# Lecture Outline

I. Introduction

A. Molecular Events Underlying Chromatographic Separations

B. Control and Monitoring Equations

II. Types of Chromatography

A. Gel Filtration

B. Ion Exchange /Chromatofocusing

C. Hydrophobic Interaction / Reverse Phase Chromatography

D. Affinity Chromatograph/ Biospecific Adsorption

E. HPLC

F. Expanded Bed Adsorption

G. Other Derivitized Phases – Filters, Solid Supports, Soluble Phases

# Lecture Outline (cont'd)

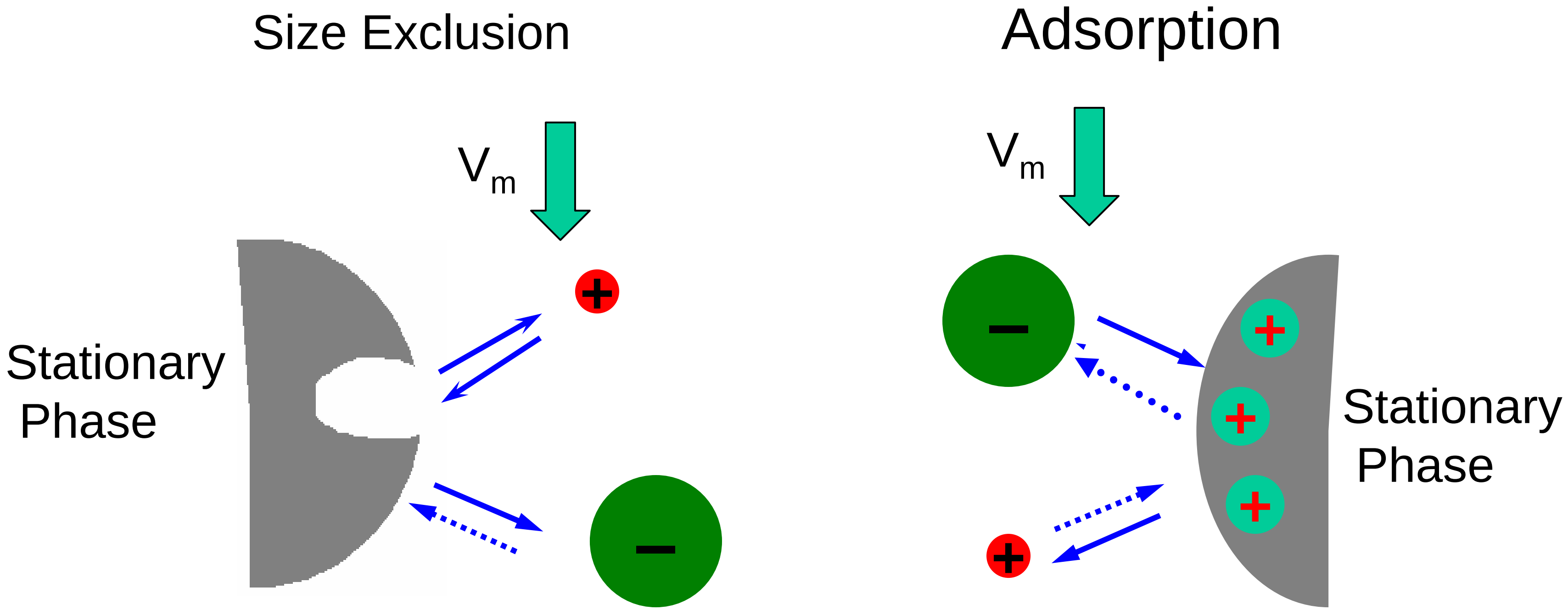
## III. Equipment

A. Columns
B. Pumps
C. Monitors (absorbance, pH, conductivity, pressure, air/liquid level, flow, activity)
D. Fraction Collectors
E. Safety Devices (bubble traps, pressure relief valves, in-line filters)
F. Controllers
G. Overall Set-up and Automation

## IV. Design of Chromatographic Separations and Integration of Multiple Steps

A. Operational Issues
B. Economics of Scale-up
C. Validation and Regulatory Issues
D. Training and Documentation
E. Trouble Shooting
F. Hygiene
G. Integration of Several Chromatographic Steps
H. Vendor Relations/ Inventory

# Molecular Basis of Chromatography




Mobility of solute, $V_p = V_m \bullet T_m$

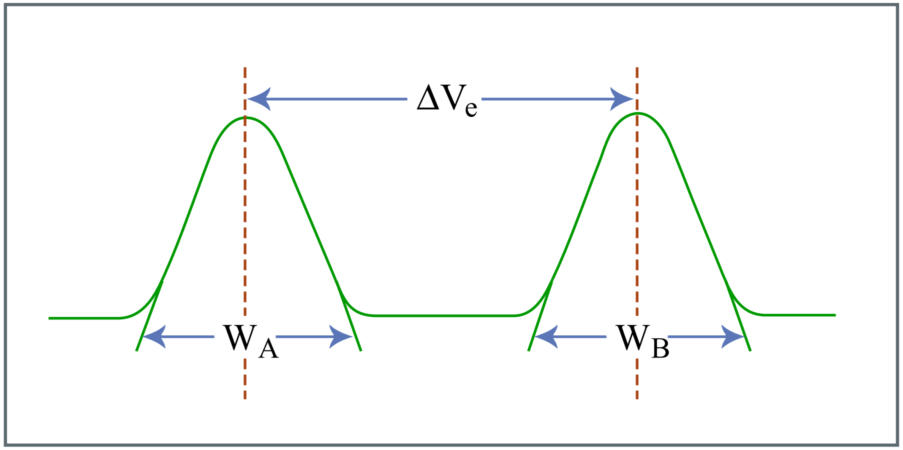
Where:

$V_m$: Velocity of mobile phase

$T_m$: proportion of time solute spends in mobile phase

# Separation Efficiency:  Resolution

Figure by
MIT OCW.




$$R = \frac{\Delta V_e}{(W_A + W_B)/2}$$

Good Selectivity → Larger ΔV –

Low Band Broadening → Larger ΔV –

→ High Resolution

# Chromatography Resolution

## Low Resolution

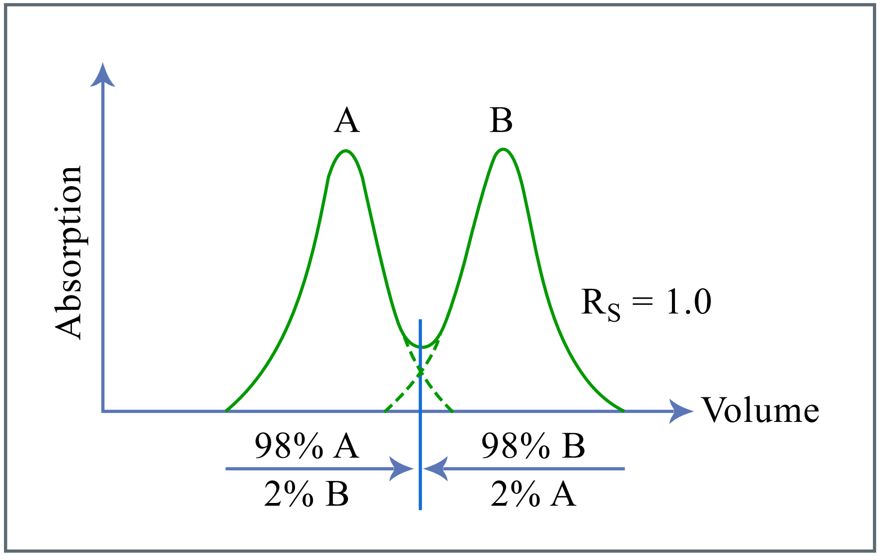



Figure by MIT OCW.

## High Resolution

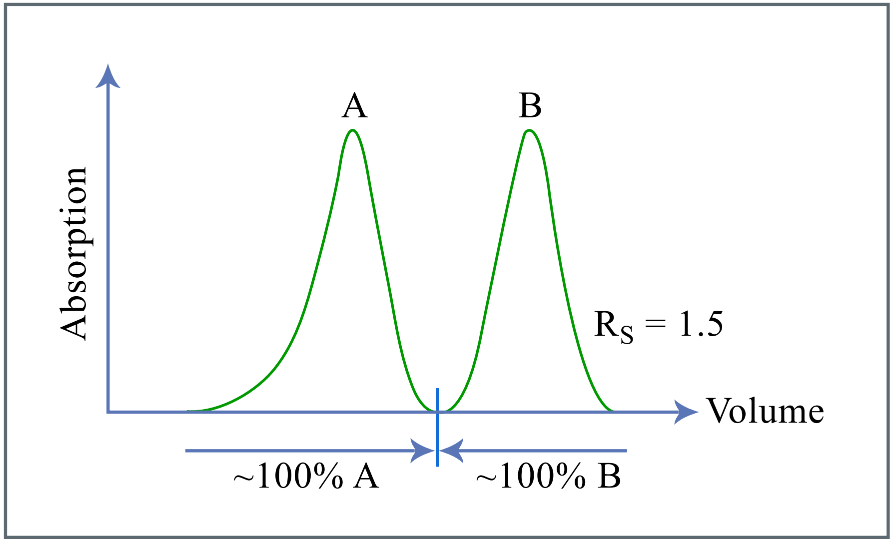



Figure by MIT OCW.

# Chromatography Selectivity

## High Selectivity

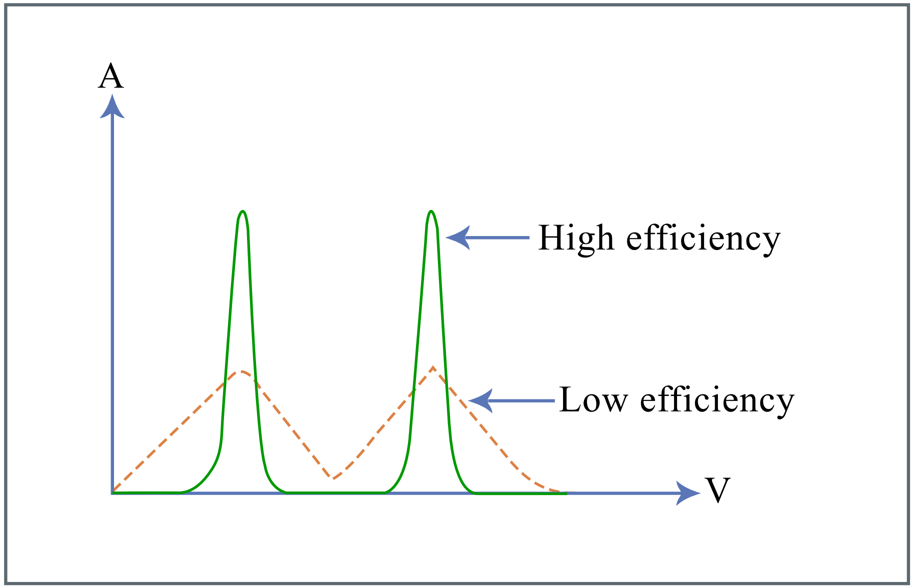



Figure by MIT OCW.

## Low Selectivity

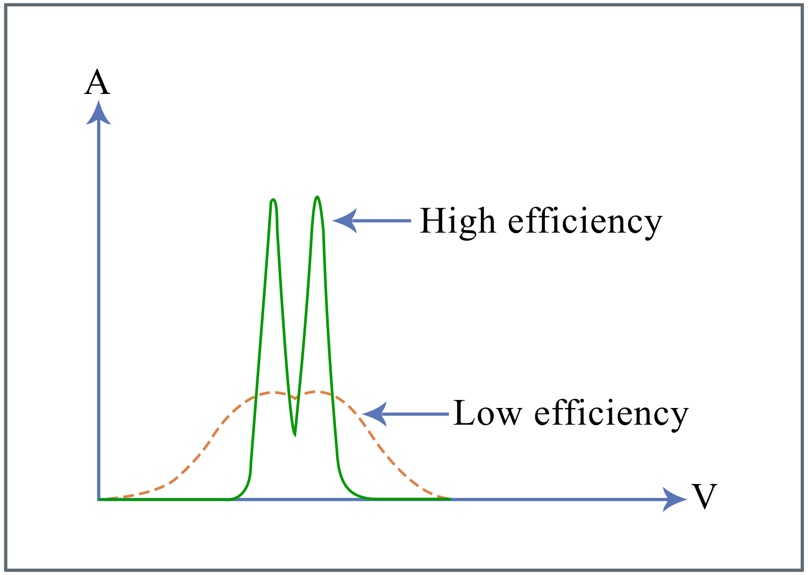



Figure by MIT OCW.

# HETP Calculation

HETP is a theoretical construct; analogous to distillation columns

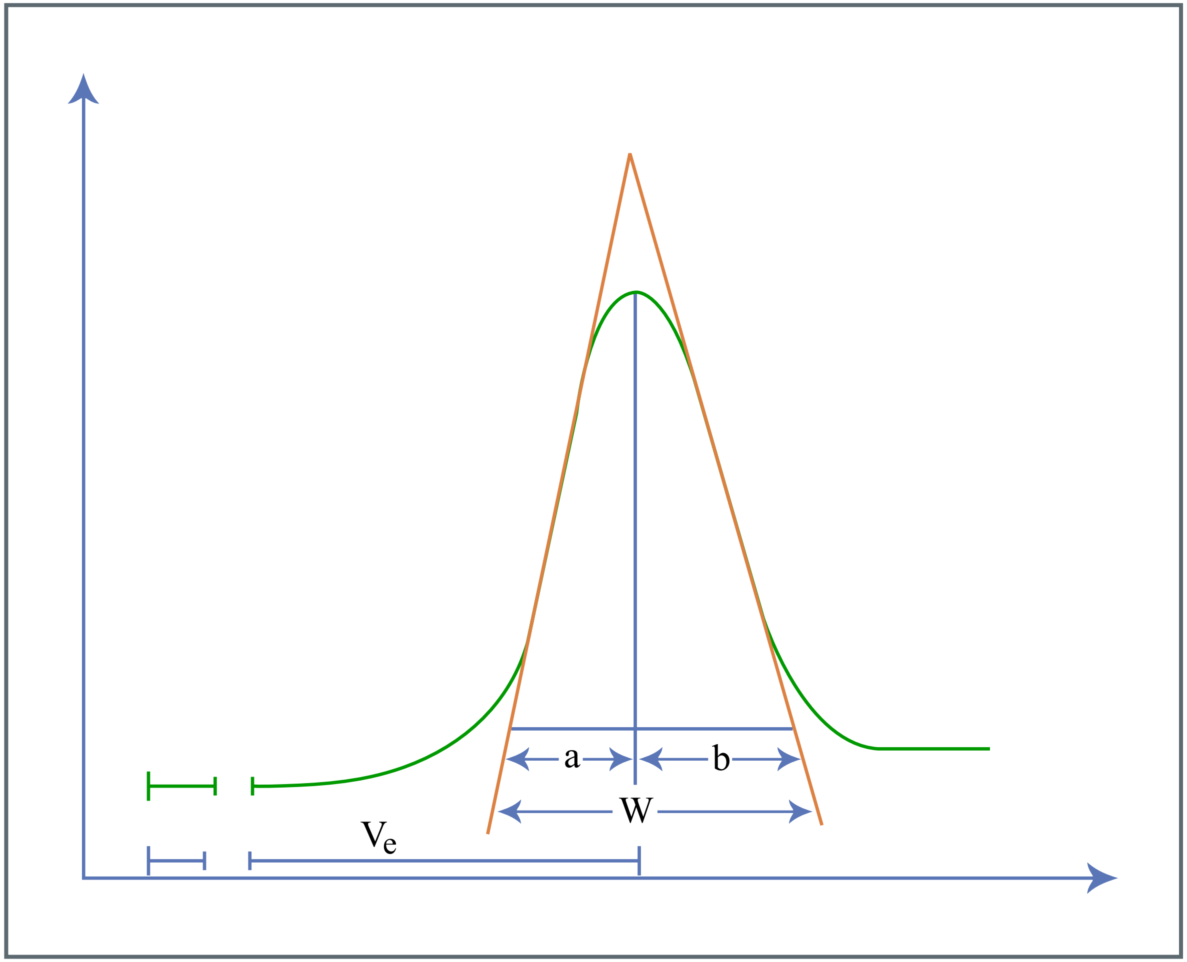



Figure by MIT OCW.

**L** = Column Length (cm)

**N** = No. theoretical plates

**$V_e$**= Elution volume (L)

**W** = Peak width (L)

**N** = $16\left(\frac{V_e}{W}\right)^2$

**HETP** = $\frac{L}{N}$ (cm)

**AF(Asymmetry Factor)** = $\frac{b}{a}$

*N is dimensionless number.

# Band Broadening

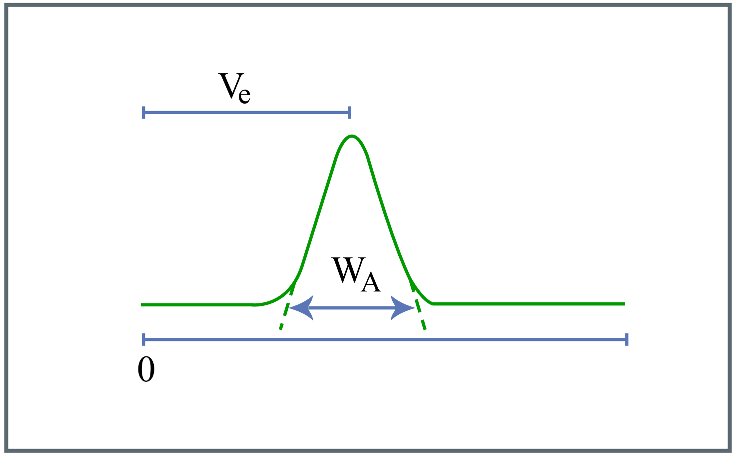



Figure by MIT OCW.

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

$$N = 16\left(\frac{V_e}{W}\right)^2$$

## Flow Optimization

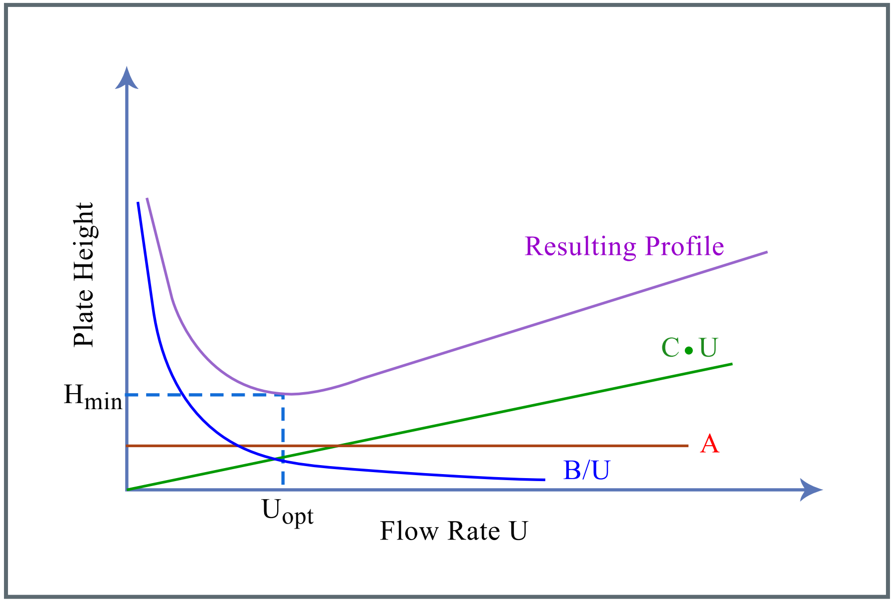



Figure by MIT OCW.

$$\boxed{H = A + B/U + C \bullet U}$$

| | |
|---|---|
| A | Eddy diffusion effects |
| B/U | Axial diffusion effects |
| C • U | Dispersion due to incomplete mass transfer |

# Some Basic Terms of Chromatography

## Capacity Factor

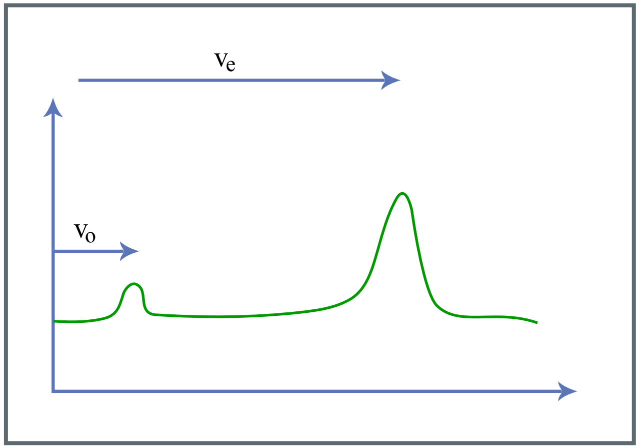


Figure by MIT OCW.

$$K' = \frac{V_e - V_o}{V_o}$$

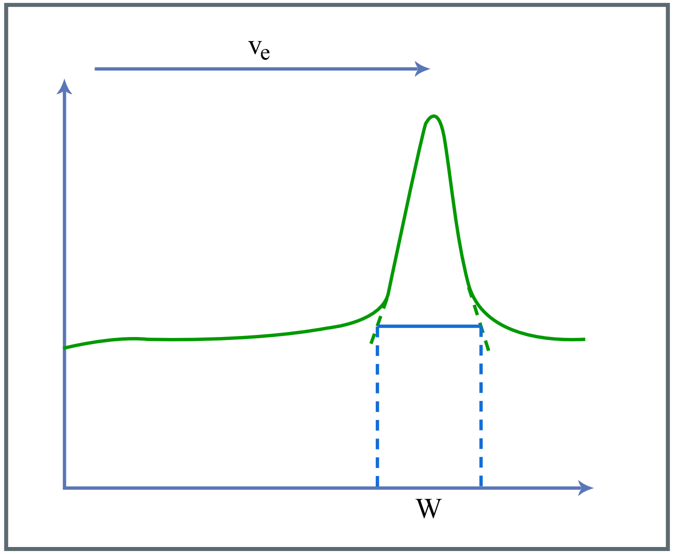


Figure by MIT OCW.

## Selectivity

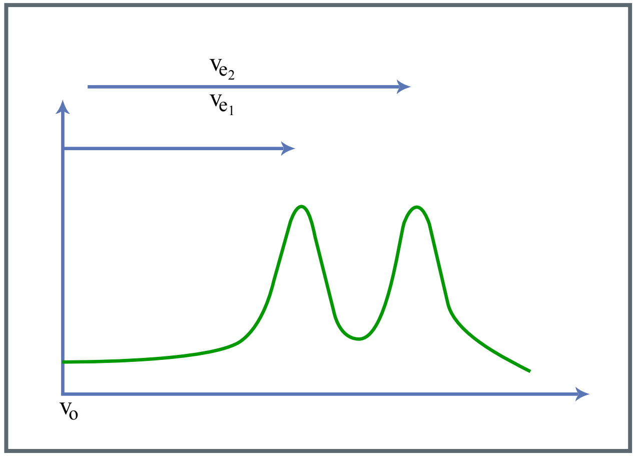


Figure by MIT OCW.

$$\alpha = \frac{V_{e2} - V_o}{V_{e1} - V_o}$$

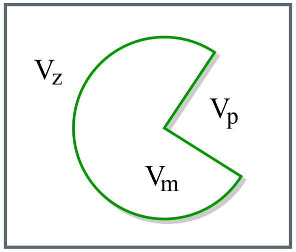
## Efficiency

$$N = 16\left(\frac{V_e}{W}\right)^2$$

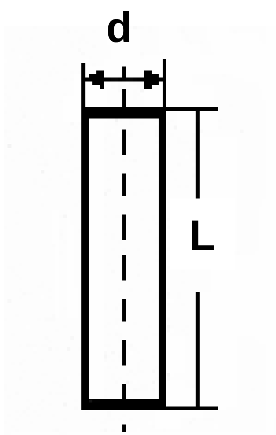
## HETP

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

# The Basic Principles of Gel Filtration




**Gel Particle**




**Column**

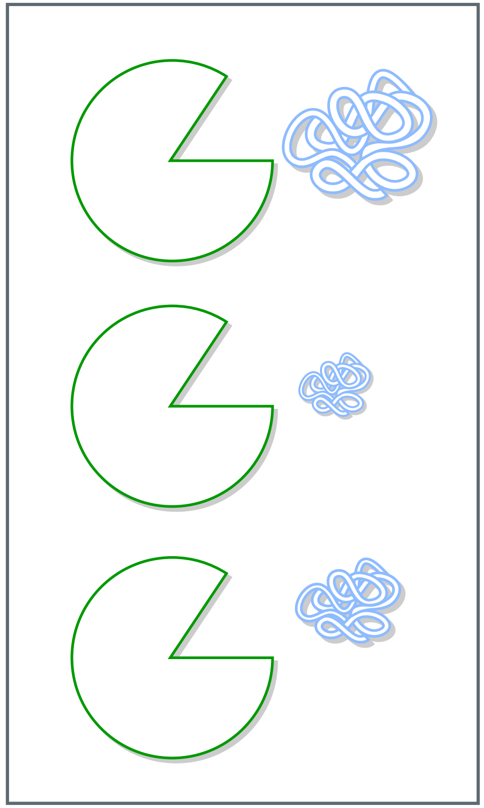


Figure by MIT OCW.

$$V_t = V_z + V_p + V_m = \frac{\pi d^2}{4} \bullet L$$

$$V_e = V_z$$

$$V_e = V_z + V_p$$

$$V_e = V_z + K_d \bullet V_p$$

$$0 \leq K_d \leq 1$$

**$V_e$ : elution volume**

**$V_z$ : interstitial volume**

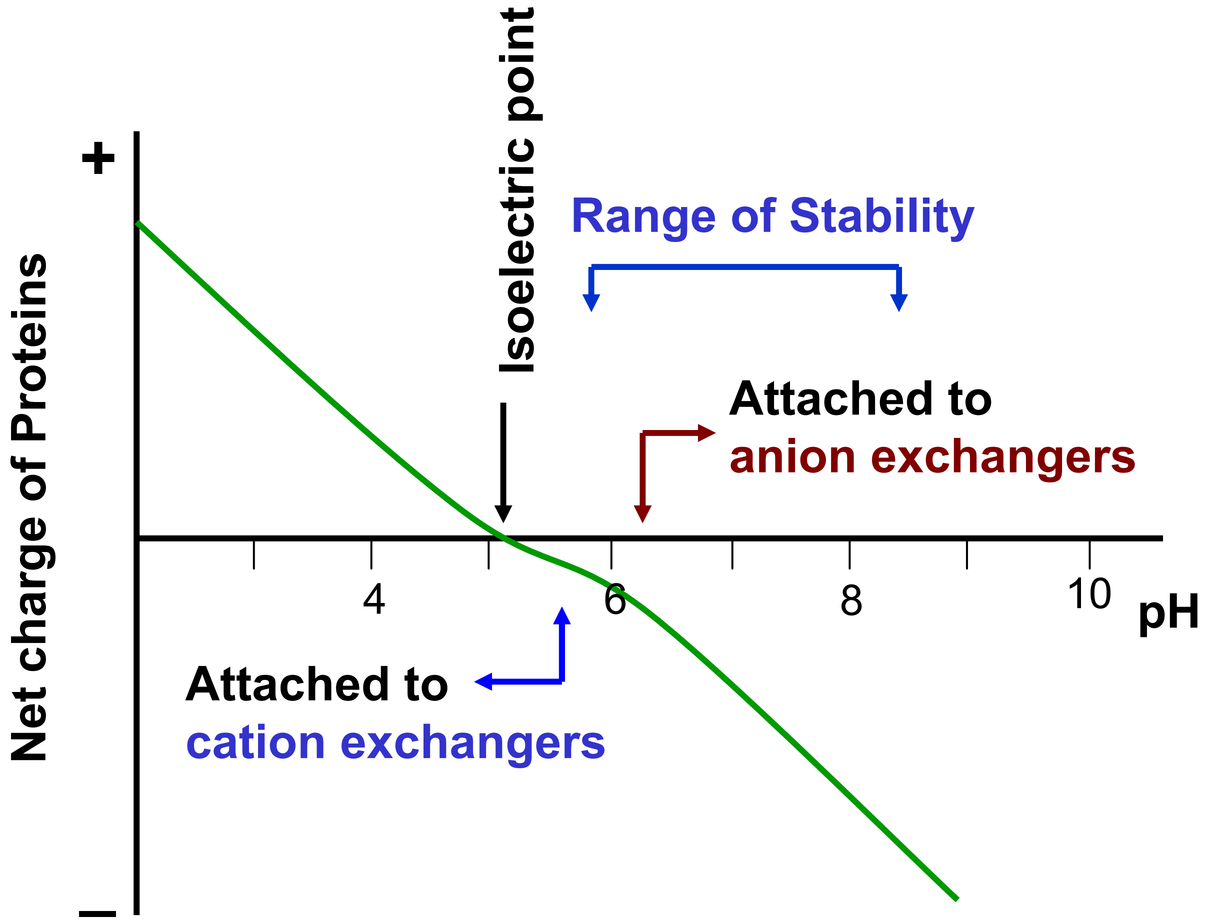
**$V_p$ : pore volume**

**$V_m$ : matrix volume**

**$V_t$ : total column volume**

# Ion Exchange Chromatography

# Amphoteric Properties of Protein




The net charge of a protein as a function of pH. The pH ranges in which the protein is bound to anion or cation exchangers and an arbitrary range of stability are shown.

# Ion Exchange Function Group Substituions

Weak Anion Exch. Diethylaminoethyl (DEAE)

$-O-CH_2-CH_2-N^{+}H(CH_2CH_3)_2 \quad Cl^{-}$

Strong Anion Exch. Quaternary aminoethyl (QAE)

$-O-CH_2-CH_2-N^{+}(C_2H_5)_2-CH_2-CHOH-CH_3 \quad Cl^{-}$

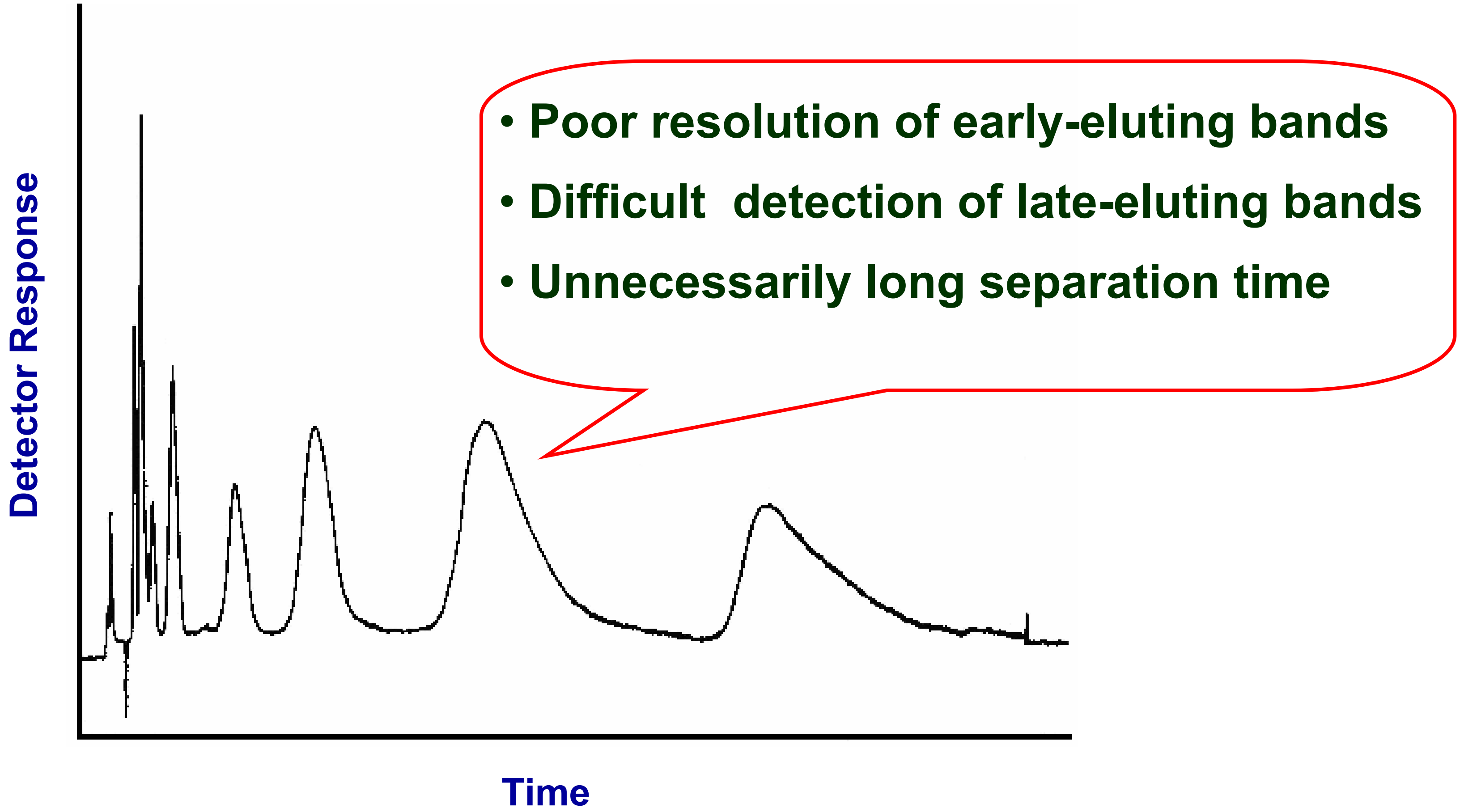
Weak Cation Exch. Carboxymethyl (CM)

$-O-CH_2-COO^{-} \quad Na^{+}$

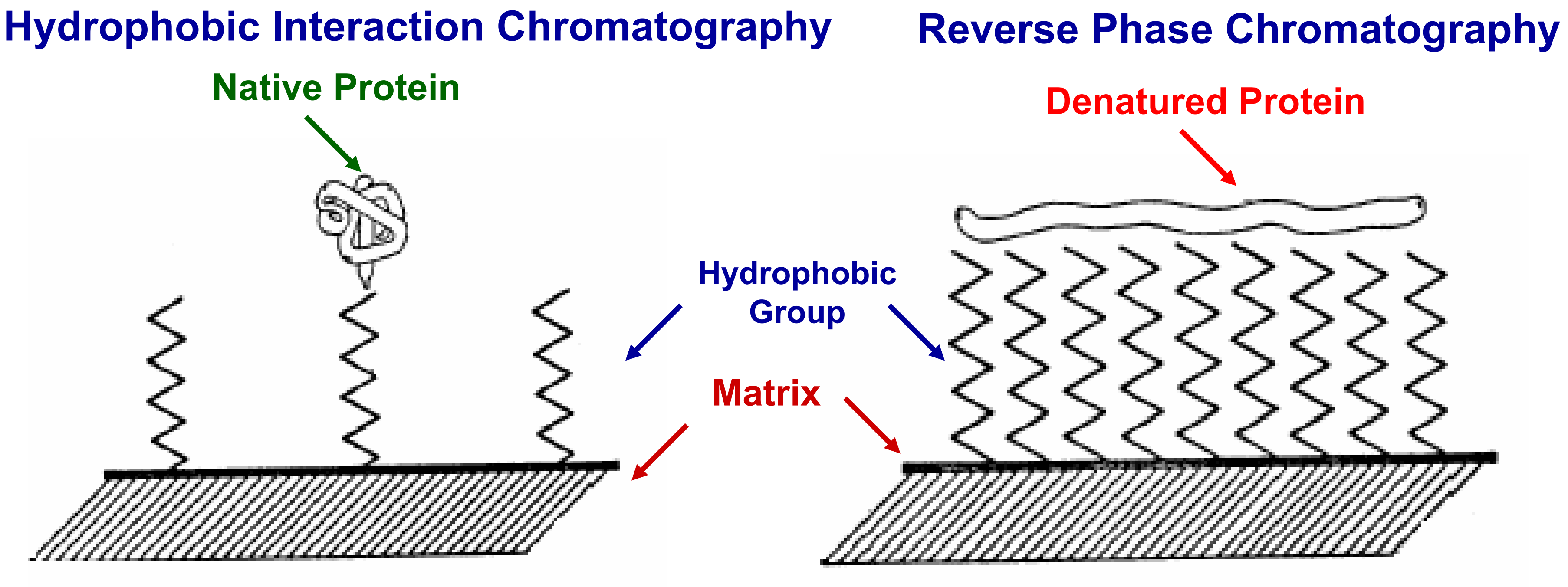
Strong Anion Exch. Sulphopropyl (SP)

$-O-CH_2-CHOH-CH_2-O-CH_2-CH_2-CH_2SO_3^{-} \quad Na^{+}$

# General Problem of Isocratic Elution



# HIC & RPC




- Low degree of substitution
- Gentle binding and elution
- Used for proteins

- High hydrophobic content
- Strong sample/matrix interaction
- Used for small molecule and peptide

# Effect of Ions in Precipitating Proteins

**<u>The hofmeister series</u>**

**<u>on the effect of some anions and cations</u>**

**<u>in precipitating proteins</u>**

**Increasing precipitation ( “salting-out”) effect**

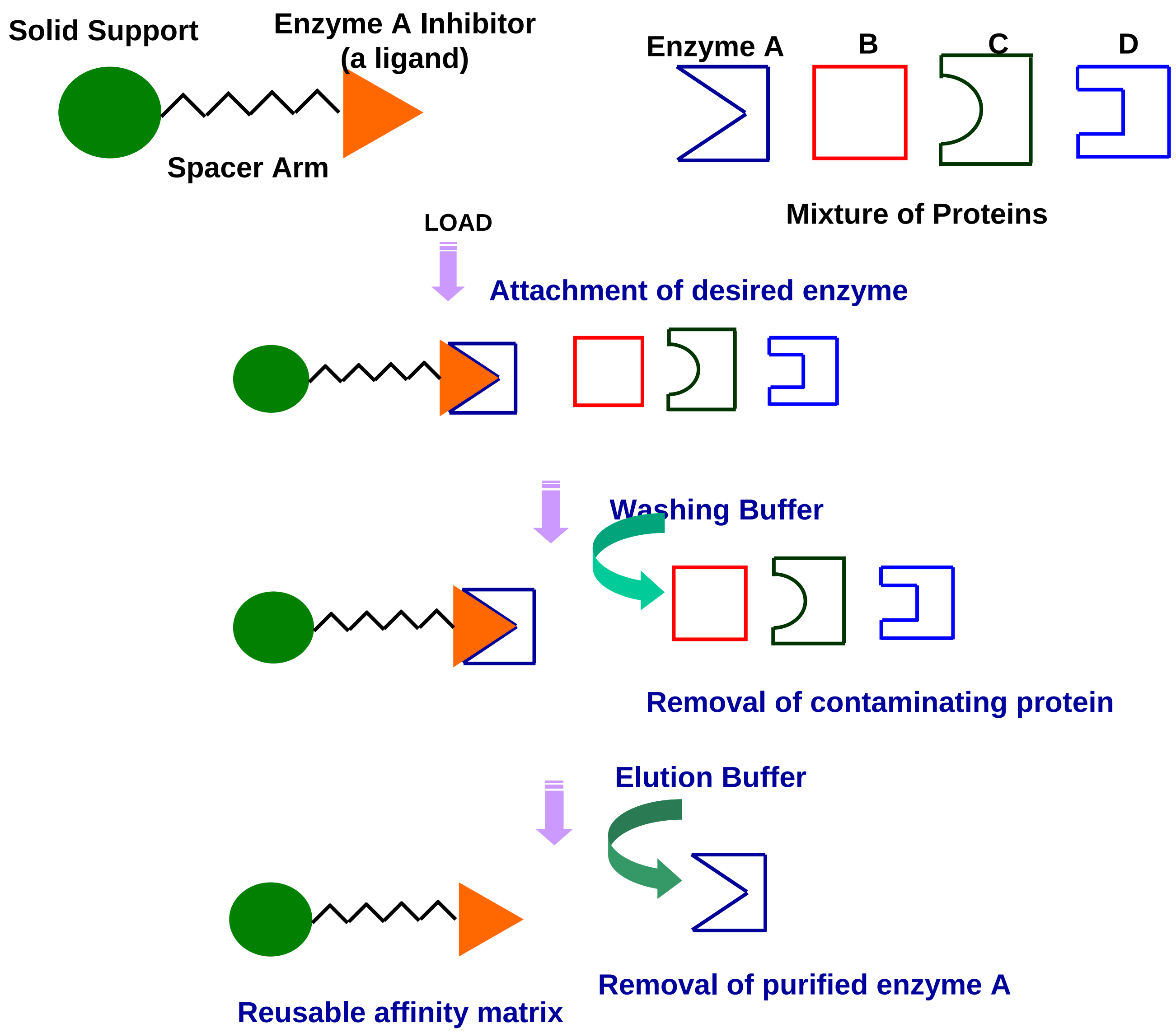
**Anions: $PO_4^{3-}$ , $SO_4^{2-}$, $CH_3{\bullet}COO^-$, $Cl^-$, $Br^-$, $NO_3^-$, $I^-$, $SCN^-$**

**Cations: $NH_4^+$, $Rb^+$, $K^+$, $K^+$, $Na^+$, $Cs^+$, $Li^+$, $Mg^{2+}$, $Ca^{2+}$, $Ba^{2+}$**

**Increasing chaotropic (“salting-in”) effect**

# Affinity Chromatography
# or
# Biospecific Adsorption

# Principle of Affinity Chromtography



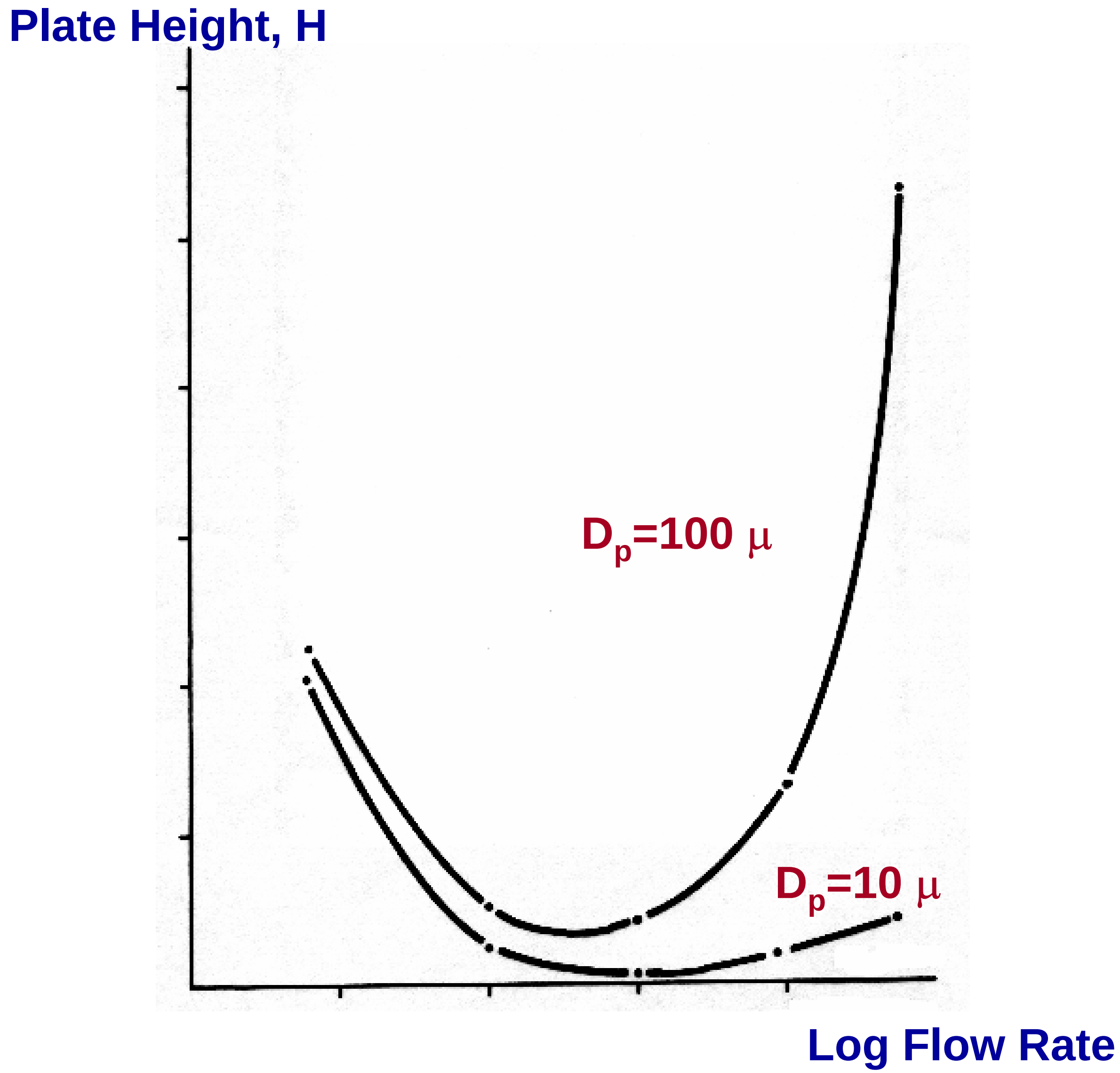
# Affinity Matrices

- Specific to The Desired Protein
  - Substrate
  - Substrate Analog
  - Inhibitor
  - Antibody
- Specific to a Group of Proteins
  - Cofactors ( 5-AMP, NAD, etc.)
  - Dyes

# HPLC

High Performance Liquid Chromatography

# Effect of Particle Size on Plate Height



# HPLC Operation

$$v = K \times \frac{d_p{}^2 \bullet \Delta P}{\eta \bullet L}$$

v: superficial (linear) velocity of eluent

K: permeability factor (friction parameter x void fraction)

$d_p$: average particle diameter

$\Delta P$: pressure drop across bed

$\eta$: eluent viscosity

L: bed height