11. Extraction

1. Extraction Principles

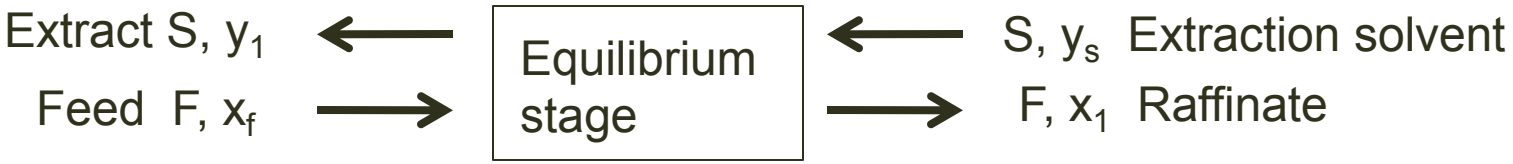
Extraction

- **Extraction**
  - Transferring a solute or particle from one phase to another
  - Usually used early in purification process
- **Types**
  - Liquid-liquid extraction
    - Use two immiscible liquids
      - Depending on the partitioning of the biomolecules between the liquid phases
    - Main methods for pharmaceutical of biotechnological applications
  - Solid-liquid extraction (leaching)

Liquid-Liquid Extraction

- **Design of the extraction process**
  - Miscibility of the two liquid phases
  - The rate of equilibration of the biomolecules between the two phases
- **Phase separation and partitioning equilibria**
  - Feed stream + extraction solvent stream

  → equilibrium extract + raffinate



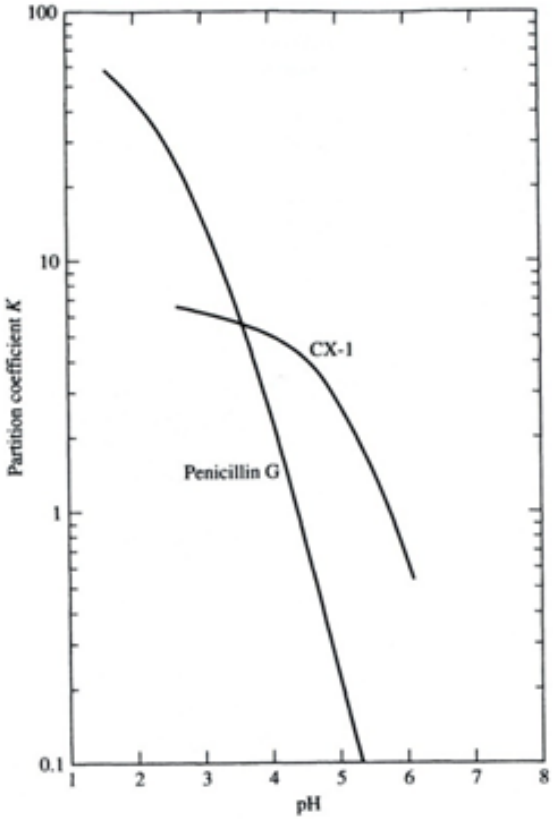
  - Partition coefficient
    - $K = y/x$

      y : solute concentration in the extract

      x : solute concentration in the raffinate phase

Partition Coefficient

- **Partition coefficient is depending on**
  - The size of the molecule being extracted
  - pH
  - Types of the solvent
  - Temperature
  - Concentration and molecular weight of polymers (salt) in the phases
- **pH dependent partition coefficient**
  - PenicillinG
  - CX1: acidic impurity
- **Bronsted model to Describe Partitioning**
  - K = exp(*Mλ/kT*)
    - Molecular weight of the partitioning molecule
    - *k :* Boltzmann constant
    - *T:* absolute temperature
    - *λ :* constant including the characteristic and the phases and partitioning molecule



Aqueous Two-Phase Extraction Systems

- **Two phases**
  - Two water-soluble polymers , PEG-dextran
  - A polymer and a salt in water, above a 'critical concentration', PEG-potassium phosphate
- **Nondenaturing and nondegrading technique**
  - For separation of proteins, enzymes, viruses, cells, and organelles
  - For removal of undesirable contaminating by-products
    - Nucleic acids, polysaccharides
- **Phase diagram**
  - PEG (top)-dextran (bottom)
    - 10% PEG, 15% dextran
    - 15% PEG, 15% salt
  - Two phases on the curve

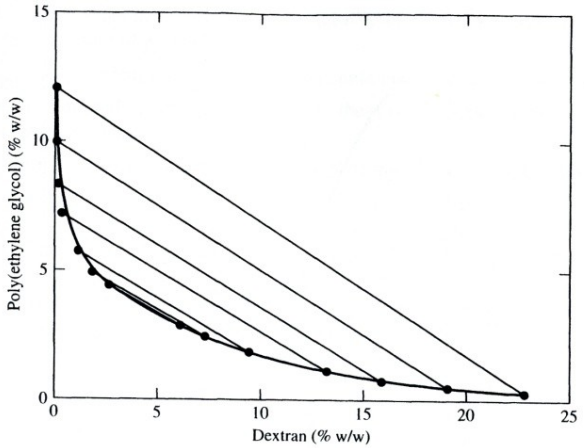


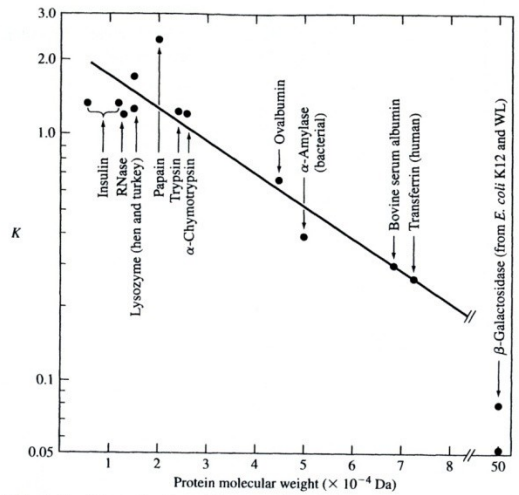
Figure 6.3 Phase diagram for a PEG 6000–dextran D48 system at 20°C. (Data from P.-A. Albertsson, *Partition of Cell Particles and Macromolecules*, 3rd ed., Wiley, New York, 1986.)

Separation of Proteins by Liquid Phase Partitioning

- **Requirement**
  - Creation of two immiscible liquid phases
  - Each liquid phase can dissolve proteins
  - Proteins should remain bioactive after dissolving
- **Phase separation**
  - Addition of certain pairs of hydrophilic polymers to aqueous solutions
    - Generation of several phases
  - Dextran and PEG
    - Phase separation
      - Dextran-rich (bottom) : Partition of most proteins
      - PEG-rich (top)
    - Useful for large scale production
      - Whole cell lysate can be applied without removing cell debris
      - High capacity : 100mg/ml

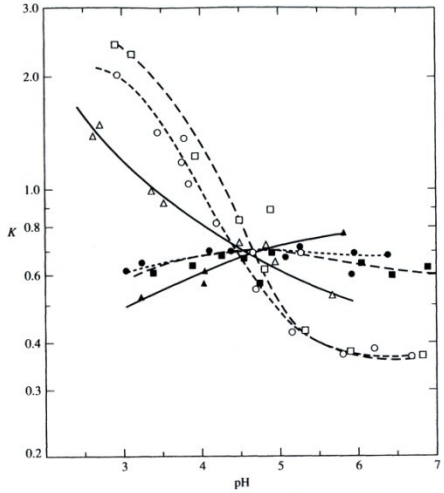
Factors Affecting Protein Partitioning

- **Difficult to predict K which is depending on..**
  - Molecular weight
    - High Mw → bottom
  - Protein charge, surface properties
  - Polymer Mw
  - Phase composition, tie line length
  - Salt effect
  - Affinity ligand attached to polymers



Mw

Figure 6.4 Effect of protein molecular weight on partitioning in a PEG 6000–dextran 500 system with pH at the isoelectric point (pI) for all proteins. (Data from S. Saskawa and H. Walter, "Partition behavior of native proteins in aqueous dextran–poly(ethylene glycol)-phase systems," *Biochemistry*, vol. 11, p. 2760, 1972.)

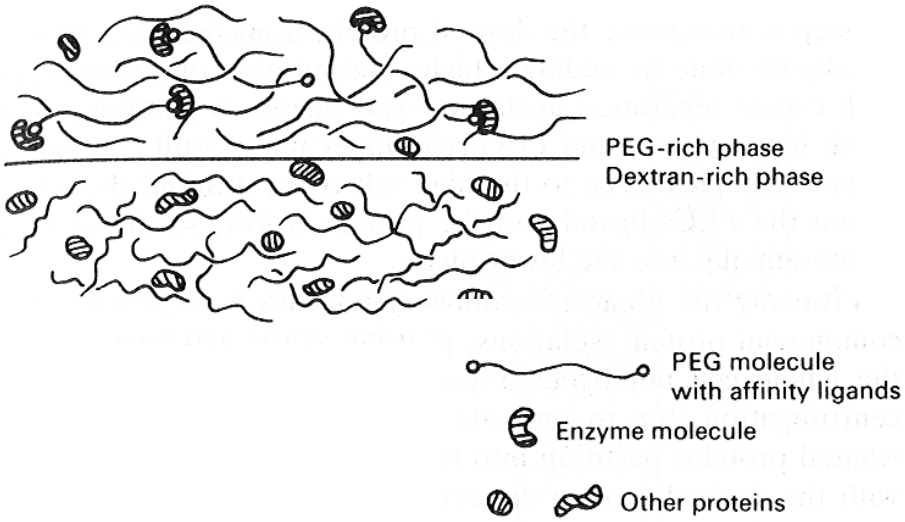


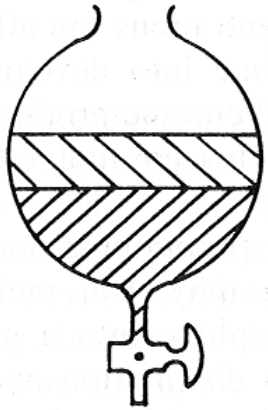
pH

Figure 6.5 Dependence of ovalbumin partitioning on solution pH and salt type in a PEG 6000–dextran 500 system. Open symbols denote chloride salts, solid symbols, sulfates (squares, potassium; circles, sodium; triangles, lithium). (Data from H. Walter, S. Sasakawa, and P.-Å. Albertsson, "Cross-partition of proteins: Effect of ionic composition and concentration," *Biochemistry*, vol. 11, p. 3880, 1972.)

Affinity Partitioning

- **Dextran and PEG + PEG-ligand**
  - PEG-ligand
    - Activation and attachment of ligand to –OH of PEG
    - Mix with the bulk PEG
  - Partitioning of the target protein in the upper phase
  - Elution of the protein from the PEG phase
    - Addition of high concentration of phosphate buffer
    - Phase separation
    - Protein partition into phosphate-rich lower phase





Affinity Partitioning

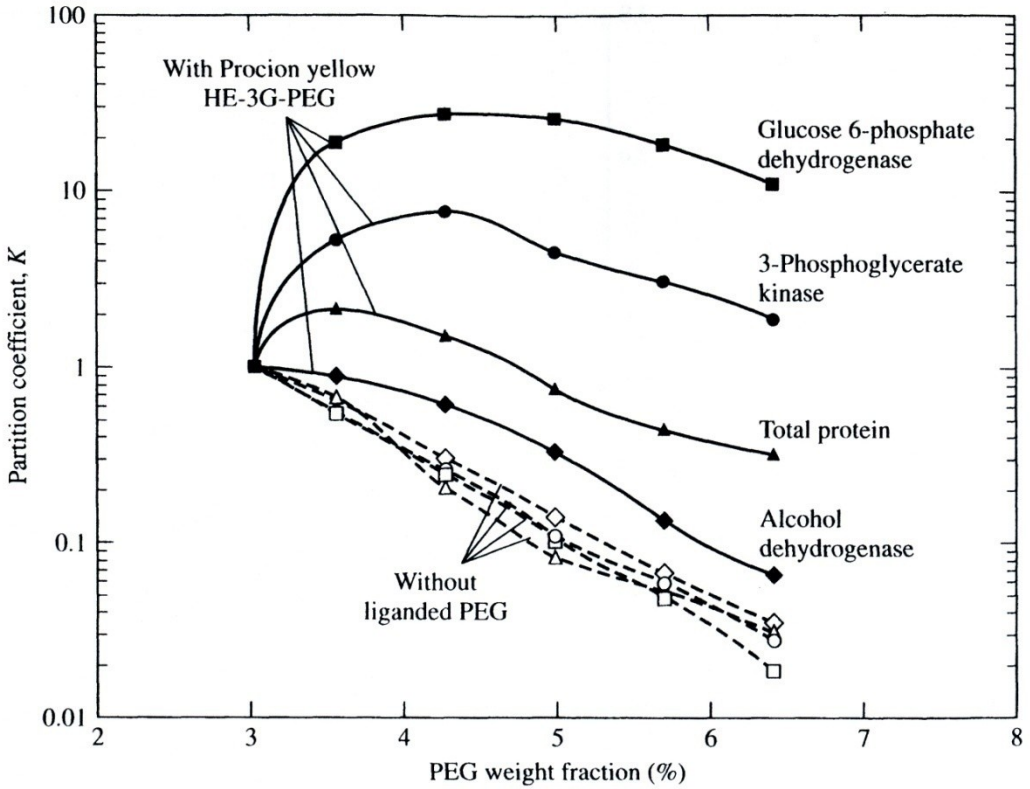
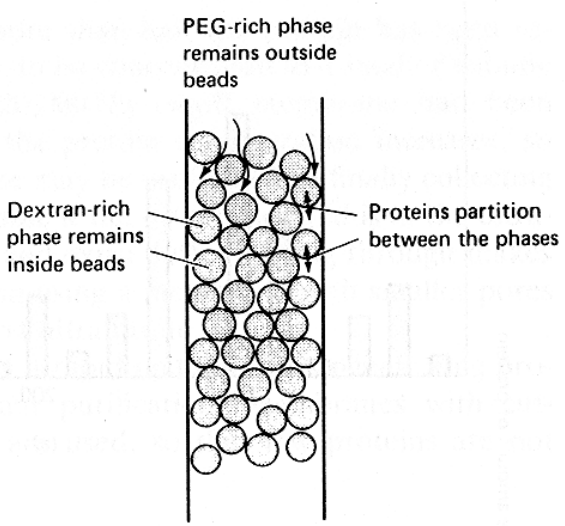


Figure 6.6 Affinity partitioning of enzymes in a PEG 6000–dextran 500 system with and without Procion yellow–PEG. (Data from G. Johansson and M. Andersson, "Parameters determining affinity partitioning of yeast enzymes using polymer-bound triazine dye ligands," *J. Chromatogr.*, vol. 303, p. 39, 1984.)

Liquid-Liquid Chromatography

- **Immobilization of one liquid phase**
  - Equilibrate agarose bead with one of the phases
    - E.g dextran-rich phase
  - Use the other phase as moving buffer in column
  - Surface tension prevents mixing of two phases
- **Problems of phase partitioning methods**
  - Handling problems with viscous solutions
  - Cost : dextran is expensive
    - Alternative: starch derivatives
    - Temperature-dependent phase separation
      - Triton X-114
      - Copolymers of ethylene an propylene oxide
- **Advantage**
  - Gentler than adsorption chromatography
    - Suitable for labile enzymes
  - Rapid
  - High capacity
  - Requires little equipment



Countercurrent Stage Calculation

- **Countercurrent extraction**
  - Extraction solvent and the feed run countercurrent to each other
  - Greater difference in solute concentration between the raffinate and extraction phases than cocurrent extraction
- **Countercurrent extraction cascade**
  - Assumption
    - The two solvents are immiscible or are already in phase equilibrium
    - The solute concentrations are sufficiently low that the flow rates of the raffinate and extract are constant
    - Equilibrium is achieved in each stage

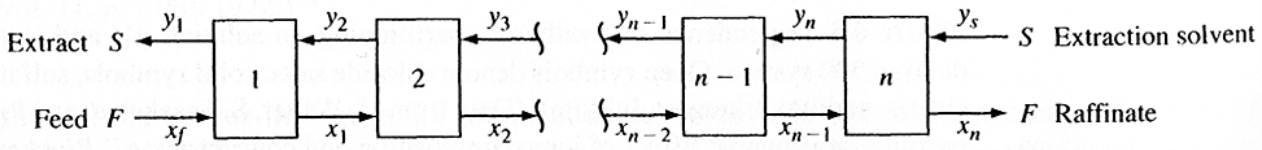


Figure 6.7 Countercurrent extraction cascade with n equilibrium stages, showing stream variables.

Material Balance

- **A material balance on the solute around the feed end of the cascade down to n-1**
  - $x_f F + y_n S = x_{n-1} F + y_1 S$
    - F: flow rate of feed or raffinate phase
    - S: flow rate of extract phase
  - $y_n = F/S \cdot x_{n-1} + (y_1 S - x_f F)S$
    - F/S : operating line
- **McCabe-Thiele-type diagram**
  - $y_s = 0$

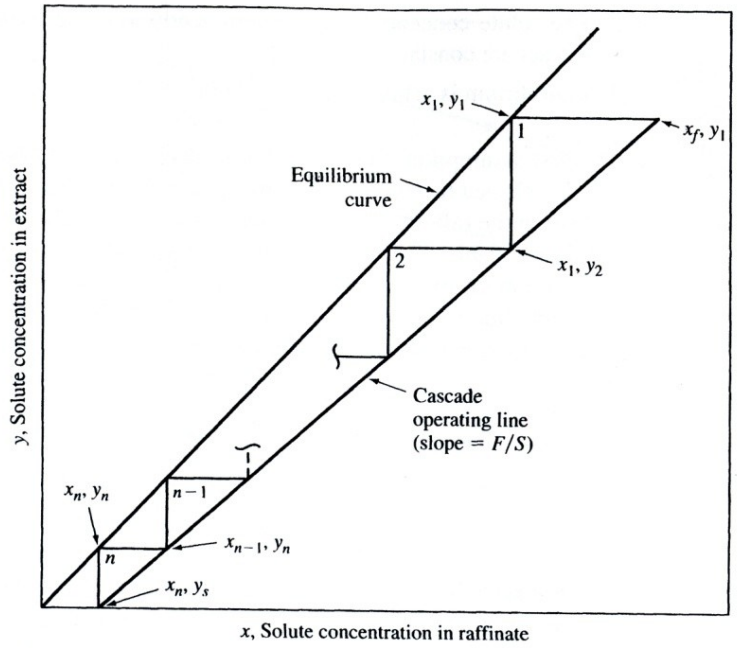


Figure 6.8 Graphical calculation of equilibrium stages for countercurrent extraction.

At constant Partition Coefficient K

- **For isothermal, dilute solutions**
- **Extraction factor E**
  - $E = K/(F/S) = KS/F$
- **Material balance on the solute for the nth stage ($y_s = 0$)**
  - $X_{n-1}F = x_nF + y_nS$
  - $X_{n-1} = (E + 1)\, x_n$
  - A material balance on the solute for the (n-1)th stage
    - $X_{n-2} = (E^2 + E + 1)\, x_n$
  - Continuing this procedure until stage 1
    - $X_f = (E^n + E^{n-1} + \ldots E^2 + E + 1)\, x_n$
    - $X_f = [(E^{n+1} - 1)/(E-1)]\, x_n$ or $X_n = [(E - 1)/(E^{n+1}-1)]\, x_f$
    - $n = \ln[(x_f / x_n)(E-1) + 1]/\ln E - 1$
    - $n \rightarrow \infty$, $x_n \rightarrow x_f$/ $E^n \rightarrow 0$ (Extraction of almost all the solute)
    - If E=1.0

      $x_n = x_f/(n+1)$
    - For E <1.0, $n \rightarrow \infty$

      $x_n \rightarrow (1-E)\, x_f$

Separation of Bioproduct and an Impurity by Countercurrent Extraction

- **Countercurrent extractor**
  - 4 equilibrium stages
  - Bioproduct K =10
  - Contaminant (10% weight of the bioproduct) K = 1
  - S/F = 0.2
  - What will be the ratio of impurity to bioproduct in the extract phase at the outlet of the extractor?
- **Solution**
  - Bioproduct
    - E=KS/F =
    - x_n / x_f =
  - Impurity
    - E=KS/F =
    - x_n / x_f =
  - ratio of impurity to bioproduct