \]

26-22. Consider the following chromatogram obtained by GC.

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.

General elution problem

b) Propose a way in gas chromatography to eliminate both problems.

Temperature programming. Early eluting peaks require lowering the temperature, while late eluting peaks require increasing temperature. A suitable temperature program can produce a nice looking chromatogram.