Chromatographic separations

Classification of chromatographic methods

Chromatographic methods are classified according to mobile phase (gas or liquid), stationary phases (solid or liquid film on support), and separation mechanism (adsorption, partition, ion exchange, permeation) (see Table 26-1 or equivalent).

Elution chromatography is the most commonly used development procedure (Fig. 26-1), other procedures (optional) include frontal and displacement (R5: Fig. 1.3).

Two theories to explain chromatographic peak shapes

Plate theory (1941) by Martin & Synge who won the Nobel Prize in Chemistry in 1952 for it: thermodynamic theory with the assumption of equilibrium.

Rate theory (1956) by Van Deemter: Kinetic theory at non-equilibrium conditions.

Plate theory

Separation of 2 solutes depends on the rates at which the 2 species are eluted. These rates are determined by the equilibrium constant (or partition coefficient) of the distribution of the solutes between the mobile phase (elution solvent) and stationary phase (column packing). The theory is based on an analogy with distillation and countercurrent extraction. Various chromatographic parameters and relationships are given in Table Table 26-4 & 5)

a) Distribution constant:

$$K = \frac{C_s}{C_M}$$

b) Retention time:

$$t_M = \frac{L}{u} \qquad t_R = \frac{L}{\bar{v}}$$

In GC, retention volume is in principle a more useful parameter than retention time for identifying chemical species because it takes into account the effects of pressure and temperature on the highly compressible gaseous mobile phase.

c) Retention (or capacity) factor: Ideal separations are achieved at which k' values lie in the range between 2 and 10. If k' is less than 1, determination of fast t_R is inaccurate. If k' is too large, t_R is very long.

$$k_A' = \frac{m_s}{m_M} = \frac{K_A V_s}{V_M}$$

d) Relation between retention time and retention factor:

$$\frac{\bar{v}}{u} = \textit{fraction of moles of the solute in the mobile phase} = \frac{c_M V_M}{c_M V_M + c_S V_S}$$

$$\therefore \frac{t_M}{t_R} = \frac{1}{1+k'}$$

e) Selectivity factor:

$$\alpha = \frac{K_A}{K_B} = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

The plate theory successfully accounts for the Gaussian shape of chromatographic peaks and their rates of movement down a column. But the theory does not explain the peak broadening phenomenon.

Rate theory

Plate height or variance per unit column length (Fig 26-5)

$$H = \frac{\sigma^2}{L} \qquad \sigma = \tau \cdot \bar{v} = \frac{W}{4} \cdot \frac{L}{t_R}$$

$$N = \frac{L}{H} = 16\left(\frac{t_R}{W}\right)^2 \quad or \quad 5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$

N and *H* are widely used to indicate column performance or efficiency.
The rate theory of chromatography zone broadening accurately predicts the shape of a plot of *H* versus *u*, which is called the Van Deemter plot (Fig 26-7 or equivalent).

$$H = A + \frac{B}{u} + (C_S + C_M)u$$

Effects of 3 factors on H
Eddy diffusion (or multipath) term, *A*: Solute molecules reach the column end over a time interval because of different residence time of the molecules in a multitude of pathways (Fig. 26-8 or R5: Fig.2-6a).

- *A* is directly proportional to the packing particle diameter, d_p (Fig. 26-10 and 28-2).
- λ is a function of packing uniformity and column geometry.
- In unpacked columns, *A* is zero.

Longitudinal diffusion term, *B/u*: Solute molecules diffuse from the concentration center of a zone to the more dilute region (ahead of and behind the zone center) (R5: Fig 2-6b).

- The longitudinal diffusion effect on *H* is inversely proportional to *u* because the solute residence time is shorter at high *u* and the extent of diffusion is less.

- *B* is directly proportional to the solute diffusion coefficient in the mobile phase, D_M.
- D_M is smaller in liquids (even smaller in more viscous mobile phase) than in gases, so *B/u* is less pronounced in LC than in GC (Fig. 26-7 or equivalent).
- The obstructive factor, γ, is lower for a packed column (i.e. $\gamma = 0.6$) than for an unpacked (capillary) column (i.e. $\gamma = 1$).

Mass-transfer term, $c_S u$ and $c_M u$: The equilibrium between the mobile phase and stationary phase is established so slowly that a chromatographic column always operates under non-equilibium conditions (hence plate theory which is based on equilibrium conditions is not adequate). So analyte molecules at the front of a band are swept ahead without equilibrating with the stationary phase and those at the trailing edge are left behind for a longer time in the stationary phase (R5: Fig. 2-6c).

- The mass-transfer effect on *H* is directly proportional to *u* because the solute residence time is longer at low u, the deviation from equilibrium is less, and zone broadening or *H* is smaller.
- $c_S u$ is less if the liquid stationary phase film thickness, d_f , is smaller, or the solute diffusion coefficient in the stationary phase, D_S is larger.
- $c_M u$ is less if d_p is smaller (hence greater surface area), or the solute diffusion coefficient in the mobile phase, D_M is larger. (See Fig. 26-10 & Fig. 28-2)

The relationships of van Deemter terms with column and analyte properties are given in Table 26-3. The *A*, *B* and *C* terms can be extracted from the plot (R5: Fig 2-7).

Column resolution

Optimization of chromatographic separation (i.e. to achieve high resolution) can be achieved by (1) reducing zone broadening, and (2) increasing the difference in migration rates of components (Fig 26-3).

$$R_S = \frac{(t_R)_B - (t_R)_A}{\frac{1}{2}(W_A + W_B)}$$

An R_S value of 1.5 gives an essentially complete (baseline) separation of the 2 components (Fig 26-11).

$$Since \quad W_A = W_B \approx W \quad and \quad N = 16\left(\frac{(t_R)_B}{W}\right)^2$$

$$\therefore \quad R_S = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_B}{1 + k'_B} \quad and \quad R_S \propto \sqrt{N}$$

N for achieving a certain R_S value (i.e. 1.5) can be determined as follows:

$$N = 16 R_S^{\,2} \cdot \left(\frac{\alpha}{\alpha - 1}\right)^2 \cdot \left(\frac{1 + k'}{k'}\right)^2 \quad and \quad N \propto R_S^{\,2}$$

kinetic effect A | equil. effect B | equil. effect C

Effects A and C depends on solutes and column properties, and B only depends on solute properties.

R_S can be enhanced
1. easily by changing temperature or mobile phase (composition, polarity and viscosity) and flow rate, and
2. less conveniently by using a different column (packing, length and stationary phase)

The effect of enhanced resolution on retention time
Unfortunately, high resolution and short retention time cannot be achieved under the same conditions, and a compromise must always be struck.
Various chromatographic parameters are summarized in Table 26-4 & 5 and E.g. 26-1.

$$u \cdot \frac{1}{1+k_B^{'}} = \bar{v}_B = \frac{L}{(t_R)_B} = \frac{NH}{(t_R)_B}$$

$$\therefore \quad (t_R)_B = \frac{16 R_S^{\,2} H}{u} \cdot \left(\frac{\alpha}{\alpha - 1} \right)^2 \frac{(1+k_B^{'})^3}{(k_B^{'})^2} \qquad \textit{and} \qquad (t_R)_B \propto R_S^{\,2}$$

The effect of k_B' on R_s and $(t_R)_B$

The effect of k_B'on R_S and $(t_R)_B$ can be visualized in Fig. 26-12, provided that Q and Q' remain approximately constant.

$$\textit{Let} \qquad R_S = Q \cdot \frac{k_B^{'}}{1+k_B^{'}} \qquad \textit{and} \qquad (t_R)_B = Q^{'} \frac{(1+k_B^{'})^3}{(k_B^{'})^2}$$

It is concluded that the optimized value of k_B', taking into account of both resolution and retention time, lie in the range of 1 to 5. (c.f. 2-10 previously)

The effect of α on N
α can be enhanced (from 1), based on modifying the relative values of K_A and K_B, by
1. changing the mobile phase composition, including changes in pH
2. changing column temperature
3. changing stationary phase composition
4. using special chemical effects, e.g. complex formation between Ag^+ and unsaturated organic compounds.

The general elution problem
Separation of fast eluted component is often achieved at the expense of zone broadening of slowly eluted component (Fig. 26-14). A common solution is to change conditions as the separation proceeds:
LC- gradient elution or solvent programming (Fig 26-13 or equivalent)
GC-temperature programming (Fig 27-5)

Exercises: 26-1 to 3, 26-6 to 10, 26-12 to16, 26-20, 26-22.