Introduction to Separations Science

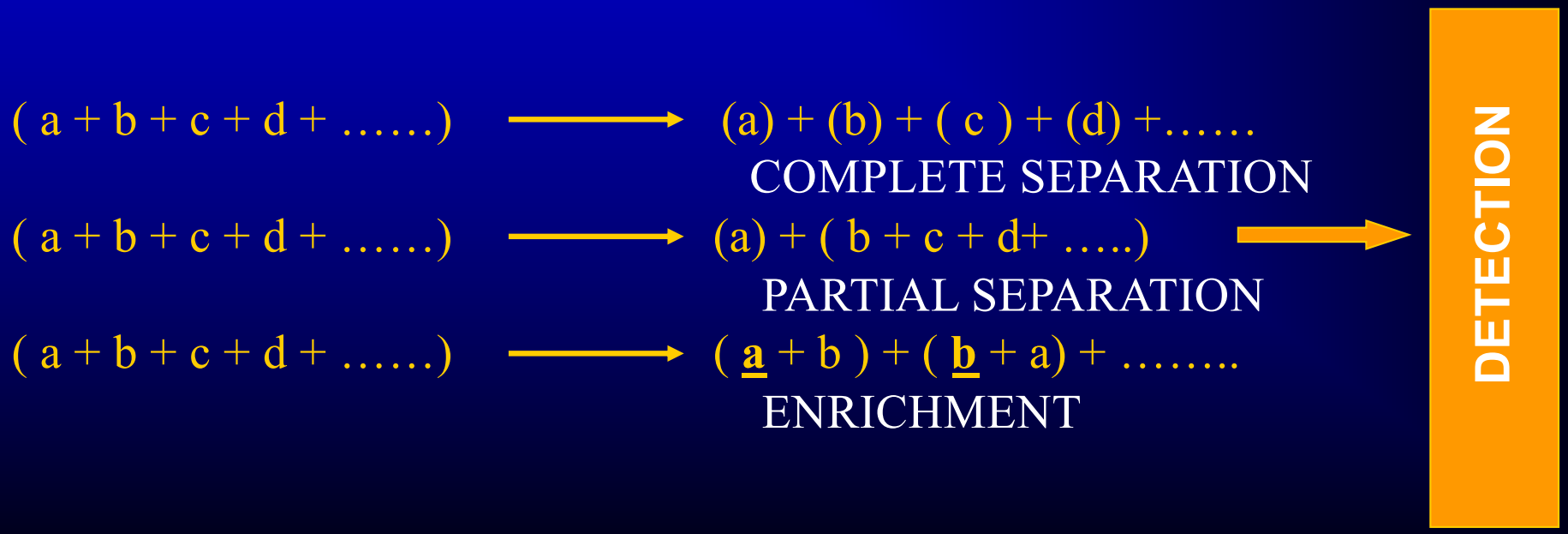
- What is separations science?
  - A collection of techniques for separating complex mixtures of analytes
  - Most separations are not an analytical technique in their own right, until combined with an analytical detector (often a type of spectrometer)

- Key analytical branches: chromatography, electrophoresis, extraction

- Reading:
  - Skoog et al. Chapter 26

What is a Separation?

- Separations are key aspects of many modern analytical methods. Real world samples contain many analytes, most analytical methods do not offer sufficient selectivity to be able to speciate all the analytes that might be present.
- Most separation methods involve separation of the analytes into distinct chemical species, followed by detection:



Basic Types of Separations

Liquid Column Chromatogrphy

Liquid- Liquid (partition) chromatography (LLC)
stationary and mobile phases (immiscible)
Liquid -Solid (adsorption) chromatography (LSC)
Ion exchange chromatography (IEC)
Exclusion chromatography (EC)
Gas-Liquid chromatography (GLC)
Gas-Solid chromatography (GSC)

Separation Methods Based on Phase Equilibria

Gas-Liquid	Gas-Solid	Liquid-Liquid	Liquid-Solid
Distillation	Adsorption	Extraction	Precipitation chrom
Sublimation	Gas- Liquid	Liq-Liq Chrom	Zone melting
Foam Fractionation	Molecular sieves	Exclusion	Fractional crystallization
			Ion Exchange
			Adsorption
			Exclusion
			Molecular sieves

Basic Types of Separations

Separation methods based on rate processes

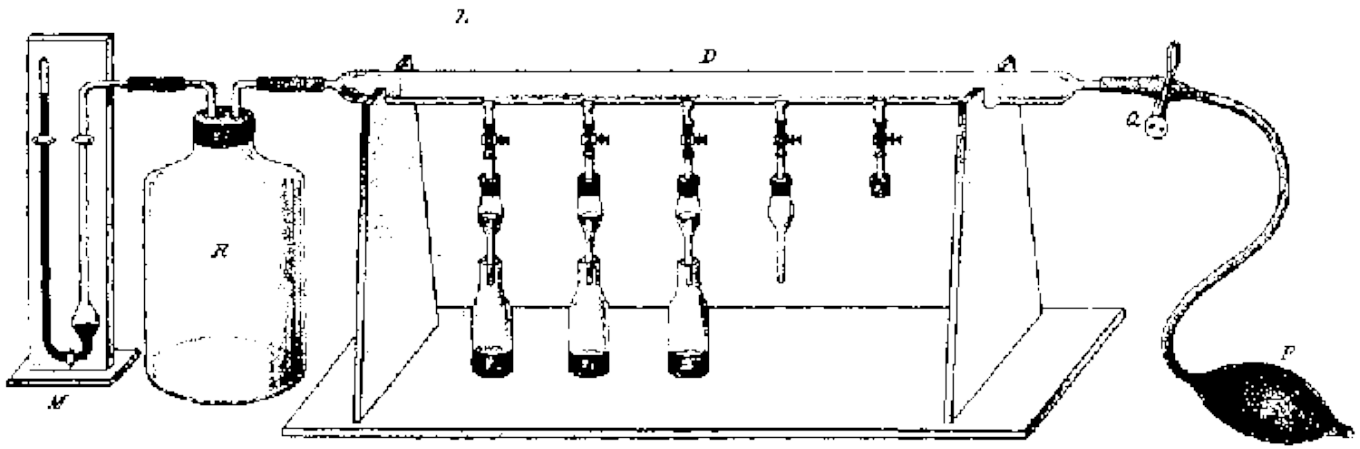
Barrier Separation	Field Separations	Other
membrane filtration	electrophoresis	molecular distillation
dialysis	ultracentrifugation	enzyme degradation
electro-dialysis	thermal diffusion	destructive distillation
electro-osmosis	electrodeposition	
reverse osmosis	mass spectrometry	
gaseous diffusion		

Particle Separation methods

Filtration
Sedimentation
Elutriation
Centrifugation
Particle electrophoresis
Electrostatic precipitation

The 100-Year History of Separations

- Russian chemist and botanist Michael Tswett coined the term “chromatography”
- Chromatography was the first major “separation science”
- Tswett worked on the separation of plant pigments, published the first paper about it in 1903, and tested >100 stationary phases
  - Separated chlorophyll pigments by their color using $CaCO_3$ (chalk), a polar “stationary phase”, and petroleum ethers/ethanol/CS_2



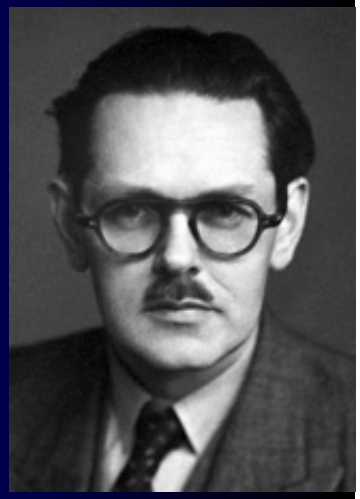
Tswett’s original adsorption chromatography apparatus

Mikhail Tswett , Physical chemical studies on chlorophyll adsorptions
Berichte der Deutschen botanischen Gesellschaft **24**, 316-23 (1906)

History of Analytical Chromatography

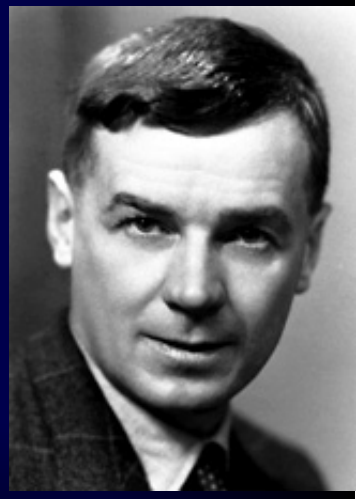
- Chromatography was “rediscovered” by Kuhn in 1931, when its analytical significance was appreciated
- Chromatography very rapidly gained interest: Kuhn (Nobel prize in Chemistry 1937) separates caretenoids and vitamins
- 1938 and 1939: Karrier and Ruzicka, Nobel prizes in Chemistry
- 1940: established analytical technique
- 1948: A. Tiselius, Nobel prize for electrophoresis and adsorption
- 1952: A. J. P. Martin and R. L. M. Synge, Nobel prize for partition chromatography, develop plate theory
- 1950-1960: Golay and Van Deemter establish theory of GC and LC
- 1965: Instrumental HPLC developed

R. Kuhn



A. J. P. Martin

A. Tiselius



R. L. M. Synge

Photographs from www.nobelprize.org

Introduction to Chromatography: Terminology

- IUPAC Definition: chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction

- Stationary phase (SP): common name for the column packing material in any type of chromatography
- Mobile phase (MP): liquid media that continuously flows through the column and carries the analytes
- Analyte: the chemical species being investigated (detected and quantitatively measured) by an analytical method

Basic Classification of Chromatographic Methods

- Column Chromatography
  - stationary phase is held in a narrow tube through which mobile phase is forced under pressure
    - Liquid chromatography
      - Mobile phase is a liquid solvent
    - Gas chromatography
      - Mobile phase is a carrier gas
    - Supercritical fluid chromatography
      - Mobile phase is a supercritical fluid
- Planar chromatography
  - stationary phase is supported on a flat plate or in the pores of a paper (e.g. TLC)

Separation of a Two-component Mixture

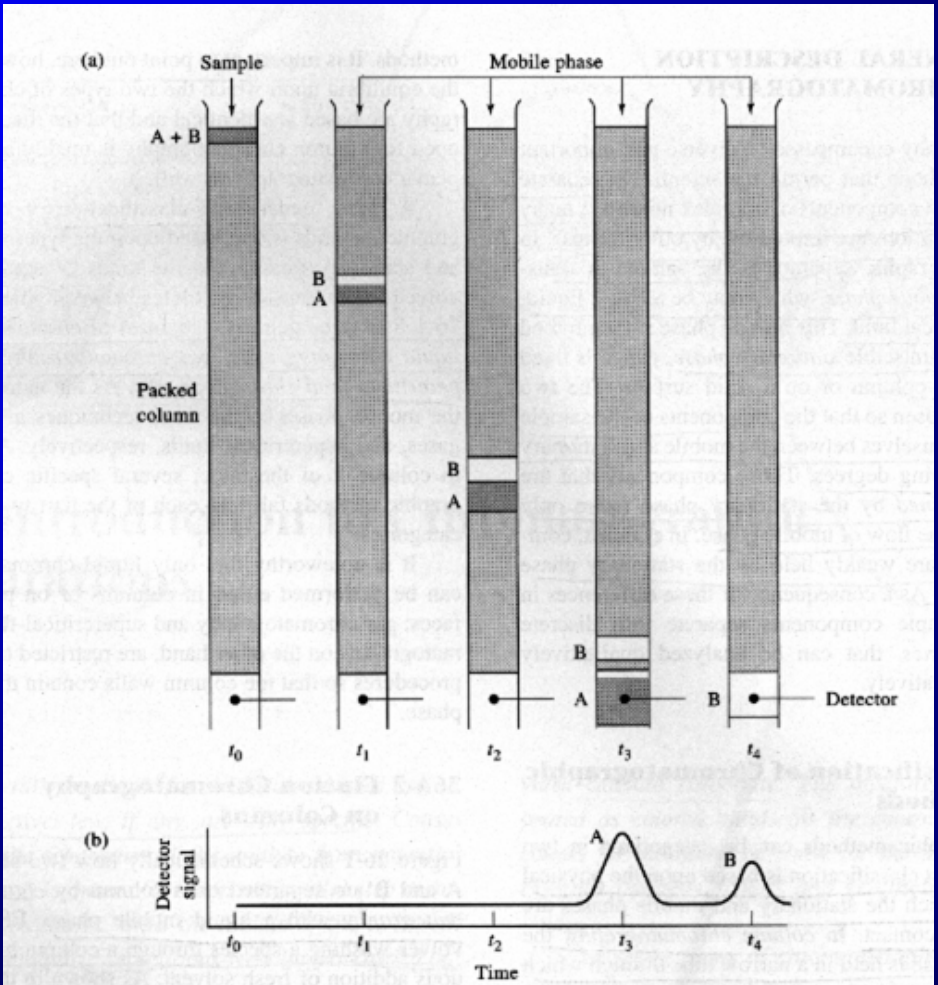
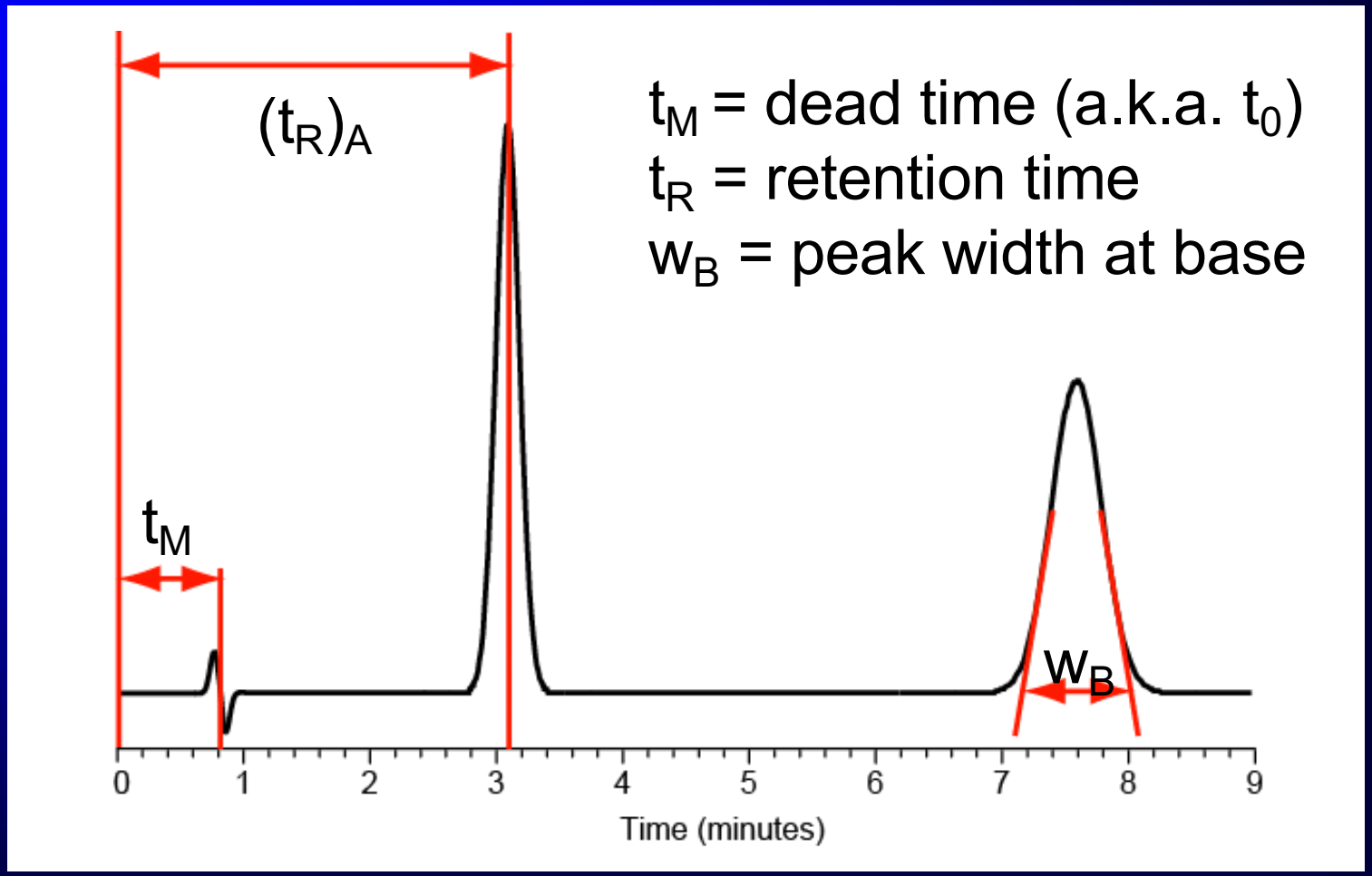


Figure 26-1 (a) Diagram showing the separation of a mixture of components A and B by column elution chromatography. (b) The output of the signal detector at the various stages of elution shown in (a).

- This demonstrates the basic concept of continuous elution

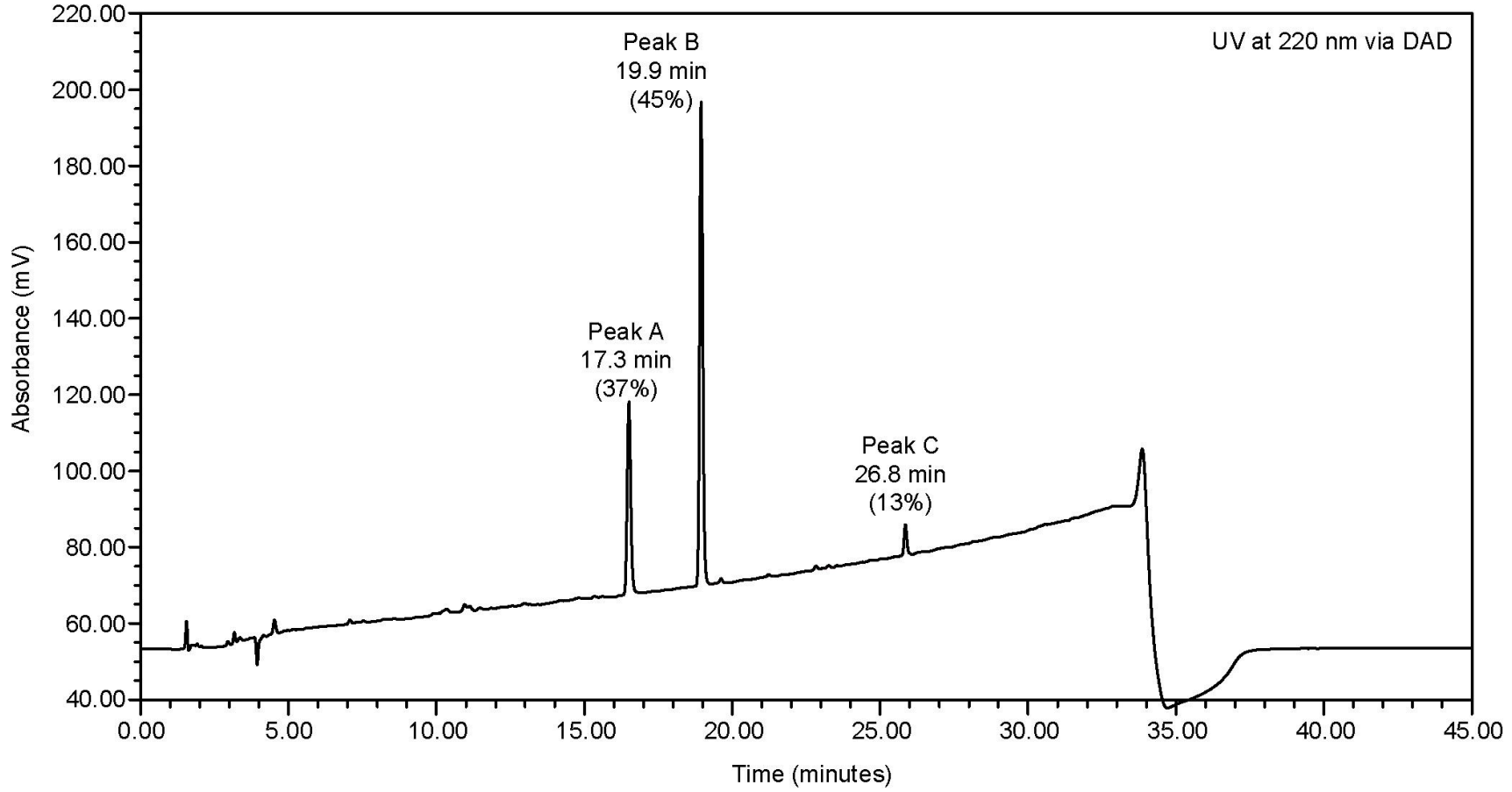
Chromatograms and Electropherograms

- A chromatogram or electropherogram shows detector response to analyte presence/concentration



- Dead time (volume): the "mobile phase holdup time", or the time it takes for an unretained analyte to reach the detector

A Typical LC Chromatogram

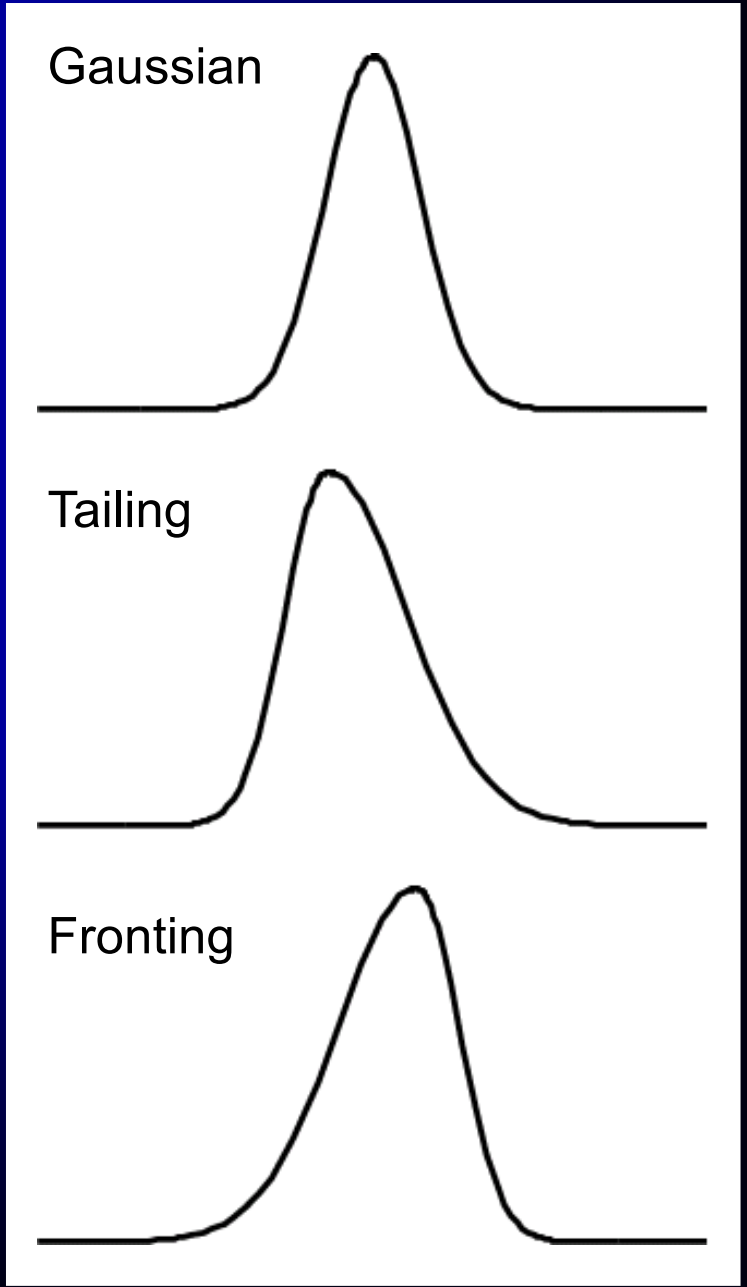


- This is a typical HPLC UV-detected chromatogram for a fairly simple mixture of a drug and a degradation product
- Note the upward-sloping baseline (we will explain when we discuss gradient elution)

Detector Peaks in Separation Sciences

- Peak shapes in separation sciences are generally Gaussian in nature, reflecting the fundamental nature of the processes at work (e.g. diffusion)

- In practice, real peaks are generally slightly asymmetric
  - Fronting peaks
  - Tailing peaks



Retention Time

- A way to characterize chromatographic retention is to measure the time between injection and the maximum of the detector response for the analyte. This parameter, which is usually called the retention time t_R, is inversely proportional to the eluent flow rate.
- Retention time is dictated by physics and chemistry:
  - Chemistry (factors that influence distribution)
    - stationary phase: type and properties
    - mobile phase: composition and properties
    - intermolecular forces
    - temperature
  - Physics (flow, hydrodynamics)
    - mobile phase velocity
    - column dimensions

Retention Volume

- The product of the retention time and the eluent flow rate (F) is called the retention volume V_R and represents the volume of the eluent passed through the column while eluting a particular analyte

$$V_R = t_R F$$

- Component retention volume V_R can be divided into two parts:
  - Reduced retention volume, which is the volume of the eluent that passed through the column while the component was stuck to the surface.
  - Dead volume, which is the volume of the eluent that passed through the column while the component was moving with the liquid phase.

Mobile Phase Velocity

- The average linear velocity of analyte migration (in cm/s) through a column is obtained by dividing the length of the packed column (*L*) by the analyte's retention time:

$$\bar{v} = \frac{L}{t_R}$$

L = length of column
t_R = retention time of analyte

- The average linear velocity of the mobile phase is just:

$$u = \frac{L}{t_M}$$

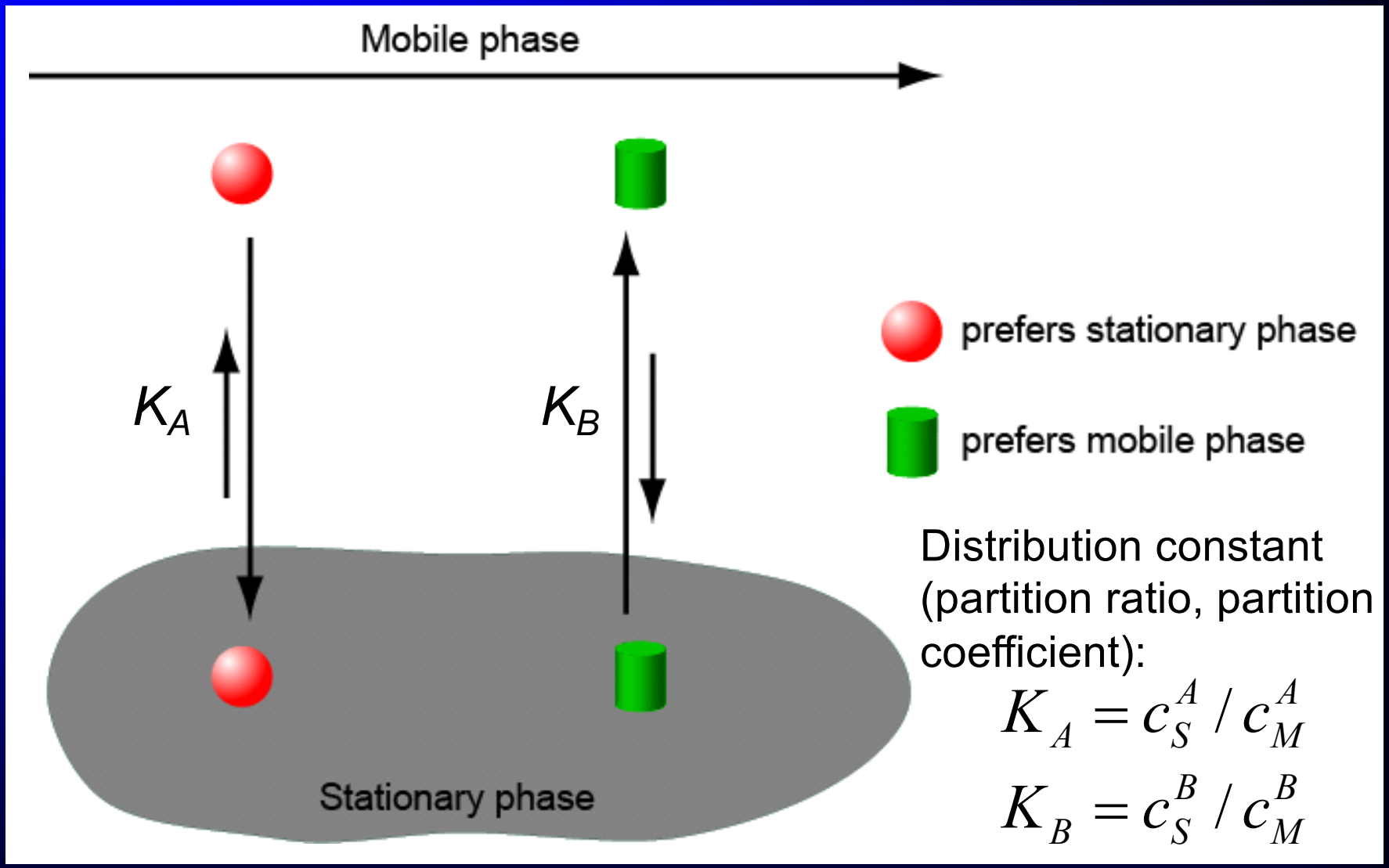
t_M = retention time of mobile phase ("dead time")

- Flow rate (mL/min) (*F*) is commonly used as an experimental parameter, it is related to the cross sectional area of the column and its porosity:

$$F = \pi r^2 u_0 \varepsilon$$

u_0 = linear velocity at column outlet
ε = fraction of column volume accessible to liquid

Retention and Differential Migration in Chromatography



Distribution constant (partition ratio, partition coefficient):

$$K_A = c_S^A / c_M^A$$

$$K_B = c_S^B / c_M^B$$

- Note: the arrows represent “approximate” equilibration

Relationship Between Retention Time and Distribution Constant

- Need to convert distribution constants into something measurable – first express rate as a fraction of mobile phase velocity:

average linear velocity of analyte migration

$$\bar{v} = u \times \frac{\text{moles of solute in mobile phase}}{\text{total moles of solute}}$$

average linear velocity of MP

$$\bar{v} = u \times \frac{c_M V_M}{c_M V_M + c_S V_S} = u \times \frac{1}{1 + c_S V_S / c_M V_M}$$

Substitute in definition of *K*

$$\bar{v} = u \times \frac{1}{1 + K V_S / V_M}$$

- Define *k*:

$$k = \frac{K V_S}{V_M} \longrightarrow \bar{v} = u \times \frac{1}{1+k}$$

Then substitute in definitions of *u* and $\bar{v}$

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1+k}$$

The Retention Factor *k*

- This leads to the definition k as the retention factor:

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1+k} \xrightarrow{\text{rearrange}} k = \frac{t_R - t_M}{t_M} = \frac{V_R - V_M}{V_M}$$

- The more universal and fundamental retention parameter is the ratio of the retention volume to the dead volume

$$k = \frac{t_R}{t_M} = \frac{V_R}{V_M}$$

- The parameter *k* is also known (especially in the earlier literature) as the capacity factor *k′*

Relative Migration Rates: The Selectivity Factor

- Selectivity factor (α): the ability of a given stationary phase to separate two components

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} = \frac{t'_{R,B}}{t'_{R,A}} = \frac{k_B}{k_A} = \frac{K_B}{K_A}$$

- α is by definition > 1 (i.e. the numerator is always larger than the denominator)
- α is independent of the column efficiency; it only depends on the nature of the components, eluent type, eluent composition, and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency.

Band Broadening (Column Efficiency)

- After injection, a narrow chromatographic band is broadened during its movement through the column.
- The higher the column band broadening, the smaller the number of components that can be separated in a given time.
- The sharpness of the peak is an indication of the efficiency of the column.

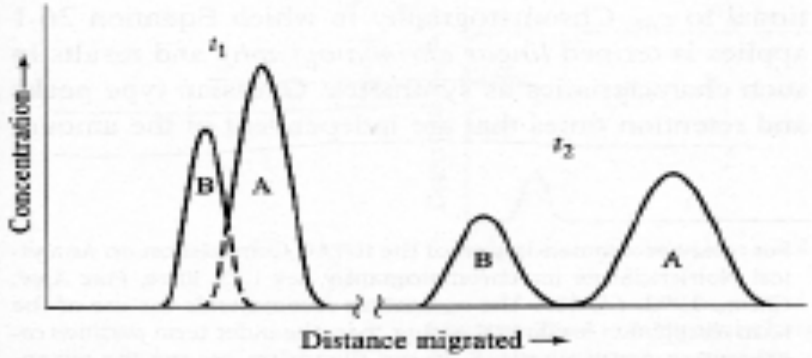


Figure 26-2 Concentration profiles of analyte bands A and B at two different times in their migration down the column in Figure 26-1. The times t_1 and t_2 are indicated in Figure 26-1.

Separation Efficiency and Peak Width

- The peak width is an indication of peak sharpness and, in general, an indication of the column efficiency . However, the peak width is dependent on a number of parameters :
  - column length
  - flow rate
  - particle size
- In absence of the specific interactions or sample overloading, the chromatographic peak can be represented by a Gaussian curve with the standard deviation σ. The ratio of standard deviation to the peak retention time σ /tR is called the **relative standard deviation**, which is independent of the flow rate.

Theoretical Plates

- A "plate": an equilibration step (or zone) between the analytes, mobile phase, and stationary phase (comes from distillation theory)
- Number of theoretical plates (N): the number of plates achieved in a separation (increases with longer columns)
- Plate "height" (H): a measure of the separation efficiency of e.g. the column
  - Smaller H is better
  - Also known as HETP (height equivalent to a theoretical plate)
  - Measures how efficiently the column is packed
- Plate equation:

$$H = \frac{L}{N}$$

Calculating Theoretical Plates

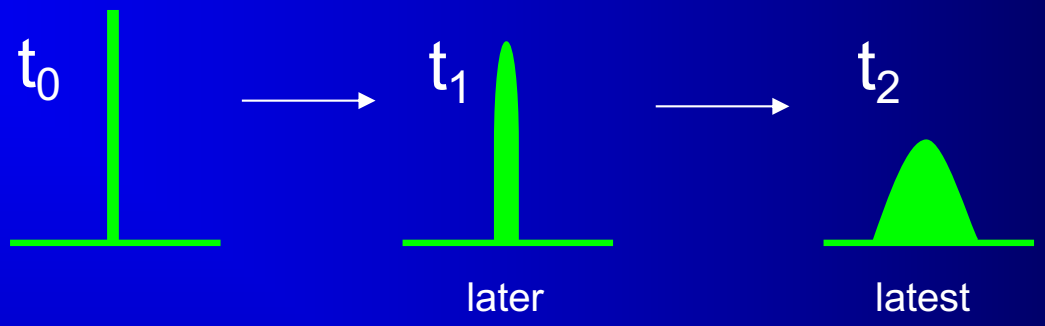
- The convention today is to describe the efficiency of a chromatographic column in terms of the plate number N, defined by:

$$N = \left(\frac{t_R}{\sigma} \right)^2$$

- In practice, it is more convenient to measure peak width either at the base line, or at the half height, and not at 0.609 of the peak height, which actually correspond to 2 σ.

$$N = \frac{16 t_R^2}{W_B^2} \qquad N = 5.545 \frac{t_R^2}{W_{1/2}^2}$$

Band Broadening Processes



- Non-column Broadening
  - Dispersion of analyte in:
    - Dead volume of an injector
    - Volume between injector and column
    - Volume between column and detector

- Column Broadening
  - Van Deemter and related model

Band Broadening Theory

- Column band broadening originates from three main sources:
  - multiple paths of an analyte through the column packing (A)
  - molecular diffusion (B)
  - effect of mass transfer between phases (C)

- In 1956, J.J. Van Deemter introduced the first equation which combined all three sources and represented them as the dependence of the theoretical plate height (H) and the mobile phase linear velocity (*u*)

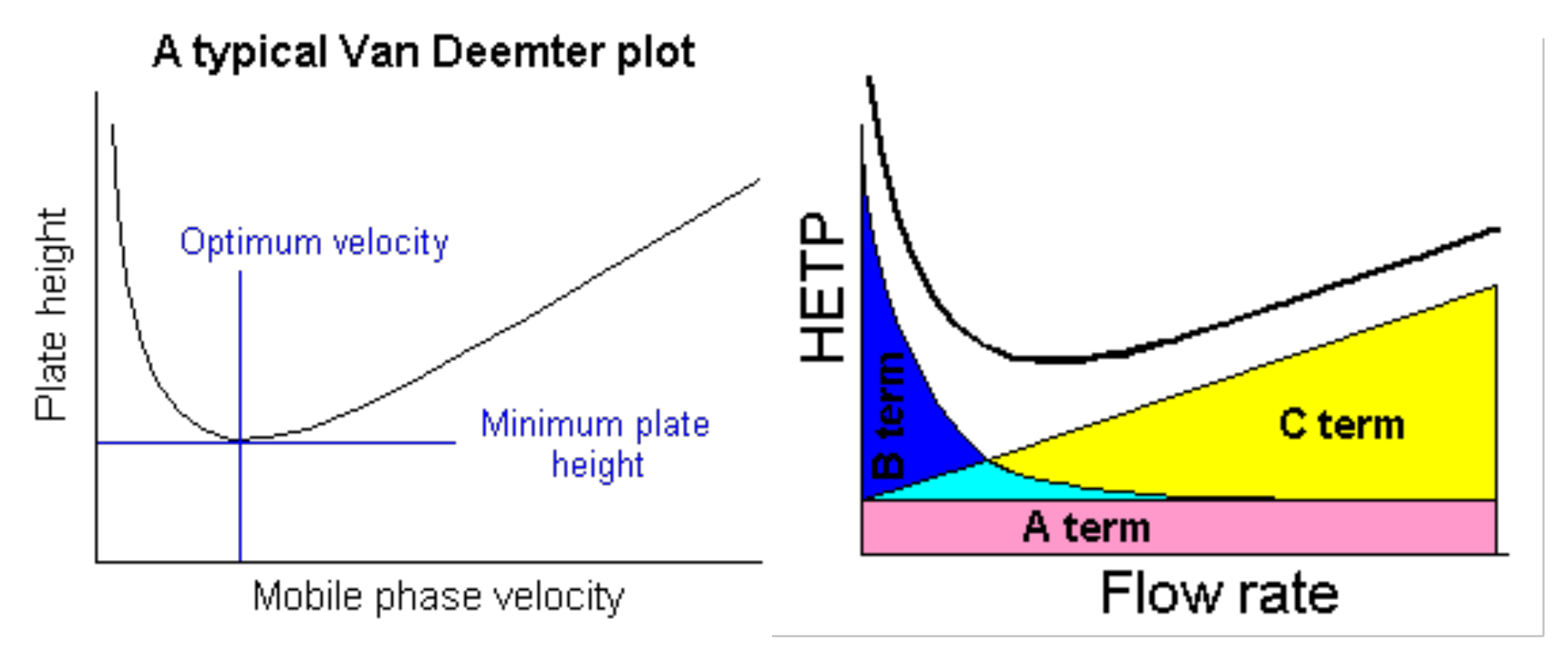
Relationship Between Plate Height and Separation Variables

- The Van Deemter equation is made up of several terms:

$$H = A + \frac{B}{u} + Cu$$

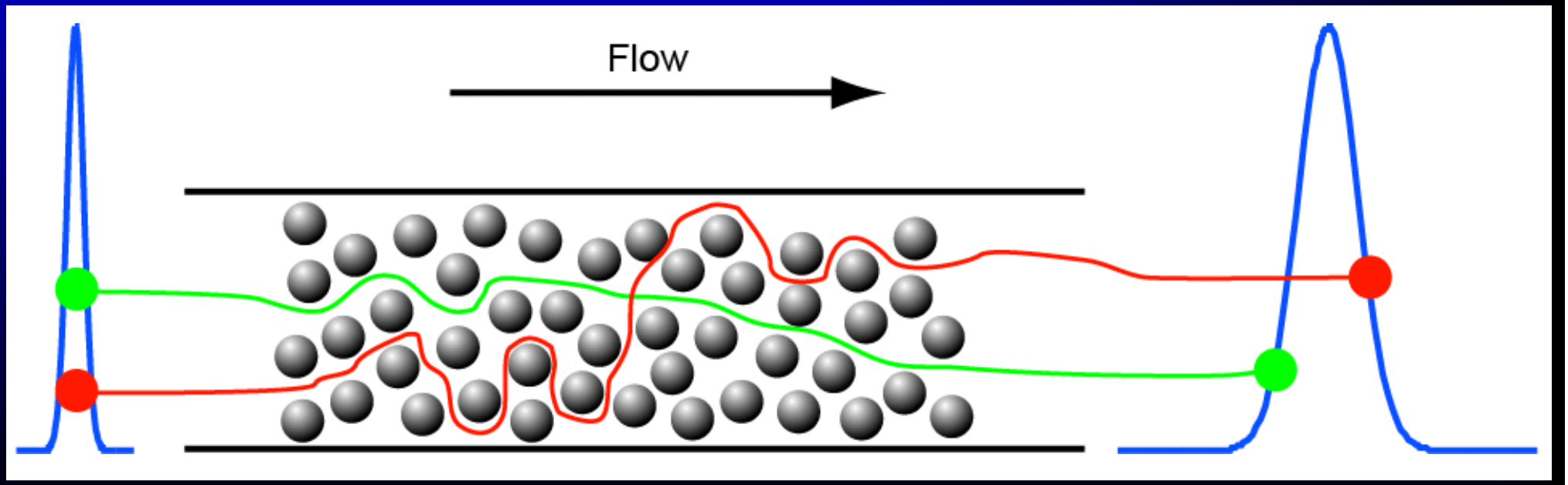
Remember: $u = \frac{L}{t_M}$

t_M = retention time of mobile phase ("dead time")



Van Deemter "A" Term

- The "A" Term: Eddy diffusion
  - molecules may travel unequal distances in a packed column bed
  - particles (if present) cause eddies and turbulence
  - "A" depends on size of stationary particles (small is best) and their packing "quality" (uniform is best)



Van Deemter "A" Term

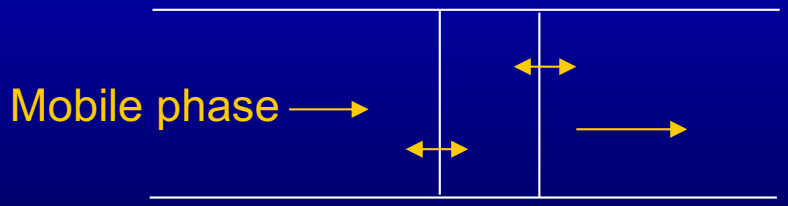
- The first cause of band broadening is differing flow velocities through the packed column
- This may be written as:

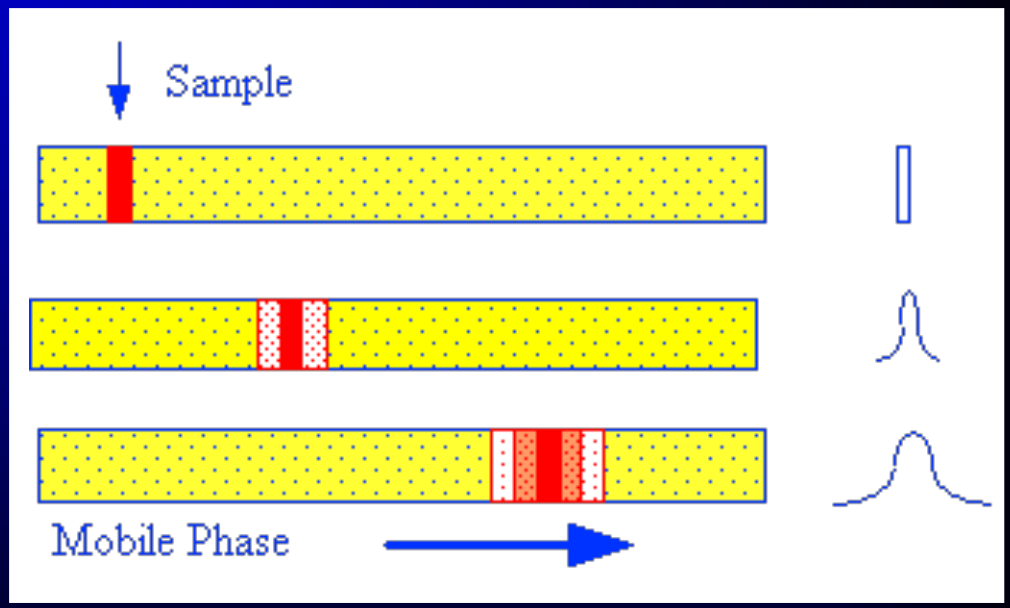
$$H = 2\lambda d_p$$

- In this equation, *H* is the plate height arising from the variation in the zone flow velocity, d_p is the average particle diameter, and λ is a constant that is close to unity
- *H* gets worse (larger) as the particle diameter increases

Van Deemter "B" Term

- The "B" Term: Longitudinal diffusion
  - The concentration of analyte is less at the edges of the band than at the center.
  - The analyte diffuses out from the center to the edges.
  - If *u* is high or the diffusion constant of the analyte is low, the "B" term has less of a detrimental effect





Note: The functional form of the term is B/u

Van Deemter "B" Term

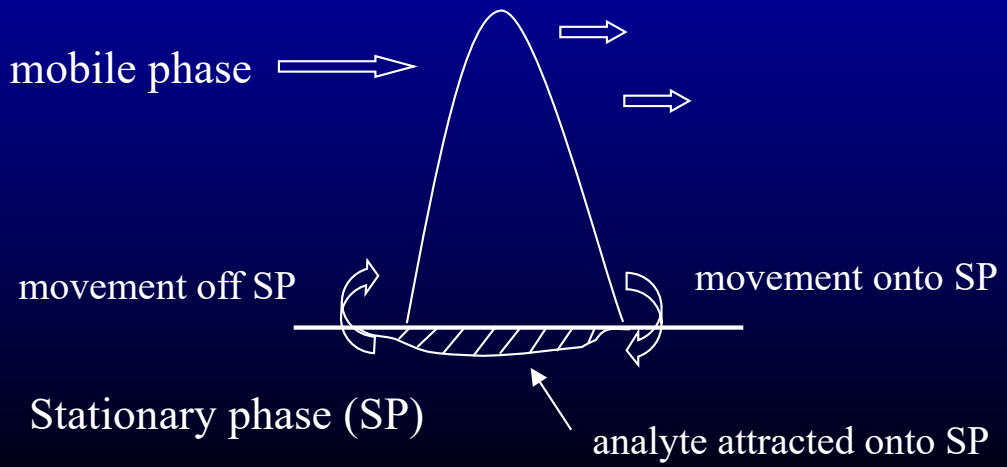
- The longitudinal diffusion (along the column long axis) leads to band broadening of the chromatographic zone. This process may be described by the equation:

$$H = \frac{B}{u} = 2\lambda \frac{D_m}{u}$$

- In this equation, D_m is the analyte diffusion coefficient in the mobile phase, λ is a factor that is related to the diffusion restriction by the column packing (hindrance factor), and u is the flow velocity.
  - The higher the eluent velocity, the lower the diffusion effect on the band broadening
  - Molecular diffusion in the liquid phase is about five orders of magnitude lower than that in the gas phase, thus this effect is limited for LC, but important for GC

Van Deemter "C" Term

- Resistance to Mass Transfer:
  - The analyte takes a certain amount of time to equilibrate between the stationary phase and the mobile phase
  - If the velocity of the mobile phase is high, and an analyte has a strong affinity for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the stationary phase
  - The band of analyte is broadened
  - The higher the velocity of the mobile phase, the worse the broadening becomes



Van Deemter "C" Term

- The C term is given by two parts (for MP and SP):

$$H = C_S u + C_M u = \frac{f(k) d_f^2}{D_S} u + \frac{f'(k) d_p^2}{D_M} u$$

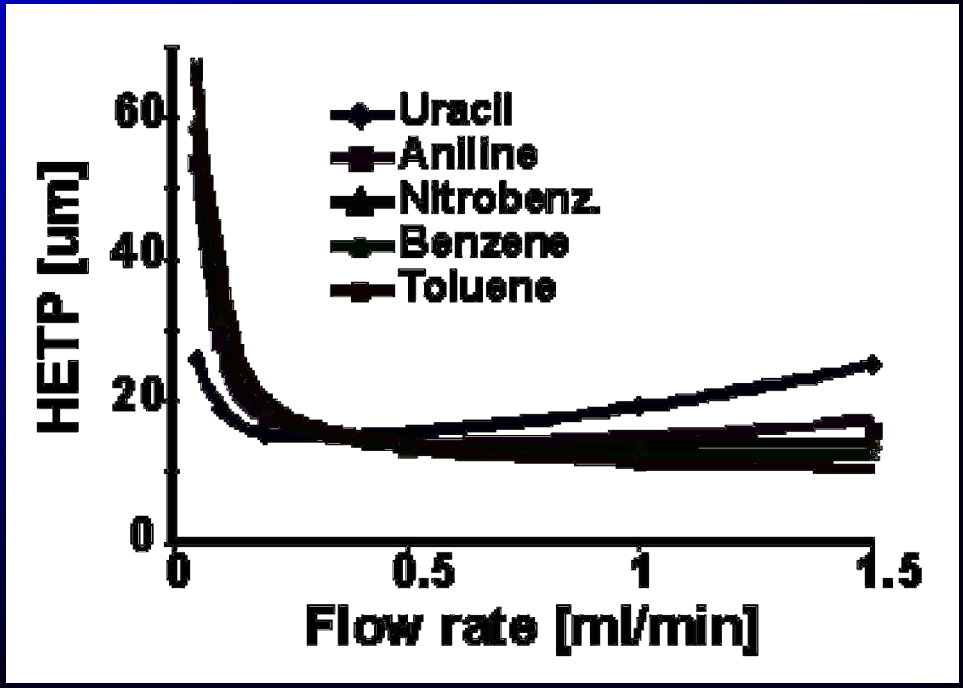
where d_p is the particle diameter, d_f is the thickness of the film, D_M and D_S are the diffusion coefficients of the analyte in the mobile/stationary phases, and u is the flow velocity

- The slower the velocity, the more uniformly analyte molecules may penetrate inside the particle, and the less the effect of different penetration on the efficiency.
- On the other hand, at the faster flow rates the elution distance between molecules with different penetration depths will be high.

The Combined Van Deemter Equation

$$H = \underbrace{2\lambda d_p}_{A} + \underbrace{2\lambda \frac{D_m}{u}}_{B} + \underbrace{\frac{f(k)d_f^2}{D_S}u + \frac{f'(k)d_p^2}{D_M}u}_{C}$$

- The most significant result is that there is an optimum eluent flow rate where the separation efficiency will be the best, and it is similar for many compounds



Alternative Models for Band Broadening

- Golay, 1958
  - open columns, no unequal pathways

    $H = B/u + Cu$

- Giddings, 1961
  - defined reduced plate height (hR) and reduced velocity (v)

    $hR = H/dP \quad v = u\ dP/DM$

- Knox et al., 1970

    $hR = Av\ 1/3 + B/v + Cv$

J. C. Chen and S. G. Weber, Anal. Chem. **1983**, *55*, 127 - 134

Resolution

- The selectivity factor, α, describes the separation of band centers but does not take into account peak widths. Another measure of how well species have been separated is provided by measurement of the *resolution*.
- The resolution of two species, A and B, is defined as

$$R_s = \frac{2\left[(t_R)_B - (t_R)_A\right]}{W_A + W_B}$$

(Eq. 26-24 in Skoog et al. 6th edition)

- Baseline resolution is achieved when $R_s = 1.5$
- The resolution is related to the number of column plates *(N)*, the selectivity factor (α) and the average retention factor *(k)* of A and B:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{1 + k}{k}\right)$$

Improving Resolution

- For good resolution in separations, the three terms can be optimized

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{1 + k}{k}\right)$$

- Increasing *k* (retention factor)
  - Change temperature (GC)
  - Change MP composition (LC)
- Increasing *N* (number of plates)
  - Lengthen column (GC)
  - Decrease SP particle size (LC)
- Increasing α (selectivity factor)
  - Changing mobile phase
  - Changing column temperature
  - Changing stationary phase