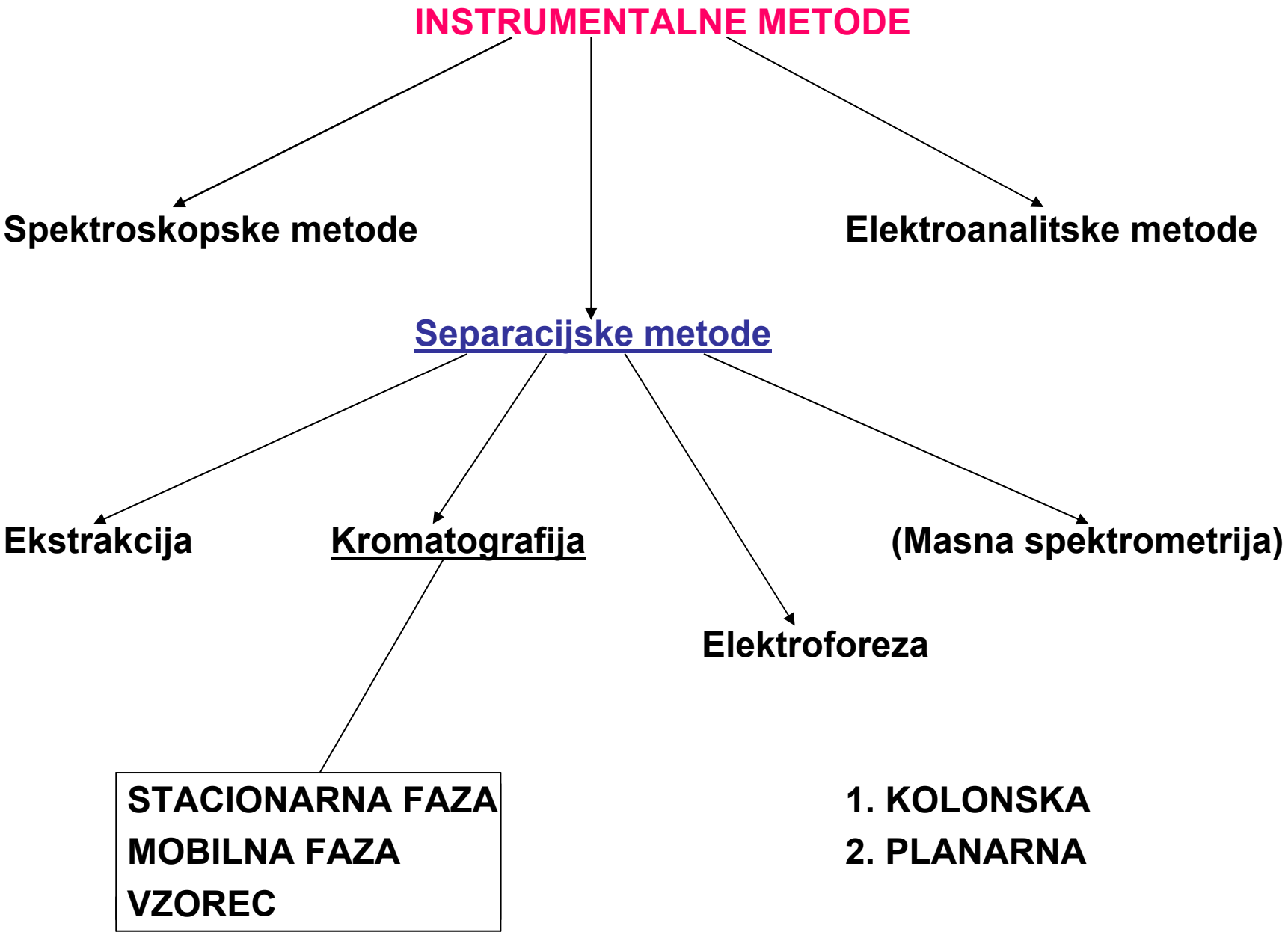

INSTRUMENTALNE METODE
Spektroskopske metode
Elektroanalitske metode
Separacijske metode
Ekstrakcija
Kromatografija
(Masna spektrometrija)
Elektroforeza
STACIONARNA FAZA
MOBILNA FAZA
VZOREC
1. KOLONSKA
2. PLANARNA

MF

1. *PLINSKA KROMATOGRAFIJA*
GC

2. *TEKOČINSKA KROMATOGRAFIJA*
LC

3. *SUPERKRITIČNA-TEKOČINSKA KROMATOGRAFIJA*
SFC

SF

ADSORPCIJSKA (GSC)

PORAZDELITVENA (GLC)

ADSORPCIJSKA (LSC)

PORAZDELITVENA (LLC)

IONSKO-IZMENJALNA (IEC)

IZKLJUČITVENA (SEC)

PORAZDELITVENA

SEPARACIJSKE METODE

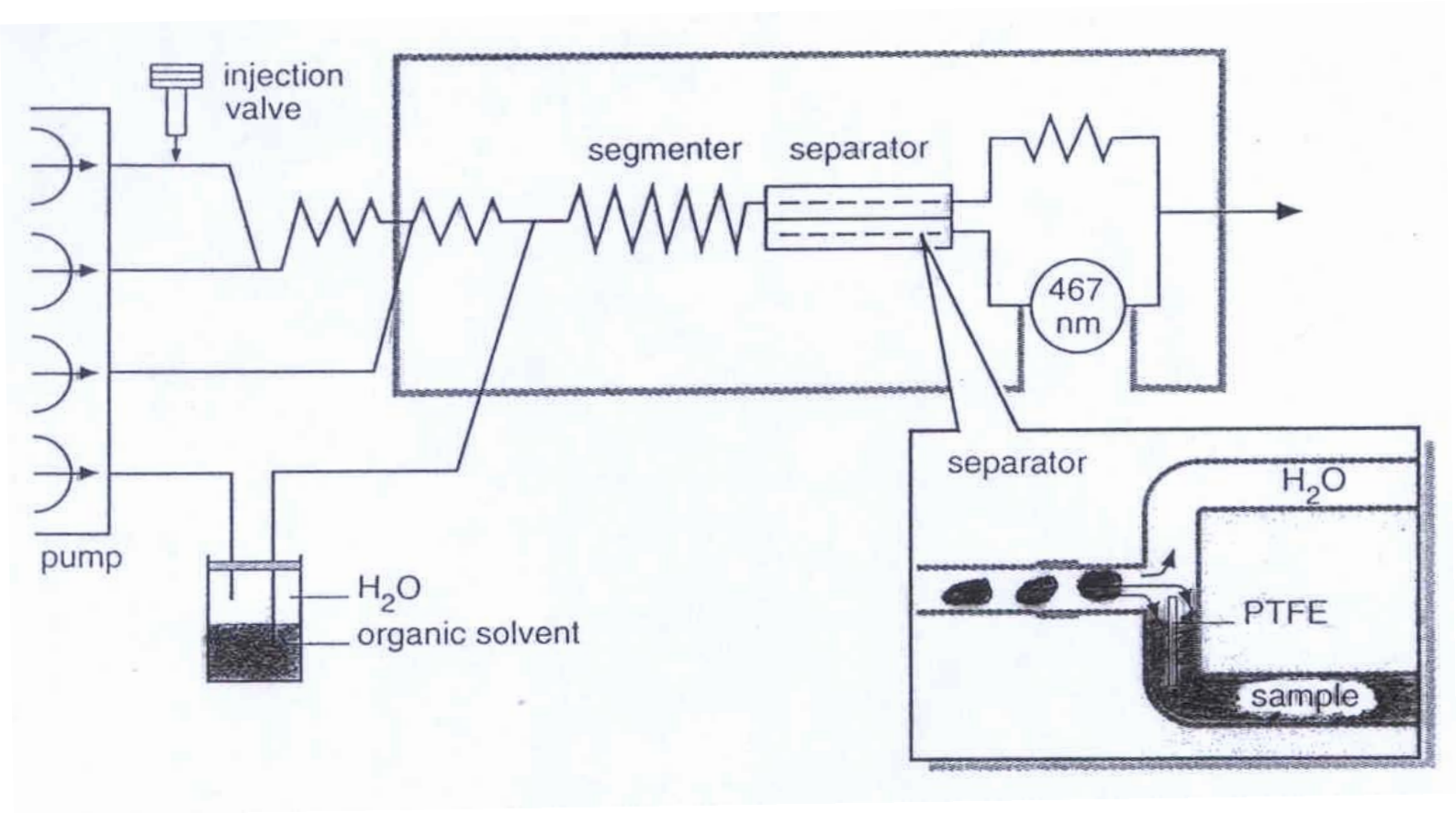
a) Separacije s transformacijo

b) Separacije brez transformacije

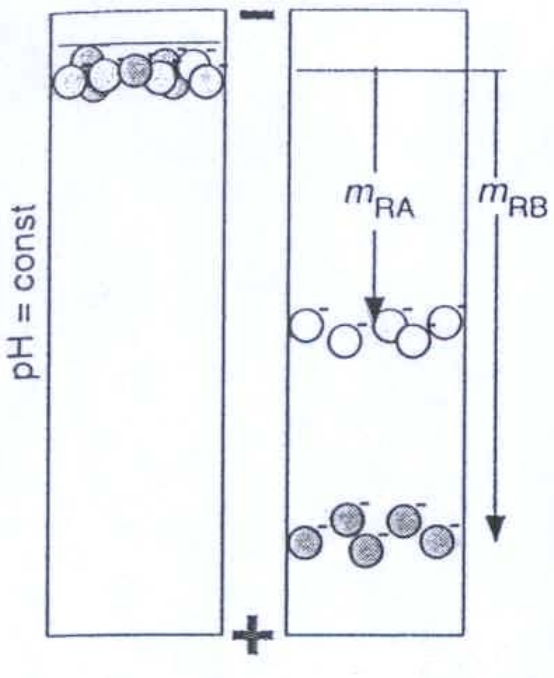
<u>Značilnost molekul kot osnova separacijskih metod</u>

- **Polarnost**
  - **- Tekočinska kromatografija**
  - **- Plinska kromatografija**
- **Ionski značaj**
  - **- Ionsko-izmenjevalna kromatografija**
  - **- Elektroforeza**
  - **- Masna spektrometrija**
- **Velikost (masa)**
  - **- Dializa in ultrafiltracija**
  - **- Izključitvena kromatografija**
  - **- Ultracentrifugacija**
- **Oblika**
  - **- Afinitetna kromatografija**
  - **- Imunokemične metode**
  - **- Encimske metode**

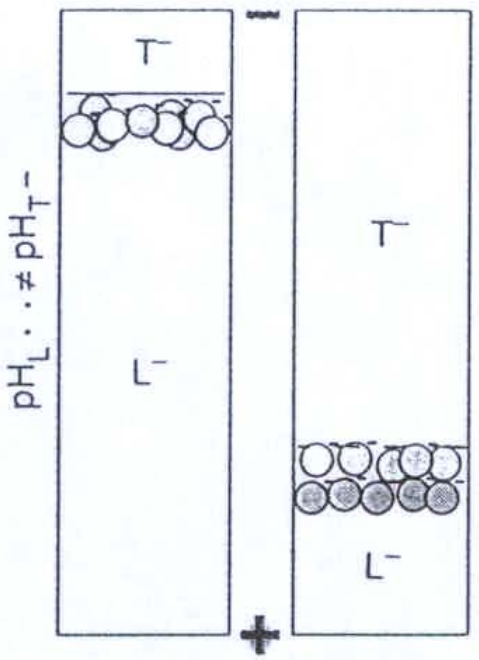
EKSTRAKCIJA



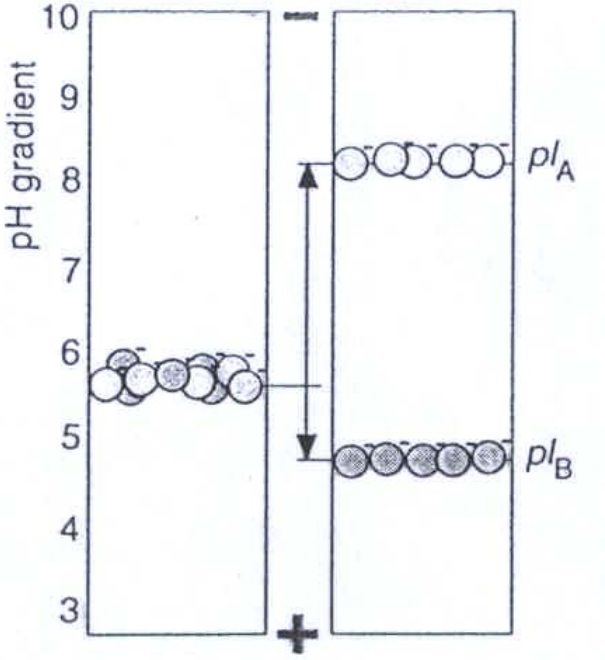
ELEKTROFOREZA



conska elektroforeza

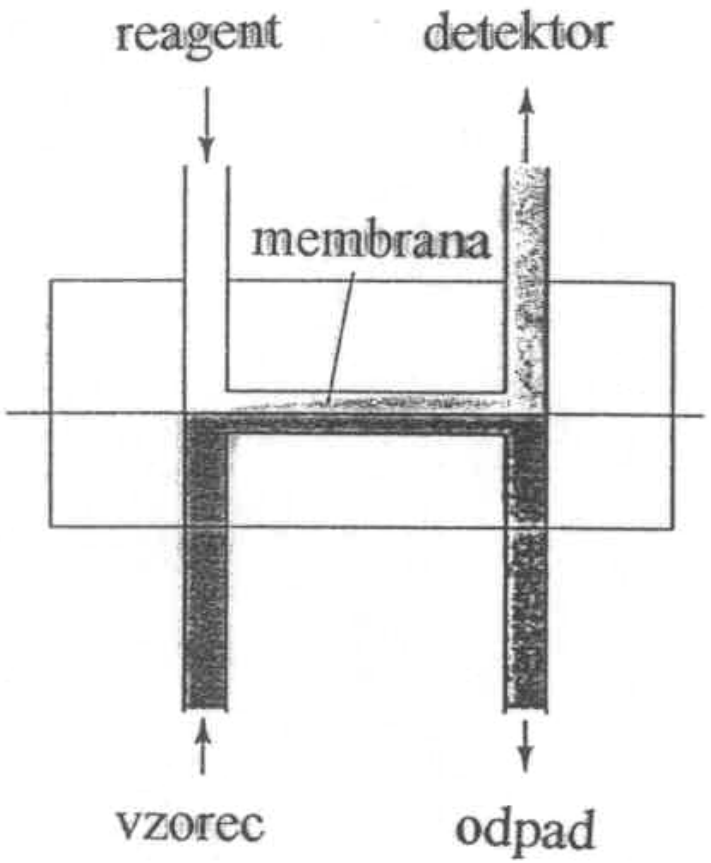


izotahoforeza

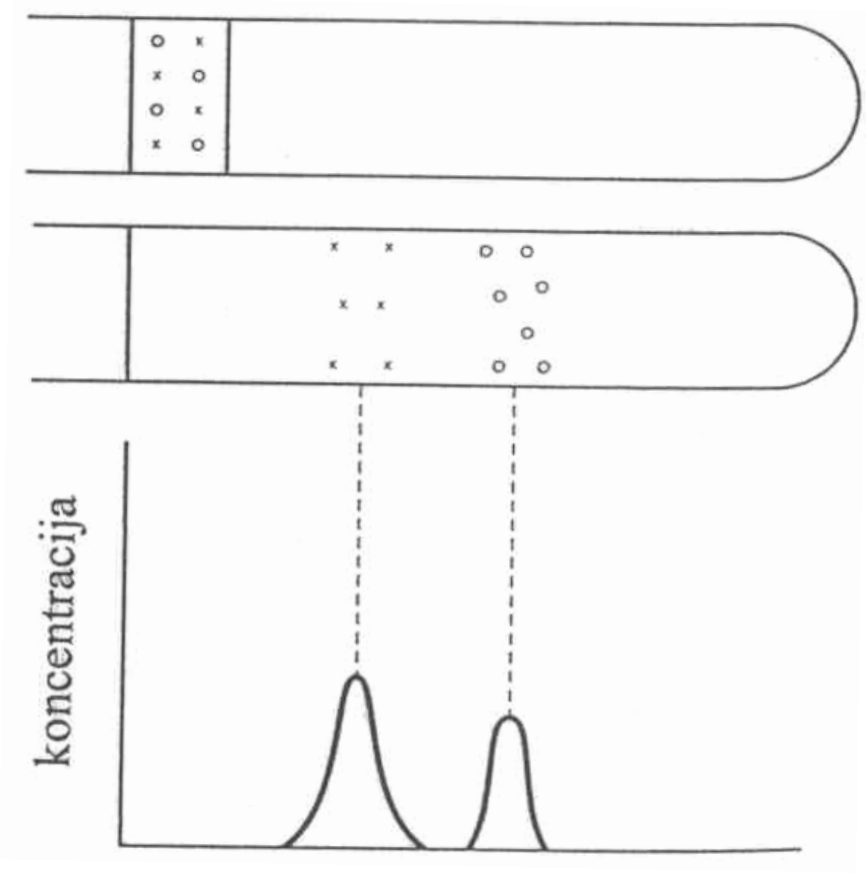


izoelektrično fokusiranje

DIALIZA

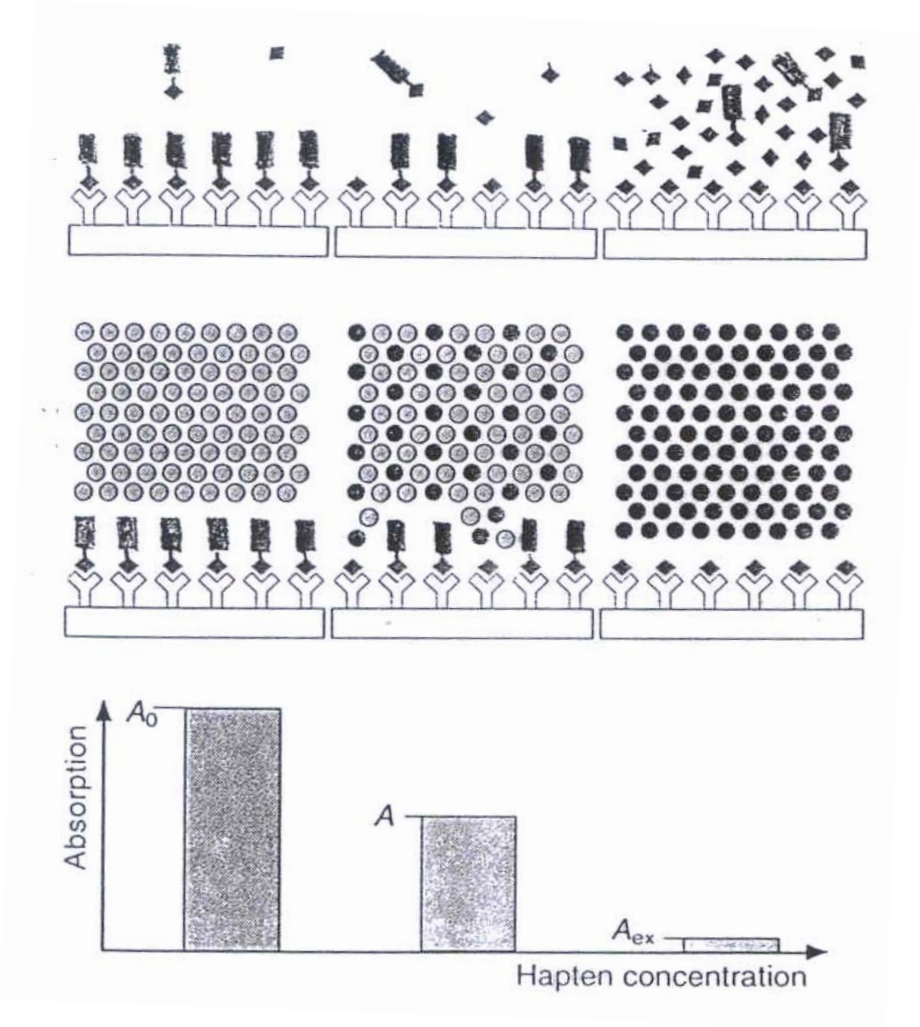


ULTRACENTRIFUGACIJA



conska ultracentrifugacija

IMUNOKEMIČNE METODE



ENCIMSKE METODE

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

$$k_m = \frac{k_{-1} + k_2}{k_1}$$

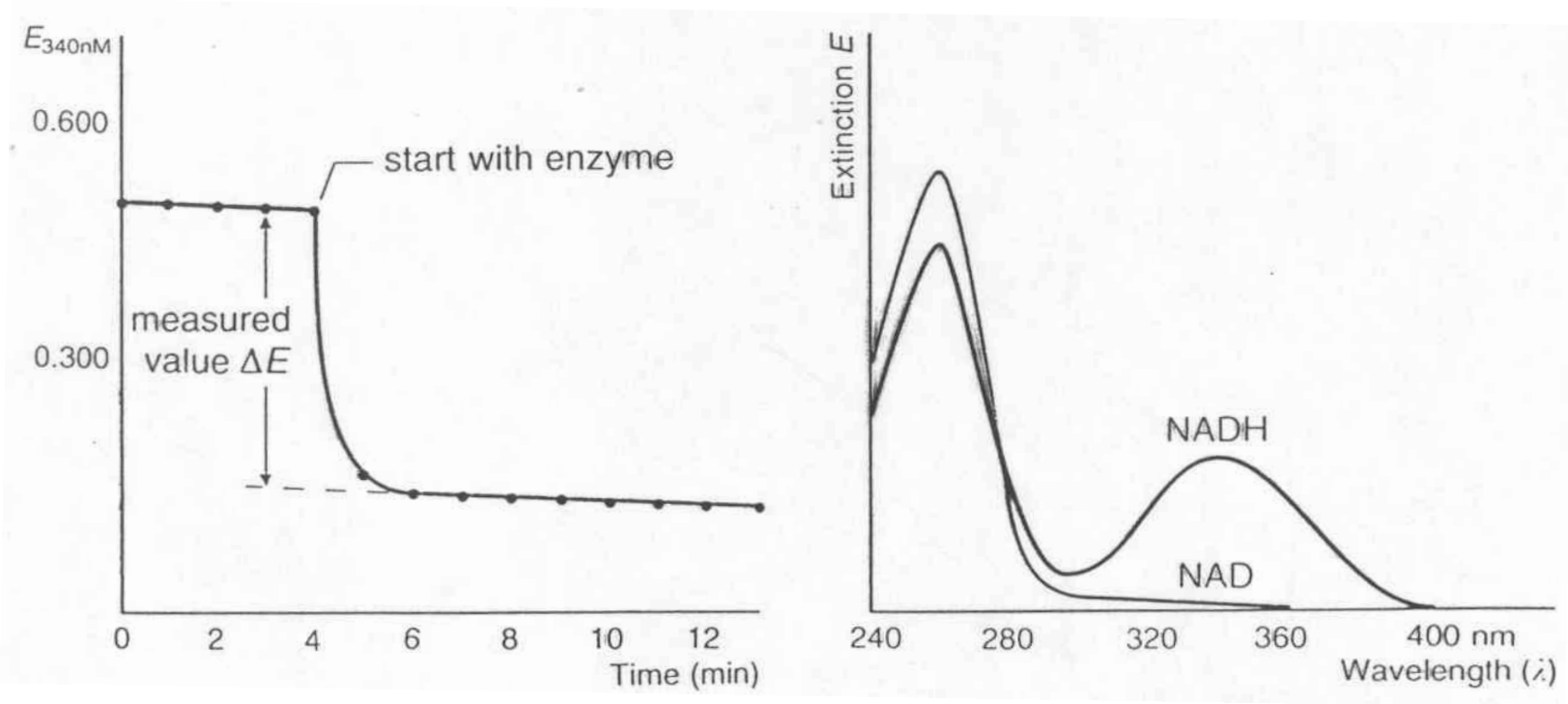
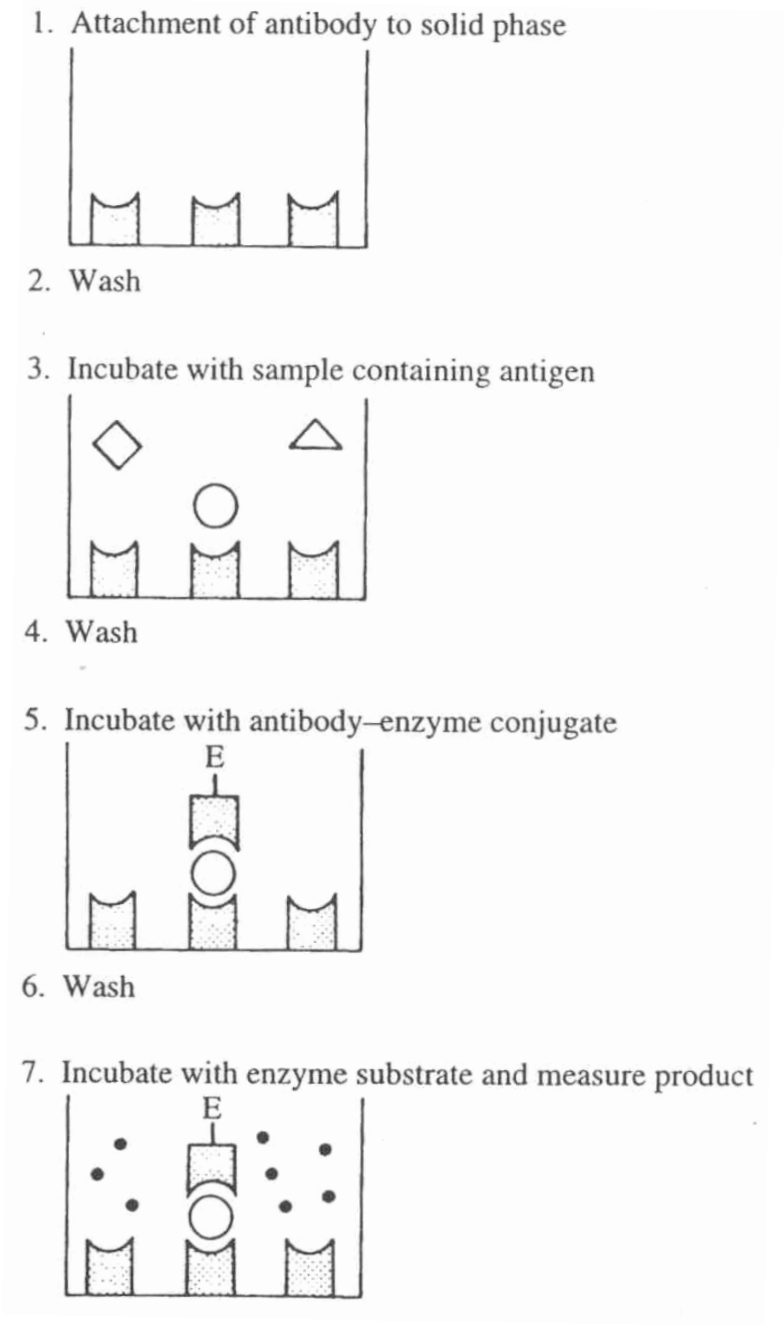
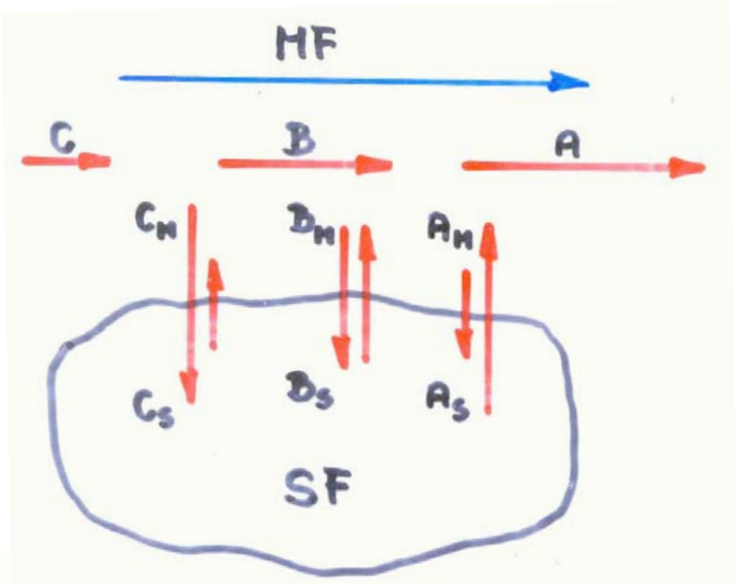
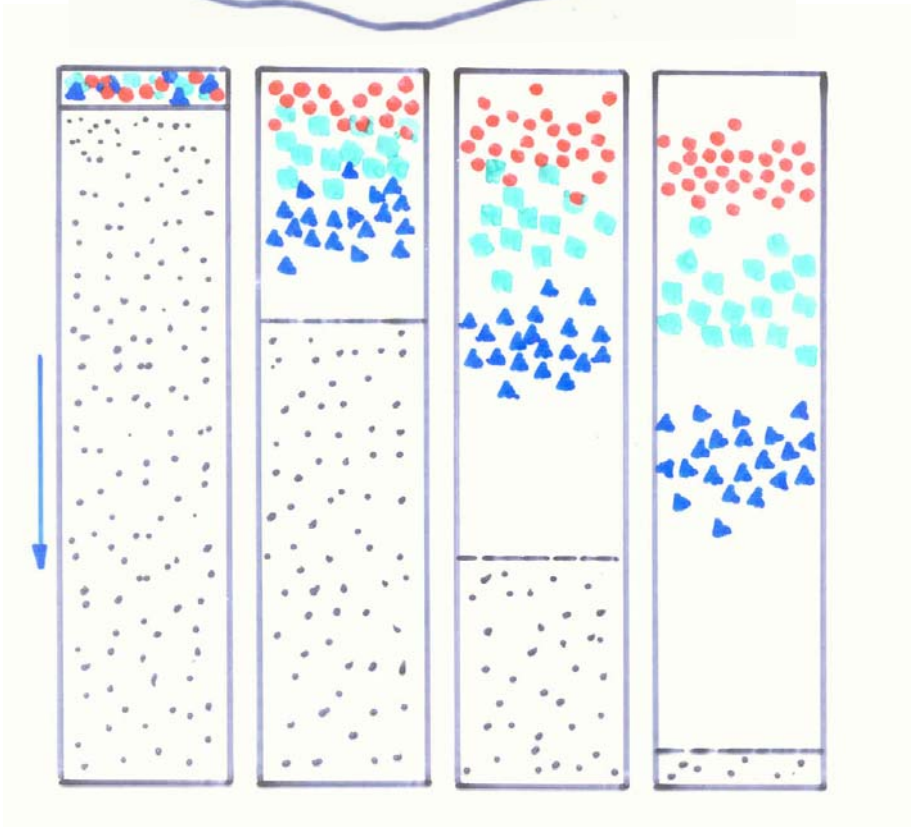


Figure 1: The two-site assay employing two monoclonal antibodies directed against two distinct epitopes.




MF
C
B
A
C_M
B_M
A_M
C_S
B_S
A_S
SF



KROMATOGRAFIJA

Zgodovina

- **Tekočinska kromatografija na koloni (Cvet 1903)**
- **Papirna in tankoslojna kromatografija (Izmailov, Shraiber 1938)**
- **Tekočinska porazdelitvena kromatografija (Martin, Synge 1941)**
- **Plinska kromatografija (petdeseta leta)**
- **Tekočinska kromatografija visoke ločljivosti (šestdeseta leta)**

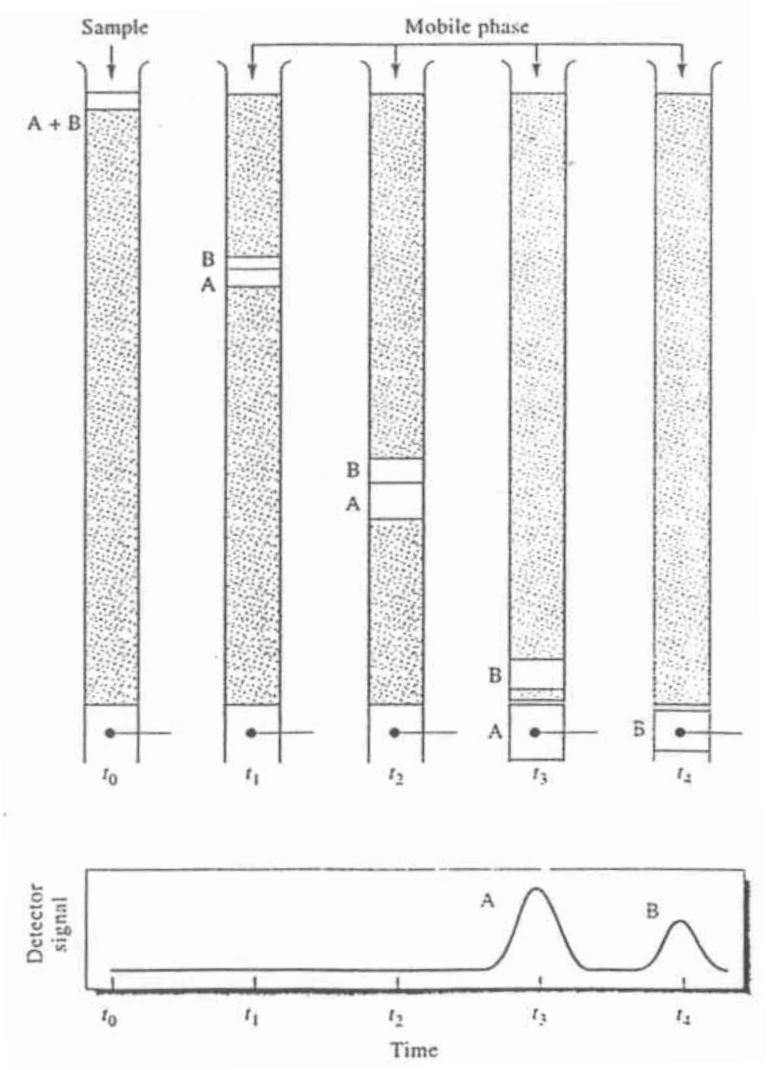
Klasifikacija kromatografskih metod

1. Plinska kromatografija (GC)
- **a) plin-trdno (GSC)**
- **b) plin-tekočina (GLC)**

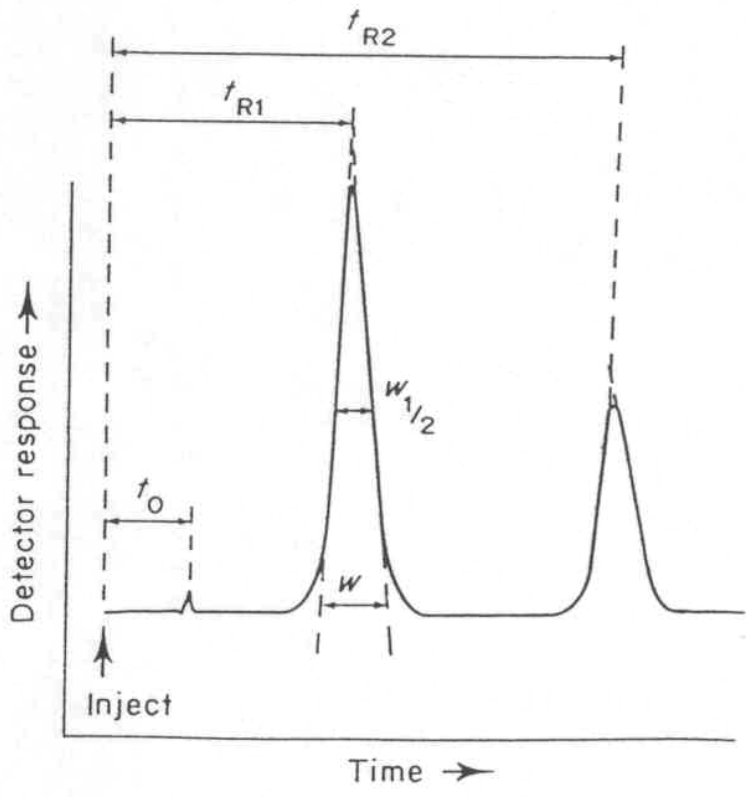
2. Tekočinska kromatografija (LC)
- **a) tekoče-trdno (LSC)**
- **b) tekoče-tekoče (LLC)**
- **c) ionsko izmenjevalna k. (IEC)**
- **d) izključitvena k. (SEC)**
- **e) afinitetna k. (AC)**

3. Superkritično-tekočinska kromatografija (SFC)

Elucija spojin skozi kolono



Kromatogram



$A_{mob} \leftrightarrow A_{stac}$

$K = c_S/c_M$ (konst. v širšem conc. območju → linearna kromatografija)

t_R – RETENCIJSKI ČAS

$\bar{v} = L/t_R$ povprečna (linearna) hitrost spojine

$u = L / t_M$ povprečna (linearna) hitrost MF (mobilne faze)

$$\bar{v} = u \times f\left(\frac{n_m}{n_t}\right)$$ f = moli spojine v MF/celokupno št. molov

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

RETENCIJSKI FAKTOR: $k' = K \cdot V_S / V_M$

$v = u \cdot 1/(1+k') \rightarrow L/t_R = (L/t_M) \cdot 1/(1+k')$

$k' = (t_R - t_M)/t_m$ optimalno: $1 < k' < 5$

Selektivnostni faktor:

$\alpha = K_B/K_A$; $\alpha > 1$

Uporabimo izraz: $k' = K \cdot V_S / V_M$

$\alpha = k'B/k'A$ **oz.**

$\alpha = (t_{R(B)} - t_M)/ (t_{R(A)} - t_M)$

Enačba Gauss-ove krivulje:

$$\frac{dN}{N} = \frac{1}{\sigma\sqrt{2\pi}} e^{-(x-\mu)^2/2\sigma^2} dx$$ **oz.**

$$\frac{dN}{N} = \frac{1}{\sigma\sqrt{2\pi}} e^{-z^2/2}\, dz$$

H [cm]: dolžina kolone, ki vsebuje frakcijo spojine, ki se nahaja med L-σ in L (34 % celokupne spojine); L ± σ ≈ 68 %

τ^2 **[sek^2] – varianca "peak-a"**

$$\tau = \sigma / v = \sigma / \left(L / t_R \right)$$

$W = 4\tau$ **(96 %, x ± 2σ oziroma 2τ)**

$$\sigma = \frac{LW}{4t_R}$$ **in ker je H = σ^2 / L →**

H = LW^2 / 16t$_R$^{2} in ker je N = L / H →

$$N = 16\left(\frac{t_R}{W} \right)^2$$ **oziroma N = 5,54 (t$_R$ / W$_{1/2}$)^2**

$R_S = (t_B - t_A)/ W$ (predpostavka $W_A = W_B \approx W$; oba t_R dovolj blizu)

$$R_S = \frac{t_B - t_A}{t_B} \times \frac{\sqrt{N}}{4} \quad \text{(ker je } W = \frac{4t_R}{\sqrt{N}} \text{)}$$

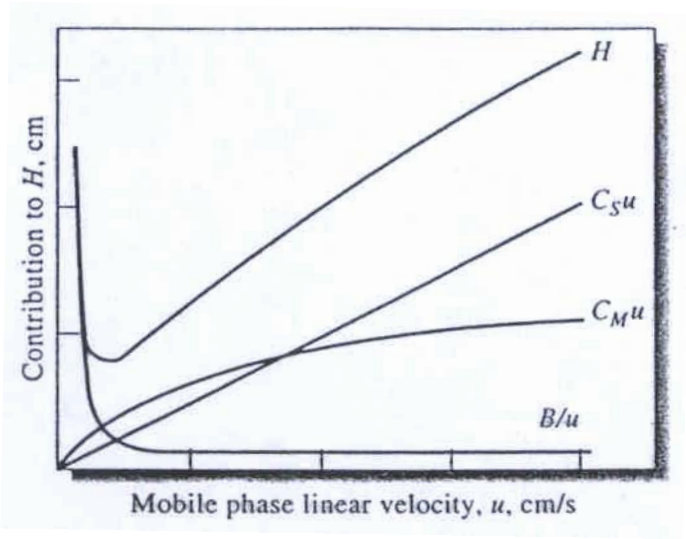
$$R_S = \frac{k_B' - k_A'}{1 + k_B'} \times \frac{\sqrt{N}}{4} \quad \text{(ker je } k' = \frac{t_R - t_M}{t_M} \text{)}$$

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B'}{1 + k_B'}\right) \quad \text{(ker je } \alpha = \frac{k_B'}{k_A'} \text{)}$$

$$N = 16R_S^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{1 + k_B'}{k_B'}\right)^2$$

Modificirana van Deemter-jeva enačba:

$$H = B/u + c_S \cdot u + c_m \cdot u$$



Resolucija R_S

$$R_S = 2 \cdot \frac{(t_B - t_A)}{W_A + W_B}$$

$$R_S = \frac{\sqrt{N}}{4} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \left(\frac{k'_B}{1 + k'_B}\right)$$

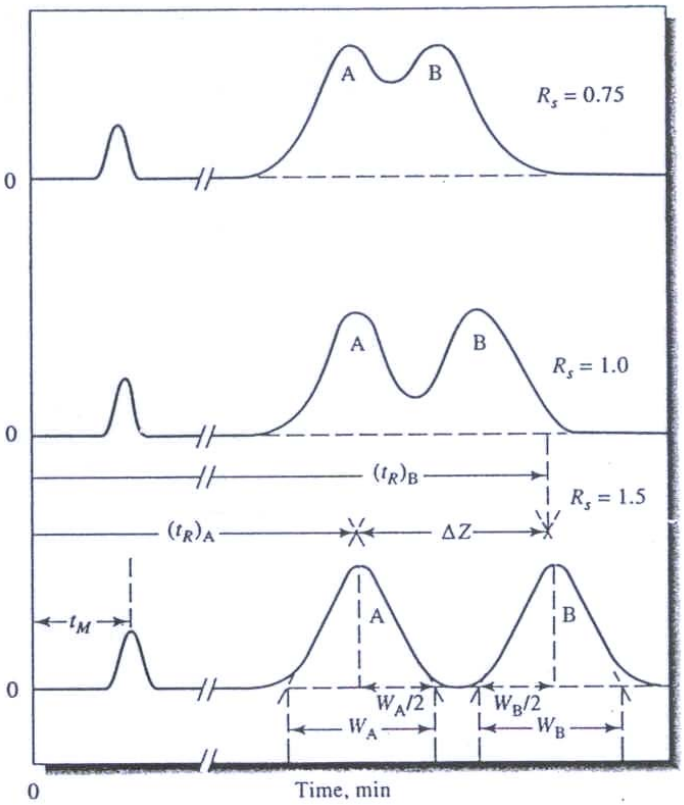


Diagram tekočinskega kromatografa

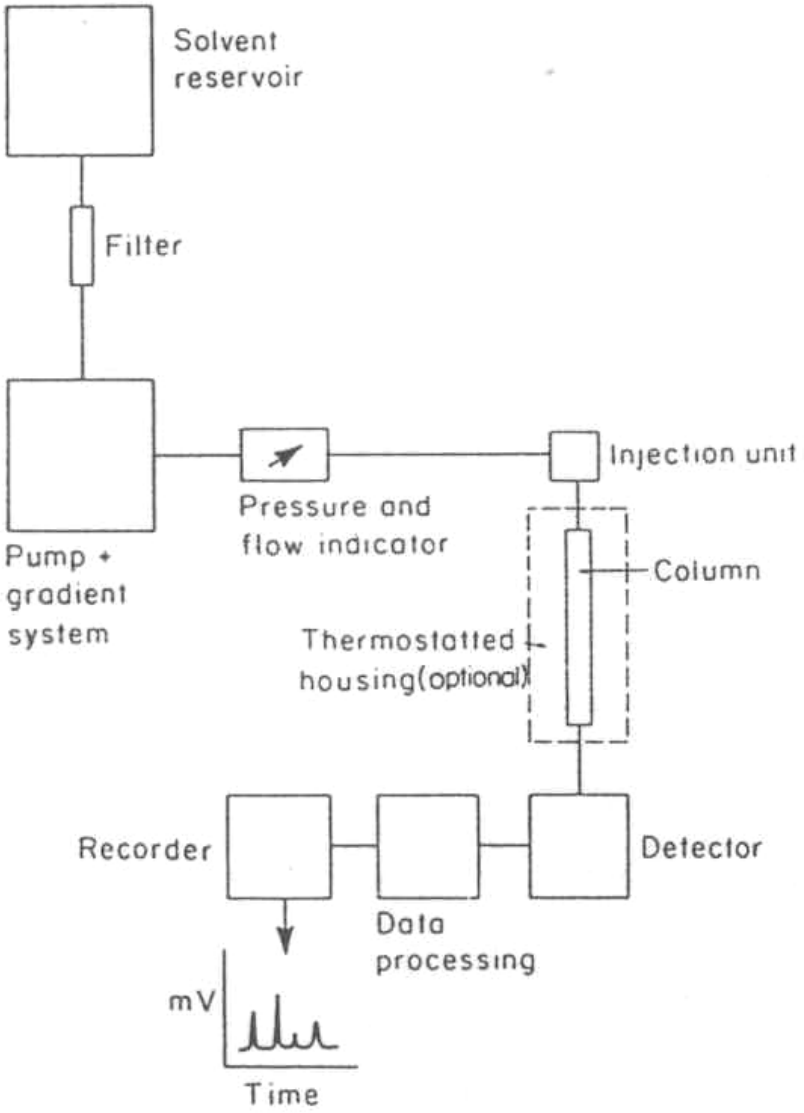


Figure 2: Concentration profiles of analyte bands A and B at two different times in their migration down the column.

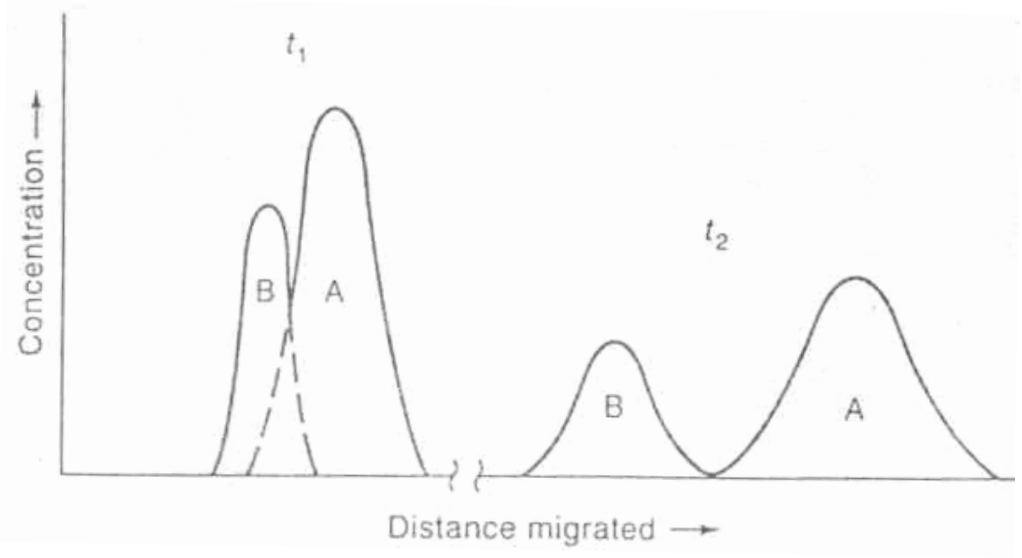


Figure 3: Definition of plate height $H = \delta^2 / L$.

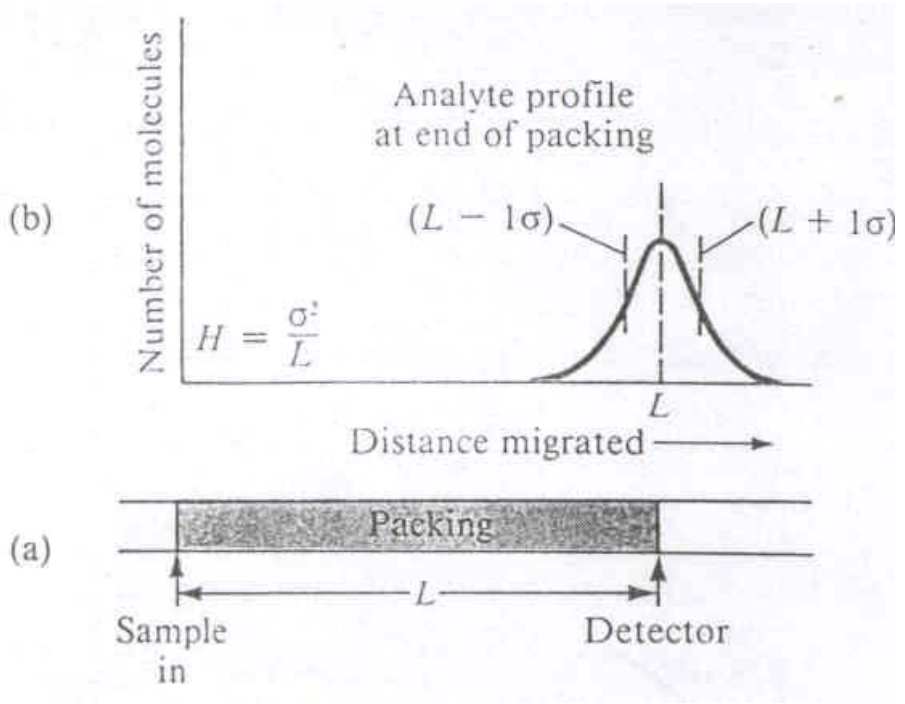


Figure 4: Two component chromatograms illustrating two methods of improving separations: (a)boriginal chromatogram with overlapping peaks, improvements brought about by (b) an increase in band separation, and (c) a decrease in band spread.

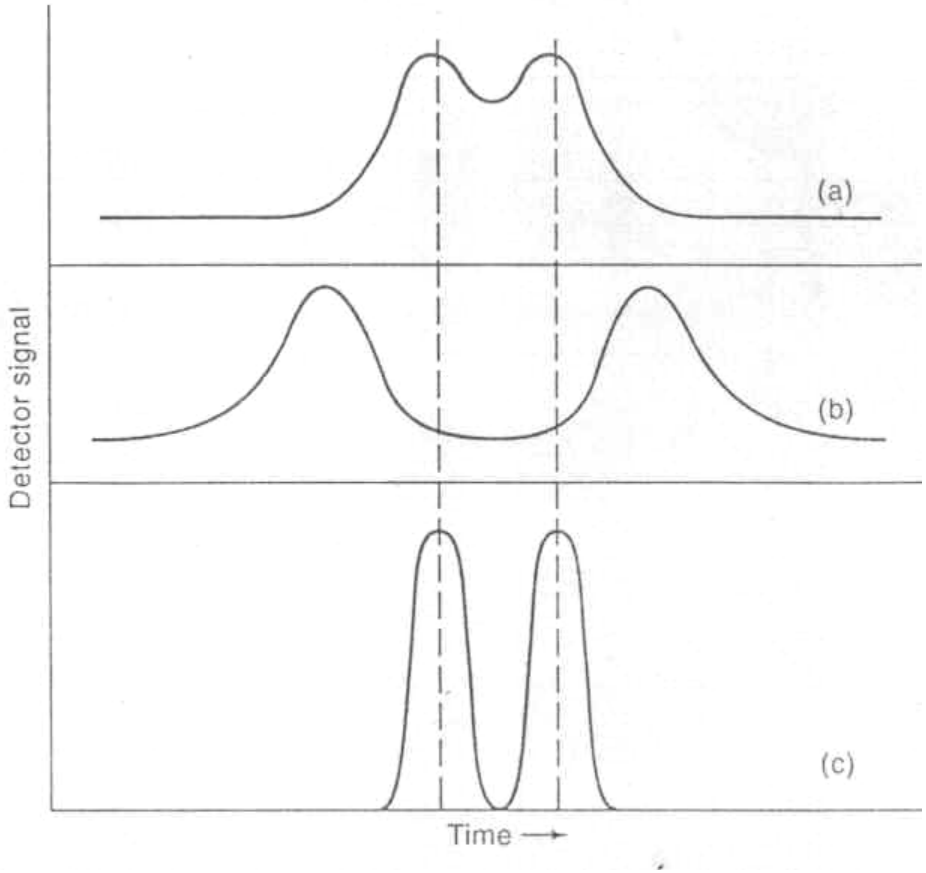


Figure 5: Determination of the standard deviation τ from a chromatographic peak: W = 4τ.

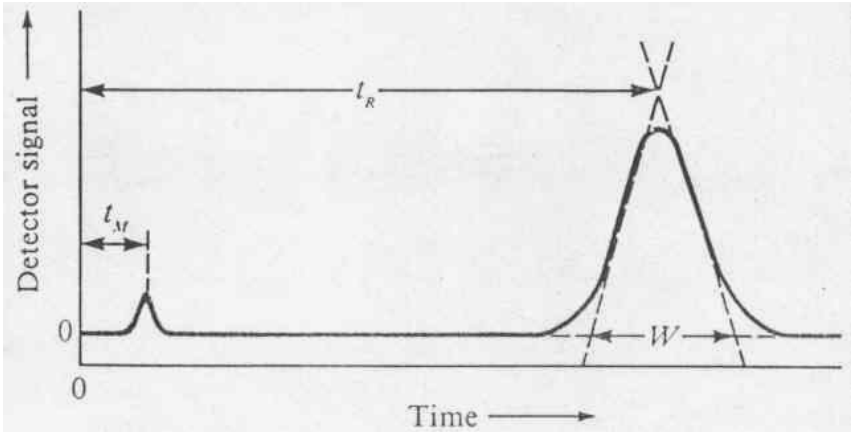


Figure 6: Effect of mobile-phase flow rate on plate height for (a) liqiud chromatography and (b) gas chromatography.

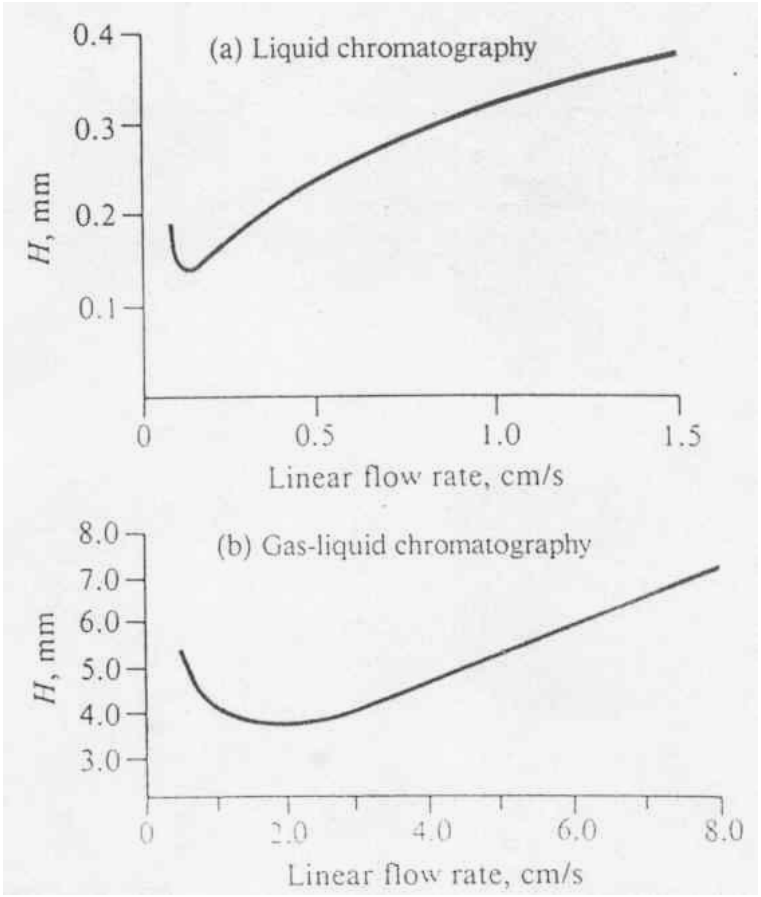


Table 1: Variables That Affect Column Efficiency

Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	cm/s
Diffusion coefficient in mobile phase*	D_M	cm^2/s
Diffusion coefficient in stationary phase*	D_S	cm^2/s
Capacity factor (Equation 24-8)	k'	unitless
Diameter of packing particle	d_p	cm
Thickness of liquid coating on stationary phase	d_f	cm

*Increases as temperature increases and viscosity decreases.

Table 2: Kinetic Processes That Contribute to Peak Broadening

Process	Term in Equation 24-19	Relation ship to Column* and Analyte Properties
Longitudinal diffusion	B/u	$\frac{B}{u} = \frac{2k_D D_M}{u}$
Mass transfer to and from liquid stationary phase†	$C_S u$	$C_S u = \frac{q k' d_f^2 u}{(1+k')^2 D_S}$
Mass transfer to and from solid stationary phase‡	$C_S u$	$C_S u = \frac{2 t_d k' u}{(1+k')^2}$
Mass transfer in mobile phase	$C_M u$	$C_M u = \frac{f(d_p^2, d_c^2, u)}{D_M} u$

u, D_M, D_S, d_f, d_p, k' are as defined in Table 1

f: function of

k_D, q: constants

t_d: average desorption time of analyte from surface; $t_d = 1/k_d$, where k_d is first-order rate constant for desorption

d_c: column diameter

B: coefficients of mass transfer in stationary and mobile phase, respectively

† Stationary phase is an immobilized immiscible liquid

‡ Stationary phase is a solid surface at which adsorption takes place

FIgure 7: Separations at three resolutions. Here, $R_S = 2\Delta Z / (W_A + W_B)$