



Chemistry 4631

Instrumental Analysis

Lecture 22

Introduction to Chromatography

History - Chromatography

Originally the separation of color (in plant pigments)

- First demonstrated in 1906 by Michael Tswett (Russian botanist) – used a column of crushed chalk and poured leaf extracts into it and separated color fractions (chlorophyll and carotene).
- Taylor and Urey – 1938 – developed ion-exchange chromatography to separate lithium and potassium isotopes on zeolites.
- Martin and Synge – 1941 – developed partition chromatography. They developed paper chromatography to separate amino acids using ninhydrin spray for detection.

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History – Chromatography, con't

- James and Martin – 1952 – developed gas-liquid chromatography, separated amines and carboxylic acids using a gaseous mobile phase.
- McWilliam, Dewar and Harley – 1958 - Gas chromatography (GC) became universally accepted with the invention of flame ionization detector.
- High performance liquid chromatography was developed in small stages by several researchers in the late 1960's.

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General Principles: Definition

IUPAC Definition of Chromatography

A method, used primarily for separation of the components of a sample in which the components are distributed between two phases, one of which is stationary while the other moves. The stationary phase may be a solid, liquid supported on a solid, or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film. The mobile phase may be gaseous or liquid.

[Notice that the definition neglects supercritical fluid chromatography (SFC)].

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General Principles: Classification Methods

- **Classify by Mobile Phase**
 - **Three areas:**
 - **Liquid Chromatography (LC)**
 - **Gas Chromatography (GC)**
 - **Supercritical Fluid Chromatography (SFC)**

Further classify by specifying stationary phase, i.e. gas-solid, gas-liquid, etc.

For LC:

A nonpolar mobile phase with polar stationary phase is called normal-phase chromatography.

A polar mobile phase with nonpolar stationary phase is called reversed-phase chromatography

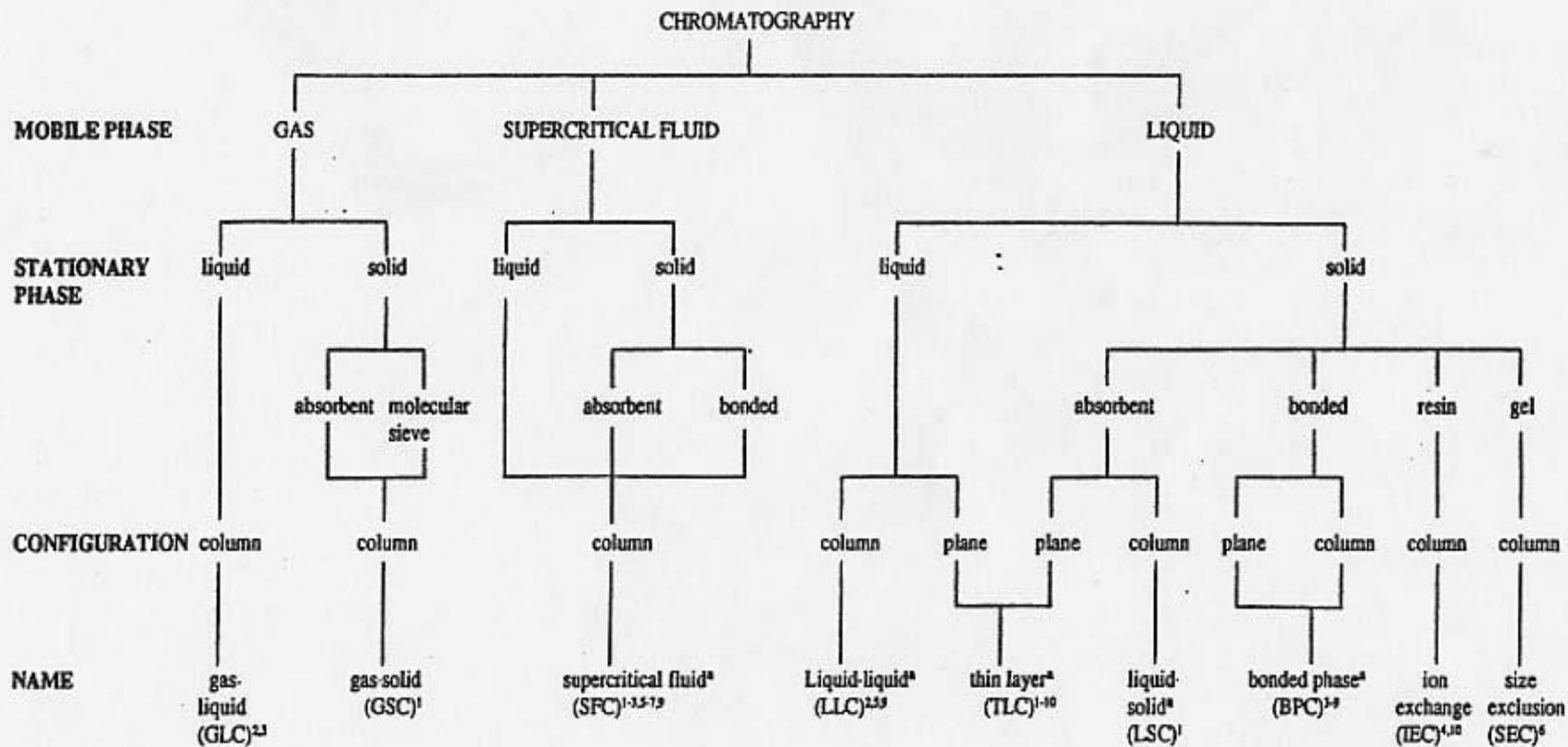
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General Principles: Classification Methods

Classify by Technique

Column or Planar (TLC)

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*For these techniques the combination of mobile and stationary phase can be varied to generate either a normal phase or reversed phase system. Mechanisms which have been exploited in the various techniques are identified as: ¹adsorption, ²partition, ³bonded phase, ⁴ion exchange, ⁵ion interaction, ⁶size exclusion, ⁷affinity, ⁸micellar, ⁹chelation, ¹⁰ion exclusion.

Fig. 1.3. Classification of chromatographic systems.

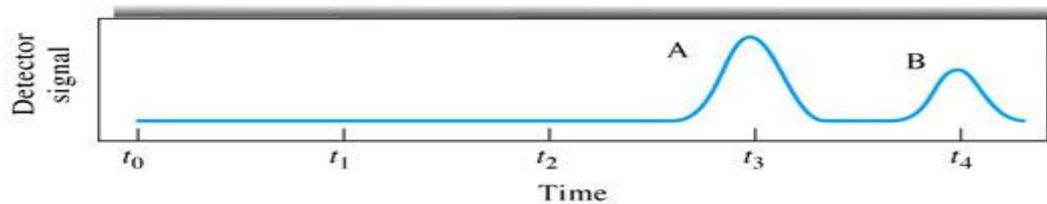
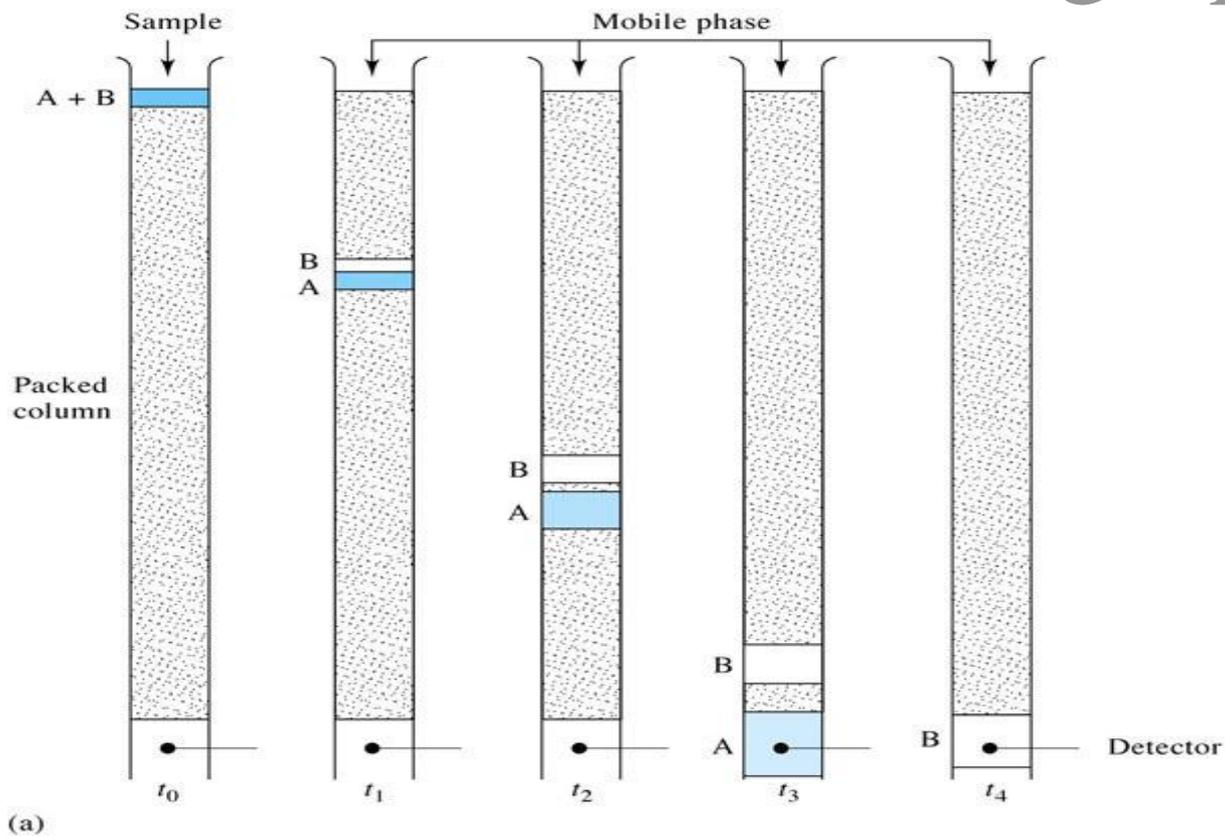
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General Principles: Classification Methods

Classify by Separation Mechanism

- **Adsorption** – solute and mobile phase molecules compete for active sites on the surface of the solid stationary phase (the adsorbent).
- **Partition** – partitioning of a solute between two immiscible liquids but one liquid is held stationary on a solid support. A solute in contact with two immiscible liquids will distribute itself between them according to its distribution coefficient, K .
- **Ion-exchange**
- **Size exclusion** – physical sieving process (gel permeation is a size exclusion process).
- **Affinity** – stationary phase is a bioactive liquid bonded to solid support. Antibody-antigen interactions, chemical, chiral, etc.
- **Micellar or pseudophase** – modifier added to mobile phase – surfactants.

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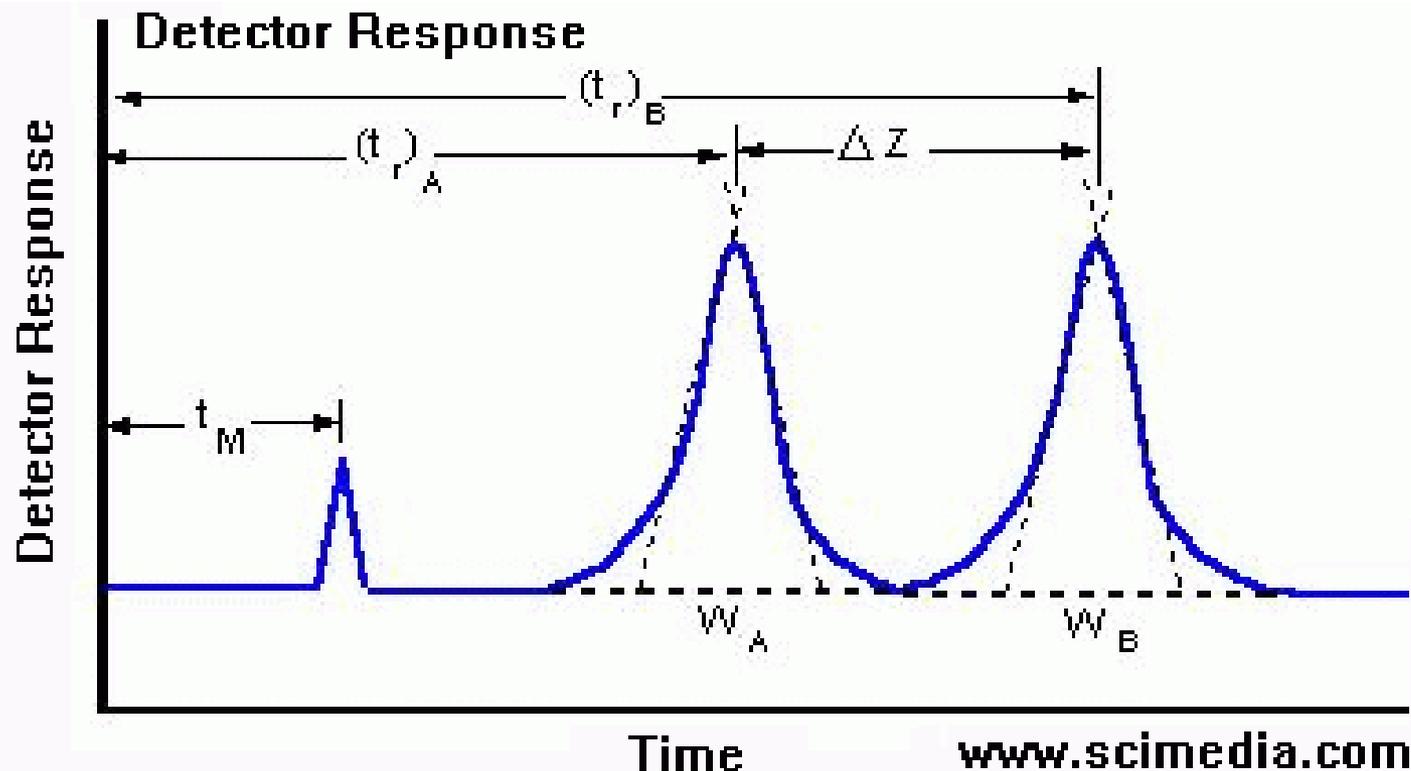


(b)
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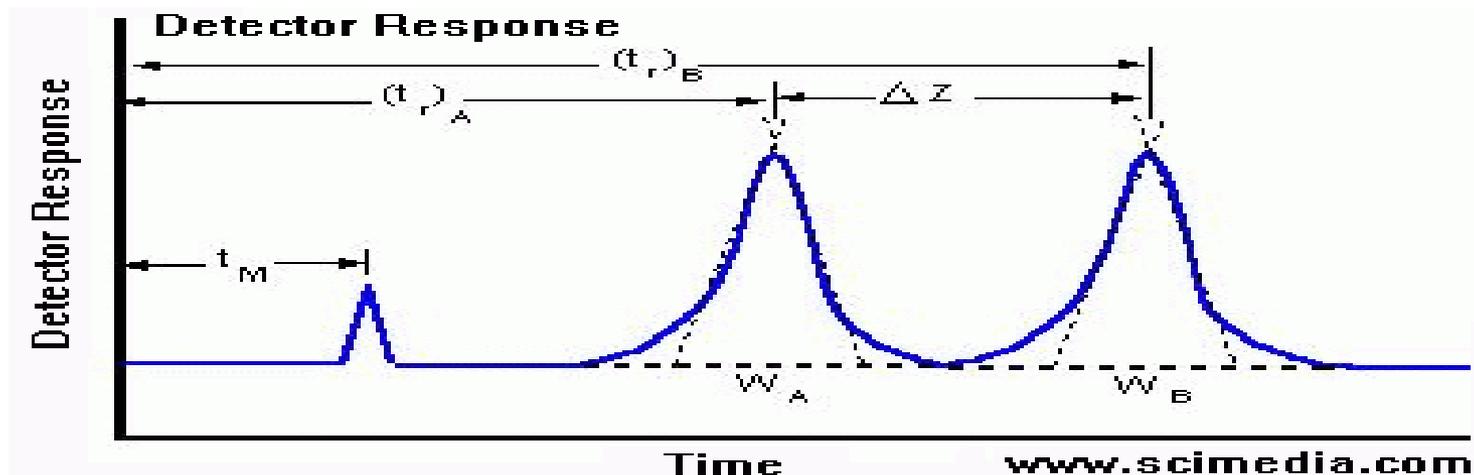
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Behavior of Solutes in Columns: Parameters Defined

Reflects the distribution of a solute between the mobile and stationary phases.



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0 sec-Injection Point

M - Unretained Solute Peak

A,B - Solute Peaks (Analyte)

V_r - Retention Volume-the volume of mobile phase necessary to convey a solute band from the point of injection, through the column, to the detector.

t_r - retention time of analyte to move from injection to detector

V_m - retention volume of the unretained solute

t_m - retention time of unretained solute

V_r' - adjusted retention volume

t_r' - adjusted retention time

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Notice the retention time an analyte spends in the system is composed of two components: t_r' and t_m

t_m - is the time it takes a solute to pass through the space occupied by the mobile phase (dead space, column void volume).

t_m is the same for all analytes in a given system – the solutes migrate with the same velocity of the mobile phase.

t_m represents no separation process.

The adjusted retention time, t_r' - represents the time the analyte spends retained by the stationary phase.

t_r' represents the separation process or interaction of analyte with stationary phase.

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Partition Coefficient, Distribution Coefficient

K – concentration of the analyte in the stationary and mobile phase

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

C_s – concentration of the analyte in stationary phase

C_m – concentration of the analyte in mobile phase

$K = 1$ when the analyte is distributed equally

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Capacity Factor, Partition Ratio, k'

- k' – solute partition ratio is an important parameter routinely used in HPLC but not as much in GC.
- k' – the ratio of the total amount of a solute in the stationary phase to the amount in the mobile phase at equilibrium.

$$k' = \frac{m_s}{m_m}$$

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Relationship between k' and K

$$K = \frac{C_s}{C_m} = \frac{m_s / V_s}{m_m / V_m} = \frac{m_s}{m_m} \cdot \frac{V_m}{V_s}$$

$$K = k' \times \frac{V_m}{V_s}$$

V_s – volume of the stationary phase

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$$K = k' \times \frac{V_m}{V_s}$$

The ratio V_m / V_s is the phase ratio called β and is used to characterize columns, especially in GC.

β is a measure of the “openness of the column.”

$K = k' \beta$ – relates the equilibrium distribution of the analyte within the column to the thermodynamic coefficient, K .

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This probability can be related to time, which is easier to measure.

$$k' = \frac{t_r - t_m}{t_m} = \frac{t'_r}{t_m}$$

In practice, accurate measure of column void volume is difficult in HPLC (difficult to find marker compounds), but for GC the practice is simple and accurate.

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Retention Time

$$k' = \frac{t_r - t_m}{t_m}$$

rearrange $t_r = t_m (1 + k')$

and substitute for k'

$$K = k' \cdot \frac{V_m}{V_s} \qquad k' = K \cdot \frac{V_s}{V_m}$$

$$t_r = t_m (1 + K V_s / V_m)$$

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Average linear mobile phase velocity is u

$u = L / t_m$ L – length of the column

$$t_r = \frac{L}{u} \left(1 + KV_s / V_m \right)$$

- equation used to predict the effect of change in column length, linear velocity of mobile phase, phase ratio and distribution coefficient on retention time of an analyte.
- equation is only valid when the mobile phase velocity is constant throughout the column.

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Column Efficiency

One goal of chromatography is to achieve sharp symmetrical peaks, thus optimizing analyte separation and improving detection.

The sharpness of the peak represents the efficiency of the chromatographic column (actually the entire system).

Two general approaches have been developed to measure column efficiency – plate theory and rate theory.

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TABLE 26-2 Variables That Influence Column Efficiency

Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	cm s^{-1}
Diffusion coefficient in mobile phase*	D_M^{**}	$\text{cm}^2 \text{s}^{-1}$
Diffusion coefficient in stationary phase*	D_S	$\text{cm}^2 \text{s}^{-1}$
Retention factor (Equation 26-12)	k	unitless
Diameter of packing particles	d_p	cm
Thickness of liquid coating on stationary phase	d_f	cm

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Plate Theory

- Models a chromatographic column as a series of narrow, discrete sections called theoretical plates.
- Assume that at each plate equilibrium of the analyte is established between mobile and stationary phase.
- Movement of analyte and mobile phase is viewed as a series of transfers from one plate to the next. Efficiency of a column increases as the number of equilibrations (i.e. theoretical plates) increases.

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Plate Theory

Efficiency of a column increases as the number of equilibrations (i.e. theoretical plates) increases.

n - # of theoretical plates or plate number

n_{theor} – measures the column efficiency

$$n = 5.54 \left(\frac{t_r}{w_{1/2}} \right)^2 \quad (\text{assumes a Gaussian peak})$$

$$n = 16 \left(\frac{t_r}{w_b} \right)^2$$

n is a dimensionless quantity

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Plate Theory

N or N_{eff} or n_{eff} – Effective plate number or number of effective theoretical plates. Used especially if void volume is large or for early eluting peaks.

$$N = 5.54 \left(\frac{t_r - t_m}{w_{1/2}} \right)^2 = 5.54 \left(\frac{t'_r}{w_{1/2}} \right)^2$$

$$N = 16 \left(\frac{(t_r - t_m)}{w_b} \right)^2 = 16 \left(\frac{t'_r}{w_b} \right)^2$$

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Plate Theory

If the peak is asymmetrical then calculation of n is more complex.

$$N = \frac{41.71(t_r / w_{0.1})^2}{A_s + 1.25} \quad (\text{for asymmetrical peak})$$

A_s – asymmetry factor

$w_{0.1}$ – peak width at 10% of peak height

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Plate Theory

Plate number and effective plate number depend on the length of the column, so other parameters have been developed.

- h or h_{etp} or h_{etp} – the height equivalent to a theoretical plate or plate height

$$h = \frac{L}{n} \quad L - \text{length of the column}$$

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Plate Theory

- H or HETP – the effective plate height or height equivalent to an effective theoretical plate.

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

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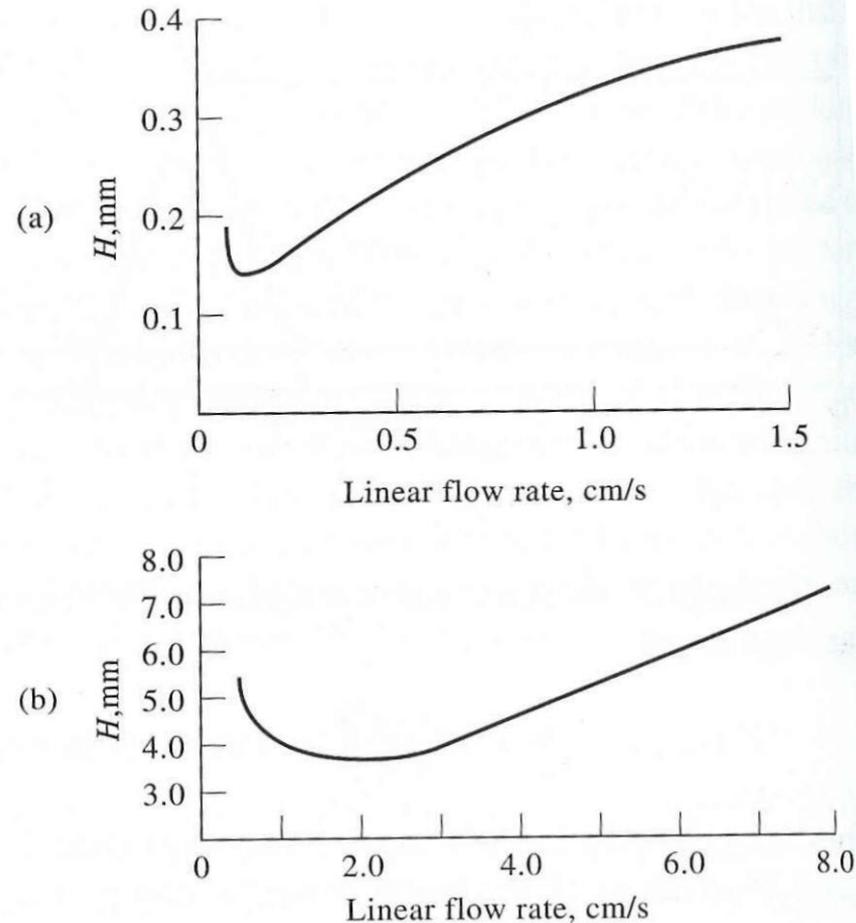


Figure 26-7 Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.

Assignment

- Read Chapter 23
- HW12 Chapter 23: 2, 4, 7, 8, and 11
- HW12 Chapter 23 Due 3/29/24

- Read Chapter 25
- HW13 Chapter 25: 1-8 and 10
- HW13 Chapter 25 Due 04/01/24

- Read Chapter 26
- HW14 Chapter 26: 1- 17
- HW14 Chapter 26 Due 04/03/24

- **Test 3 – PPT Lectures 15-21 - Friday April 5th**

