8. Separation by Adsorption I: General Principles

8.1. Adsorption Equilibrium

Adsorption of Proteins

- **Adsorbents for proteins**
  - Ion exchangers
  - Hydrophobic materials
  - Inorganics : calcium phosphate gels
  - Chemically synthesized ligand adsorbents
  - Affinity adsorbents (Biological compounds)
    - Substrates, enzyme inhibitors, antibodies
- **Matrix**
  - Cellulose
  - Agarose
  - Carbohydrate polymers
  - Synthetic matrix

Partition Coefficients

- **Protein-matrix interaction**
  - Cannot be describe by simple parameters
    - Multiple interaction sites in a protein
    - Heterogeneous array of binding sites in adsorbent
  - Chromatographic theory for proteins
    - Use approximations and assumptions

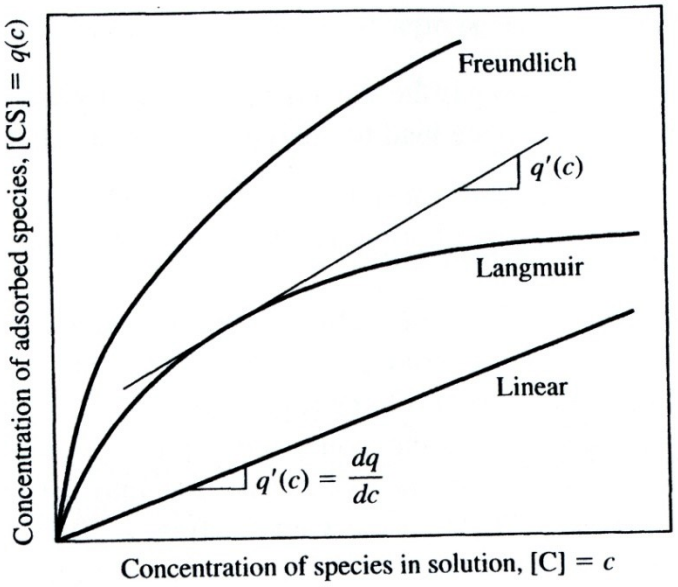
- **Chromatography**
  - Separation of solutes according to their different partitioning between two phases
    - Solid (stationary) and liquid (mobile) phases
    - → different mobility down a column
  - Partition coefficient (α)
    - Fraction of the solute adsorbed on the solid
    - α =0.8 : 80% is adsorbed, 20 % is free
    - Solute will move at 20% of the speed of the liquid flow
  - Mobility of the solute relative to the liquid (Rf)
    - 1- α

Adsorption Equilibrium

- **C + S ↔ CS, K_{eq} = [CS]/{[C][S]}**
  - C: dissolved chemical species
  - S: adsorption site
- **Assumptions**
  - Reversible and chemical's interaction with the adsorption sites does not affect solution properties or solution state
  - Specific binding of one chemical to one binding site
  - Only one mode of binding, can be described as a single K_{eq}
  - **Linear equilibrium (no saturation)**
  - when [S]>>[C]
  - K_{eq} = [CS]/[C], [CS] = K_{eq} [C],
    - Common in analytical liquid chromatography
    - Small sample volume and dilute solutes
    - Suitable for analytical chromatography
      - To measure the concentrations of the various components of a mixture

Adsorption Equilibrium

- **Langmuir isotherm**
  - Consider all the adsorption sites including empty site
    - S_t (total site concentration)= [CS]+[S]
  - [CS] = $\{K_{eq} S_t [C]\}/ \{1+ K_{eq}[C]\}$
  - Saturation of binding site at high [C]
  - Useful for preparative and industrial chromatography
- **Freundlich isotherm**
  - [CS] =$K_{eq}[C]^{1/n}$, n>1
  - Results of energetic heterogeneity of the surface of the adsorbent
  - Superimpose of a set of Langmuir isotherms with different K_{eq}



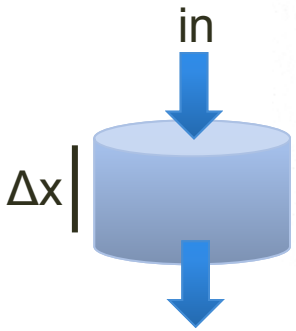
Specified Theory of Adsorption

- **Dissociation constant for protein – matrix interaction**
  - Multiple binding sites → multiple dissociation constant
  - Assume "apparent dissociation constant"
- **Partition coefficient vs. K_p**
  - K_p = [S] [C]/ [CS]
    - S_t = [CS]+[S] : total effective concentration of adsorption sites
    - p_t = [CS]+[C] : total protein concentration
    - α = [CS]/p_t, [CS] = $p_t\,\alpha$
  - $K_p = (S_t - p_t\,\alpha)(p_t - p_t\,\alpha)/(p_t\,\alpha) = (S_t - p_t\,\alpha)(1-\alpha)/\alpha$
  - $P_t\,\alpha^2 - \alpha(S_t + p_t + K_p) + S_t = 0$
    - α is the solution of this quadratic equation
  - When $S_t \gg p_t$, large excess of binding sites compared with protein to bind
    - [S] $\approx S_t$, [CS] $\approx p_t$, [C] $= p_t -$ [CS] $= p_t(1-\alpha)$
    - K_p = [S] [C]/ [CS] = $S_t(1-\alpha)/\alpha$
    - α, p_t, S_t can be determined experimentally
- **Typical concentrations of effective binding sites and p_t**
  - ~ 0.01 mM for affinity adsorbents
  - > 1 mM for ion exchangers
- **For useful adsorption on column chromatography**
  - $\alpha \geq 0.8$, $K_p \leq 0.1$ mM

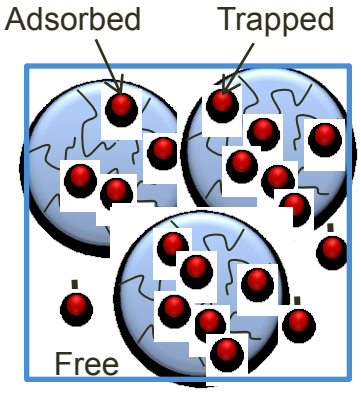
Adsorption Column Dynamics

Fixed-Bed Adsorption

- **Mass Balance for Fixed-Bed Adsorption**
  - Rate of separand in – rate of separand out = rate of accumulation of separand



$$A\varepsilon\Delta t\left(\frac{v}{\varepsilon}c_i - \mathscr{D}_{\text{eff}}\frac{\partial c_i}{\partial x}\right)\Big|_{x,t} - A\varepsilon\Delta t\left(\frac{v}{\varepsilon}c_i - \mathscr{D}_{\text{eff}}\frac{\partial c_i}{\partial x}\right)\Big|_{x+\Delta x,t} = A\varepsilon\Delta x(c_i|_{t+\Delta t} - c_i|_t) + A(1-\varepsilon)\Delta x(q_i|_{t+\Delta t} - q_i|_t)$$



V_t : total volume

V_o : void volume,

- A: cross sectional area
- v: mobile phase superficial velocity (Q/A)
- c_i : concentration of separand i in the mobile phase = $[C]_i$
- q_i: concentration of separand i in the stationary phase averaged over an adsrobent particles = $[CS]_i$
- ε : void fraction , V_o/V_t , commonly 0.3 to 0.4
- $\mathscr{D}_{\text{eff}}$: effective dispersivity of the separand
- T: time
- X : longitudinal distance in the column

Fixed-Bed Adsorption

- **Dividing by $A\varepsilon\Delta x\Delta t$, limit as Δx Δt go to zero**

$$\mathscr{D}_{\text{eff}}\frac{\partial^2 c_i}{\partial x^2} - \frac{v}{\varepsilon}\frac{\partial c_i}{\partial x} = \frac{\partial c_i}{\partial t} + \frac{1-\varepsilon}{\varepsilon}\frac{\partial q_i}{\partial t}$$

Diffusion or mechanical dispersion

convection

Accumulation rate of solute in any section of the column

- $\delta q_i / \delta t = f(c_i, c_j, \ldots..., q_i, q_j) = K_a (c_i - c_i^*)$
  - $\delta q_i / \delta t$: rate of mass transfer of the separand to an average particle in the stationary phase
  - K_a : overall mass transfer coefficient
  - c_i^* : liquid phase concentration that would exist at equilibrium with q_i

Assumption of Local Equilibrium and Negligible Dispersion

- **Assumption**
  - Local equilibrium, $q_i = f(c_i)$
  - Ignore dispersion

$$\mathscr{D}_{\text{eff}} \frac{\partial^2 c_i}{\partial x^2} - \frac{v}{\varepsilon}\frac{\partial c_i}{\partial x} = \frac{\partial c_i}{\partial t} + \frac{1-\varepsilon}{\varepsilon}\frac{\partial q_i}{\partial t}$$

$$\longrightarrow \quad \frac{\partial c_i}{\partial t} + \frac{v}{\varepsilon + (1-\varepsilon)q_i'(c_i)}\frac{\partial c_i}{\partial x} = 0 \qquad (1)$$

  - If $$u_i = \frac{v}{\varepsilon + (1-\varepsilon)q_i'(c_i)}$$
    - u_i: Effective velocity of component *i*
    - *For langmuir isotherm,*
      - q_i' decreases with increase in c_i → increase in u
  - (1) becomes

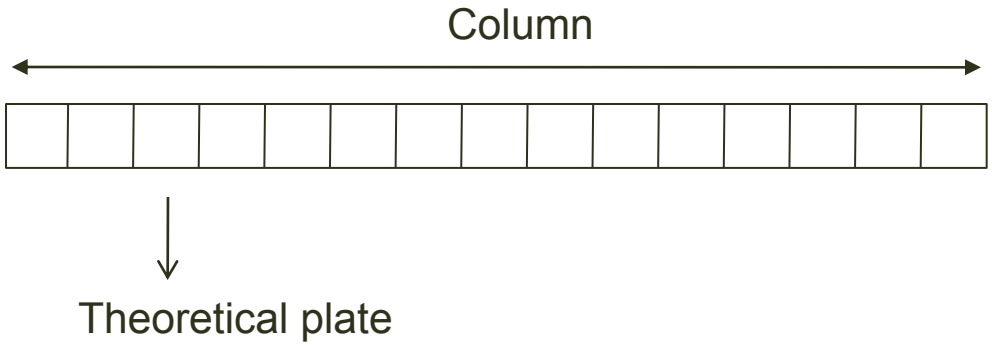
$$\frac{\partial c_i}{\partial t} + u_i \frac{\partial c_i}{\partial x} = 0$$

Chromatography Column Dynamics

Theoretical Plate Model of Chromatography

- **Plate**
  - The largest uniform zone able to accommodate the solute (theoretical)
  - Consider column chromatography as a number of discrete steps
    - Transferring solute from plate to plate
  - Number of theoretical plate
    - The more the better

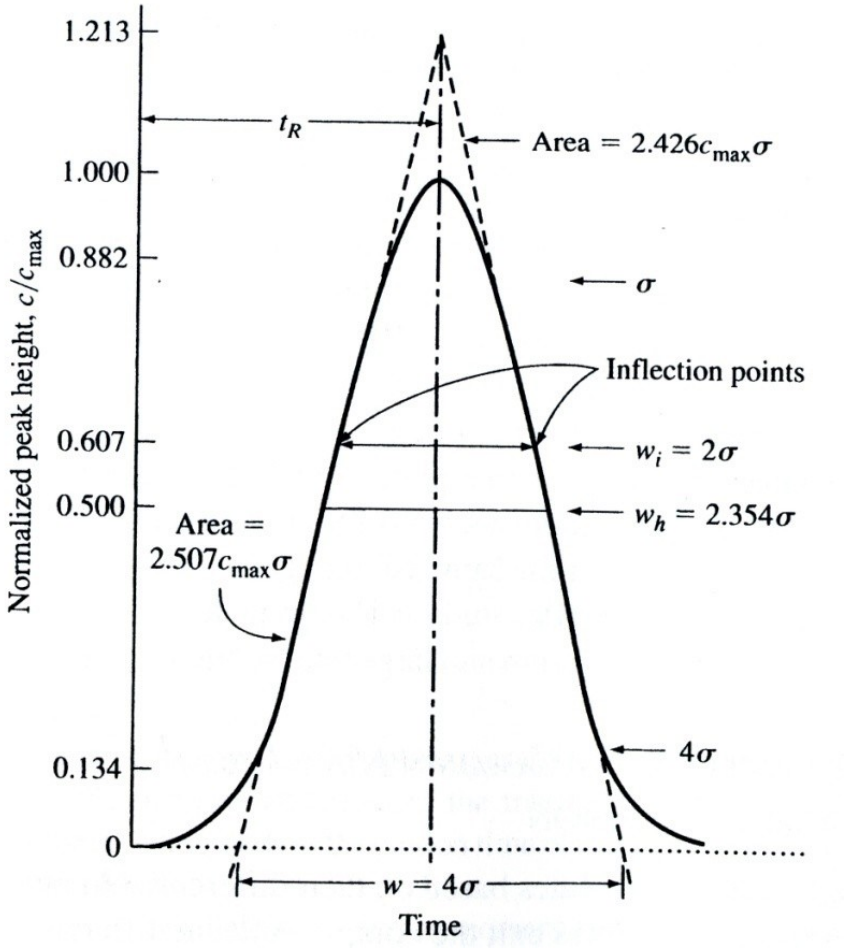
- **Plate height**
  - H = L/N
    - H: plate height
    - L: Length of the column
    - N: the number of plates



  - Smaller plate height → more discrete steps along the column → better separation

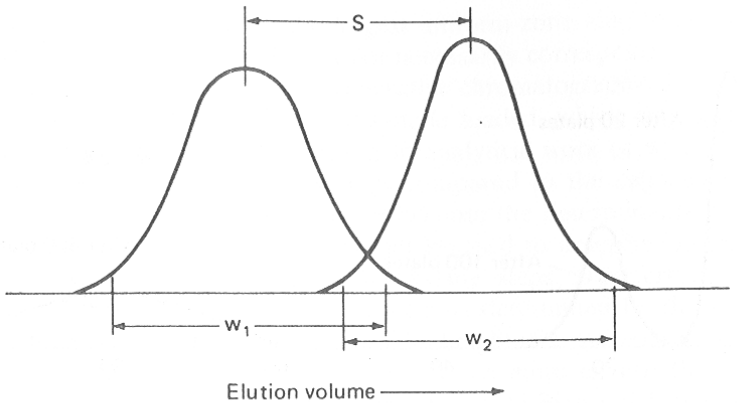
Concentration Profile : Gaussian Peak

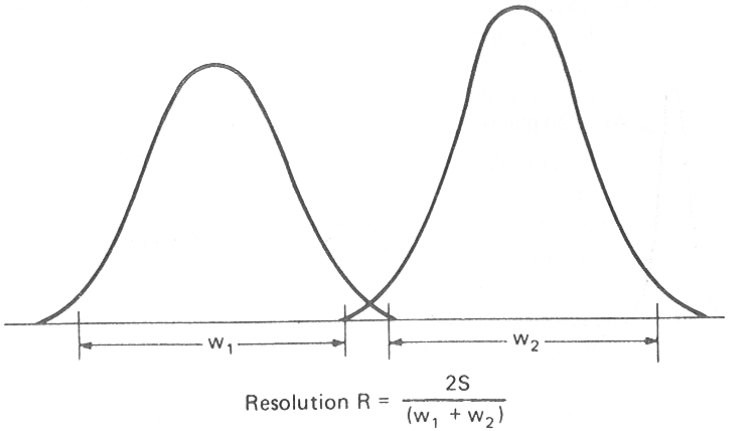
- $N = t_R^2/\sigma^2 = t_R^2/(\omega/4)^2$
  - t_R : average retention time
  - σ : standard deviation
  - ω : peak width



Resolution (R)

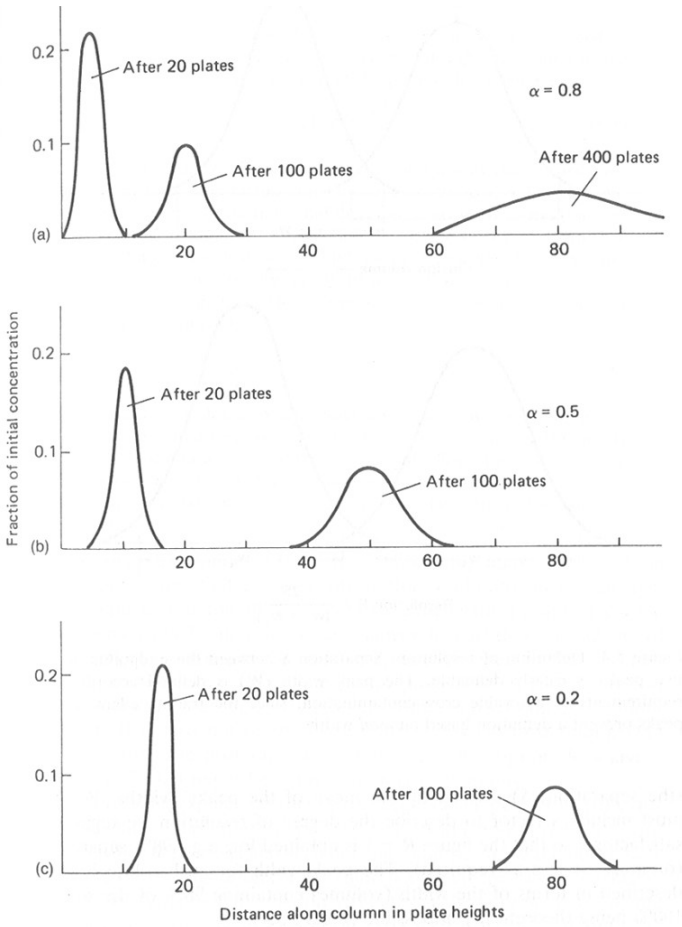
- **R = 2($t_{R\,2}$ - t_{R1}) /(ω1 + ω 2)**
  = 2S /(ω1 + ω 2)
  - S : Separation
    - The distance between two neighboring peak maxima
  - ω : Peak width
    - Defined according to requirements of allowable cross-contamination
    - Cannot defined based on total width because of trailing edges





Zone Spreading

- **Zone spreading**
  - Increases with number of plate transfers rather than distance travelled down the column
  - Higher α : solute emerge later in a larger volume
  - Emerging of the center peak
    - After 1/(1-α) X column volume
- **Separation of the three solutes (α=0.8, 0.5, and 0.2)**
  - Separation after 100 plate transfer
  - Column with a length of 20 plates could be used to separate two solutes (α=0.8 and 0.2)



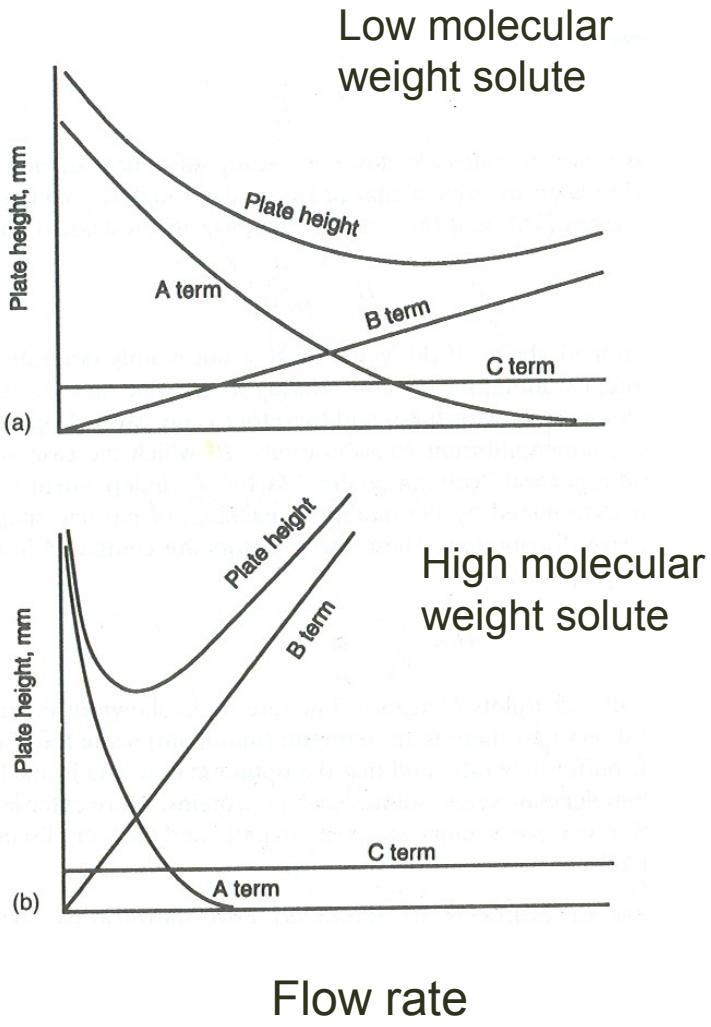
- α : fraction of protein adsorbed
- Buffer traveled the defined number of plate heights

Plate Height (H)

- **$H = \sigma^2/L$**
  - σ : Variance of the Gaussian concentration profile
  - L : Column length
- **$H = Lw^2/(16V_e)$**
  - V_e: Total volume of solvent passed through column as the peak emerges
  - w: peak width
- **Factors affecting H**
  - A : diffusion , inversely proportional to flow rate
  - B : Nonequilibrium considerations, increase with flow rate
  - C : Column quality factor, independent flow rate
    - Quality of packing
    - Particle shapes
    - Particle size distribution

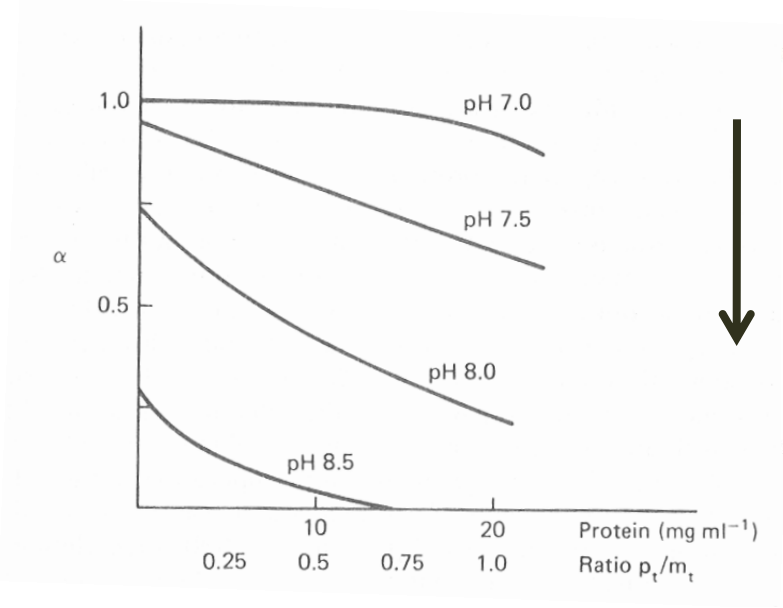
Plate Height (H)

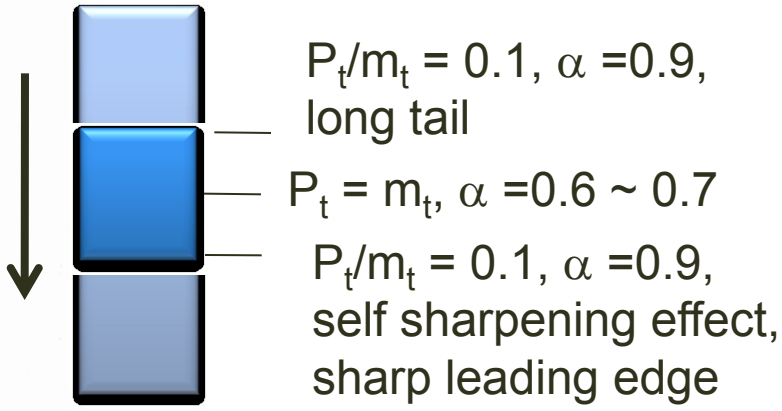
- **Van Deemter equation**
  - H =A/v + Bv + C
    - v: flow rate
  - Optimum (minimum) value for H in terms of linear buffer low rate
  - Based on isocratic operation
    - Not very relevant to protein chromatography using gradient elution

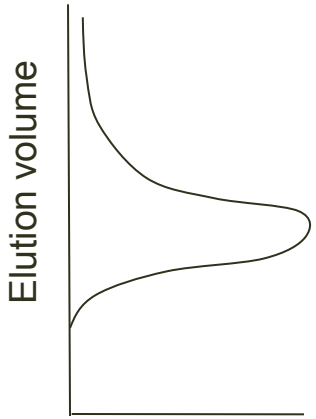


Partition Coefficient Depending on Protein Concentration

- **Higher protein concentration → lower α**
  - Approaching saturation
  - Lower average affinity → lower α value
    - Stronger binding site (low K_p) are occupied first
    - Weaker binding sites have to be used at higher protein concentration







Other Factors

- **Penetration factor**
  - Presence of less accessible binding sites
    - It takes a long time for a protein to bind and to come off
    - Delayed or no elution
  - Adsorption occurs mainly on the outer portions of bead particles
    - Outer 20% of bead represents about 50% of the total bead volume
    - Better not to have proteins going inside to the center of the beads
- **Inhomogeneity of adsorbents**
  - m_t is an average value
  - Effective value of m_t may be much higher in local areas