Thermodynamic modeling of protein separation

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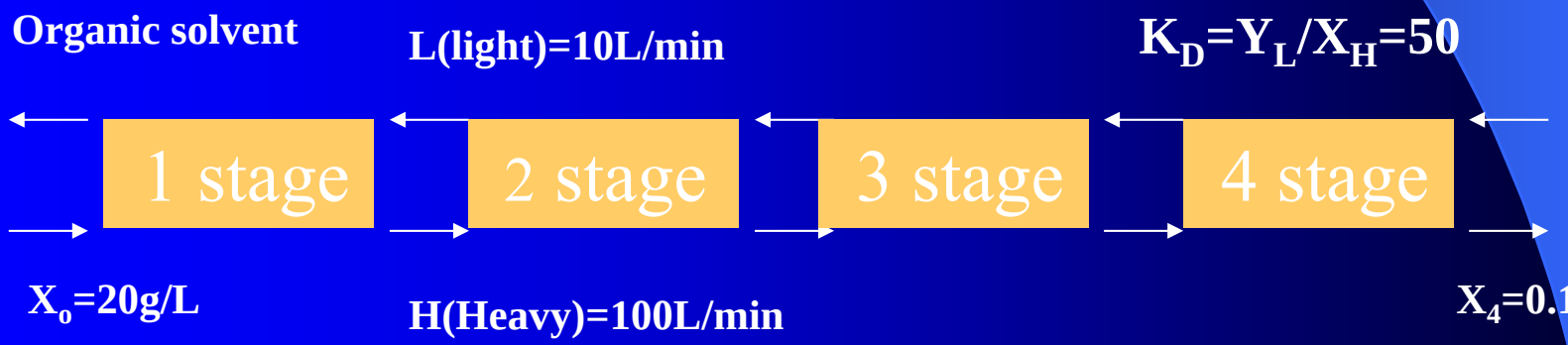
Ph. D. Course

Scope

- Roles of thermodynamics in biochemical engineering
- Factors in protein purification
- Purification of protein
- Thermodynamic modeling of protein separation and precipitation.

Roles

- For rational, efficient and rapid process development and equipment design in bioprocesses.



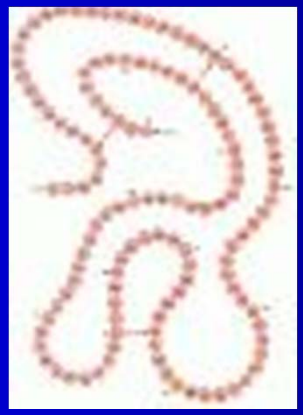
[Multistage countercurrent liquid extraction of Penicillian-G]

Potential role of thermodynamics

- Predictions of **product behavior** (aggregation, degradation) given T,pH,I
- Predictions of **chromatographic** behavior based on product properties
- Prediction of **solvent-product interactions**
- Prediction of **two-phase aqueous extraction** via equilibrium

Factors affecting protein properties

- Ionic Strength
- pH
- Temperature
- Solubility
- Organic Solvents
- Primary, Secondary, tertiary, quarternary structure



Purification process of protein

- Precipitation

  Solid-Liquid Equilibria

  Liquid-Liquid Equilibria

- Aqueous two phase system
  - Polymer-Polymer-protein-Water
  - Polymer-Salts-protein-Water

Precipitations.

- By adding Salts ($(NH_4)_2SO_4$, Na_2SO_4)
  - $\log S/S_o = -K_s(I)$

    (S_o : solubility of protein at I=0)
- By adding Polymers(PEG, DEX)
  - reducing the amount of water acting on protein molecules

Aqueous two phase systems(I)

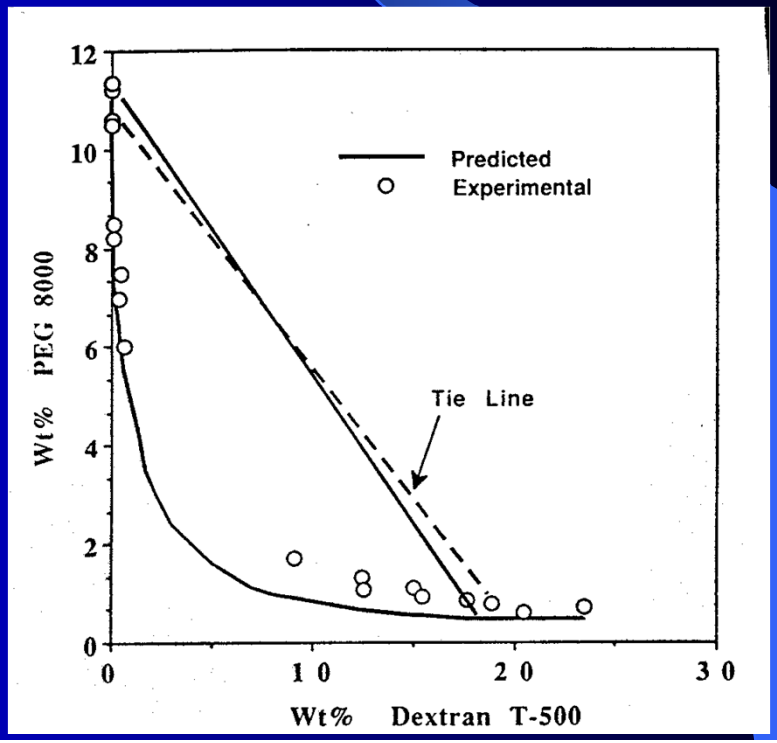
- Polymer-Polymer-water-protein system

Phase : PEG(Dextran)

Dilute solution of protein i,j,k…, and salt

Phase : Dextran(PEG)

Dilute solution of protein i,j,k…, and salt



Aqueous two phase systems

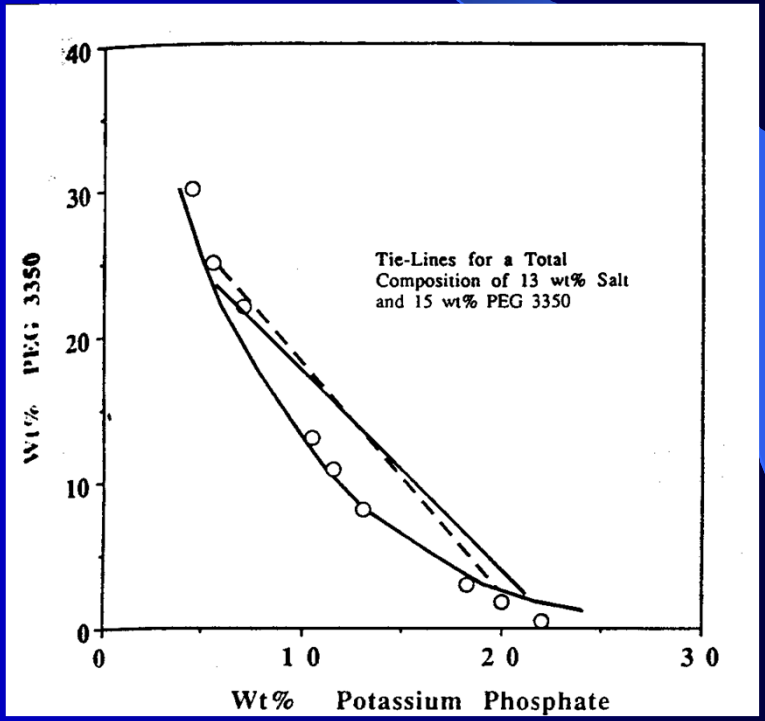
- Polymer–Salt–water–protein system

Phase : PEG

Dilute solution of protein i,j,k…, and salt

Phase : Phosphate salts

Dilute solution of protein i,j,k…, and salt



Modeling(I) : Salt-Induced Protein Precipitation

- Potentials of mean force for aqueous protein-protein interactions
- (C.J.Coen et al, 1995)
- $W(r) = W_{disp}(r) + W_{q-q}(r)$ (DLVO)
- $+ W_{q-\text{dipole}}(r) + W_{\text{dipole}-\text{dipole}}(r)$
- $+ W_{OA}(r)$

$$B_2(a_1^0, T) = B_2^{hs} - \frac{N_A}{2}\int_{d_2+3\text{Å}}^{\infty} \{\exp[-W(r, a_1^0, T)/kT] - 1\} 4\pi\}^2 dr$$

$$\frac{Kc_2}{\overline{R_\theta}} = \frac{1}{M_{w,2}} + 2B_2c_2 \quad (By\ LALLS)$$

Modeling(I) : Salt-Induced Protein Precipitation

- Approach using EOS (Prausnitz et al, 1996)

Equation of States approach

- RPA(Random-Phase Approximation) equation of state

$$Z = \frac{P}{\rho kT} = \left(\frac{P}{\rho kT}\right)_{ref} + \frac{\rho U}{2kT}$$

$$\left(\frac{P}{\rho kT}\right)_{ref} = \frac{1+\eta+\eta^2+\eta^3}{(1-\eta)^3}$$

$$U = 4\pi \int W_{pp}(r) r^2 dr$$

Protein–Protein Potential

- $W_{ij}(r)=W_{elec}(r)+W_{disp}(r)+W_{osmotic}(r)+W_{specific}(r)$

$$W_{elec}(r)=\frac{z_i z_j e^2 (1/r)\exp[-\kappa(r-\sigma_{ij})]}{4\pi\pi_o\varepsilon_r(1+\kappa\sigma_{ij}/2)^2}$$

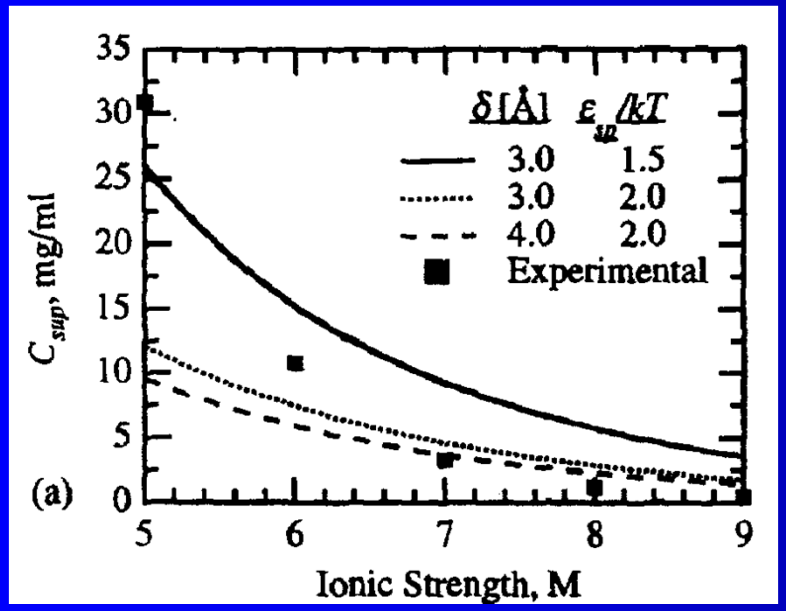
$$W_{disp}(r)=-\frac{H_{ij}}{12}\{\frac{\sigma_{ij}^2}{r^2-\sigma_{ij}^2}+\frac{\sigma_{ij}^2}{r^2}+2\ln(1-\frac{\sigma_{ij}^2}{r^2})\}$$

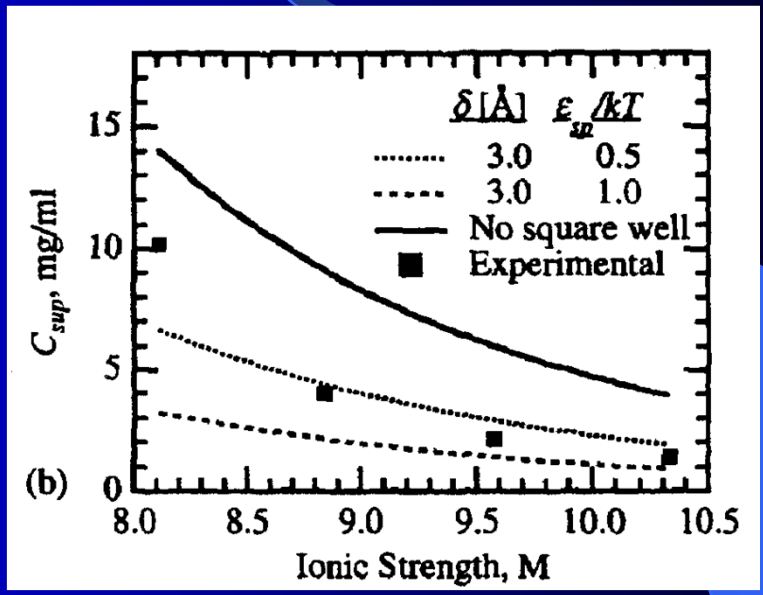
$$W_{osmotic}(r)=-\frac{4}{3}\pi\sigma_{ij,s}^2(\rho_s kT)[1-\frac{3r}{4\sigma_{ij,s}}+\frac{r^3}{16\sigma_{ij,s}^3}]$$

$$W_{speific}(r)=-\varepsilon_{sp}(\sigma_{ij}<r<(\sigma_{ij}+\delta))$$

$$=0(r>(\sigma_{ij}+\delta)$$

Correlation of experimental supernatant phase protein





- a)Hen-egg-white lysozyme in ammonium sulfate
- b)a-chymotrypsin in ammonium sulfate

Modeling(II) : Protein-Electrolytes

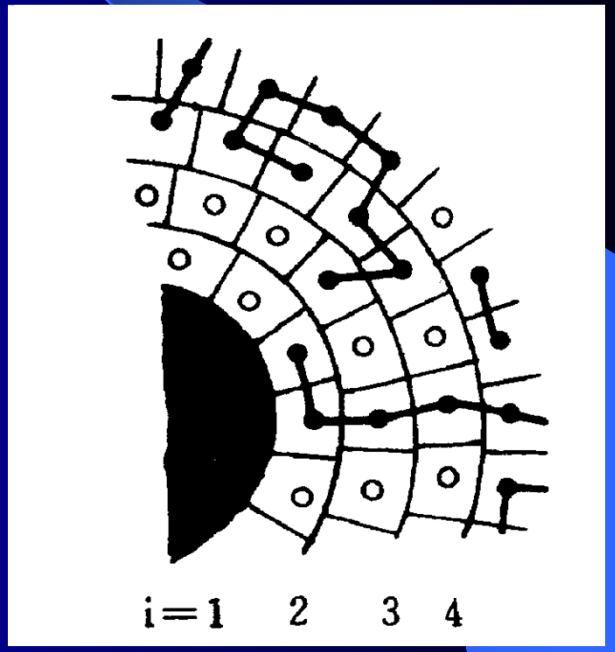
- Adsoprtion lattice model(Baskir, 1987)
- + Pitzer long-range electrostatic term
- (Qunhua Peng et al,1995)

$$\ln\gamma_4 = \frac{g - g^*}{kT} = -\ln\left(\frac{\Omega}{\Omega^*}\right) + \frac{\Delta U}{kT} - \frac{(n_1^{\sigma}\Delta\mu_1 + n_2^{\sigma}\Delta\mu_2)}{kT}$$

$$\ln\gamma_4^{LR} = \frac{2A_x I_x^{3/2}}{1 + \rho I x^{1/2}}$$

Characteristics of Model

- Assumptions
- ► Protein : neutral molecule
- ► No electrolyte in the
- adsorption layers of protein
- ► No interaction between
- proteins



Modeling(III) : Protein-Polymer/electrolyte

- Integral-Equation Theory
- (C.A. Haynes et al, 1993)

$$h_{ij} = C_{ij}(r) + \sum_{k} \rho_k \int_0^r C_{kj}(|r - r'|) h_{ik}(r') dr$$

$$+$$

$$C_{ij}(r) = -u_{ij}(r)/kT \quad r > d_{ij}$$

$$h_{ij}(r) = -1 \qquad\qquad r < d_{ij}$$

Characteristics of Model

- $A`_{Ex} = A`_{Ex,hs} + A`_{Ex,na} + A`_{Ex,ic} + A`_{Ex,cc} + A`_{Ex,ve}$

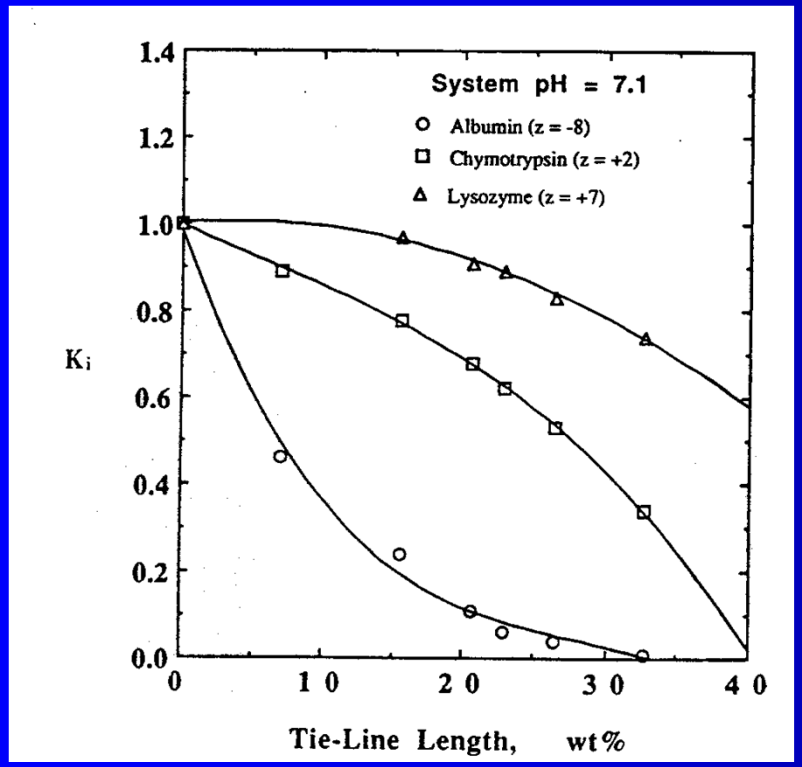
$$A'_{EX} = RTN_{Av} \sum_{i\neq 0} \sum_{j\neq 0} n_i \frac{n_j}{V} \beta^*_{ij}(\mu_o, T)$$

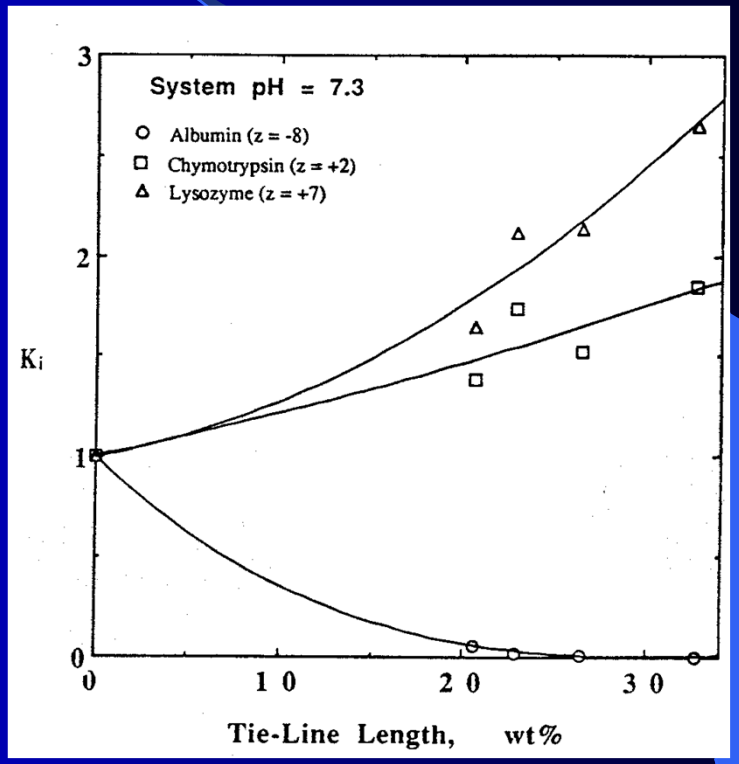
$$\frac{Kc_i}{R_\theta} = \frac{1}{M_{w_i}} + 2B_{ii}c_i \quad \text{(for Polymer and protein)}$$

$$\frac{K'(c_i + c_j)}{R_q} = m(c_i + c_j) + b, \quad m = m(B_{ii}, B_{ij}, B_{jj})$$

(Cross osmotic second virial coefficients for macromolecules)

Comparison between exp and calc





- a) PEG3350-Dextran T70-potassium phosphate
- b) PEG3350-Dextran T70-potassium chloride

Conclusion.

- 현재까지 실제 공정에 적용할 수 있는 정량적인 예측능력을 가진 모델은 없다.
- 단백질의 상평형을 모사하기 위해서는
  - 계에 존재하는 힘들을 적절하게 묘사하는
  - 항들이 포함되어야 한다.
- Lattice fluid를 Bio-separation에 적용하기 위해서는 segment들의 크기를 고려한 항들이 추가되어야 한다.