Chapter 11: Product Recovery and Purification

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Presentation Outline:

- Overview of Bioseparations
- Separation of Insoluble Products
- Primary Isolation / Concentration of Product
- Purification / Removal of Contaminant Materials
- Product Preparation

Introduction to Bioseparations

Characteristics of Bioseparations vs Chemical Separations

Characteristics	Biochemical	Chemical
Environment	Aqueous Media	Organic Media
Concentration Range	v. Dilute Product	Concentrated Product
Temperature Sensitivity	Product Vulnerable	Product Not Vulnerable

Traditional chemical separations are unsuitable or must be augmented

Biochemical Separations Technologies

Technologies

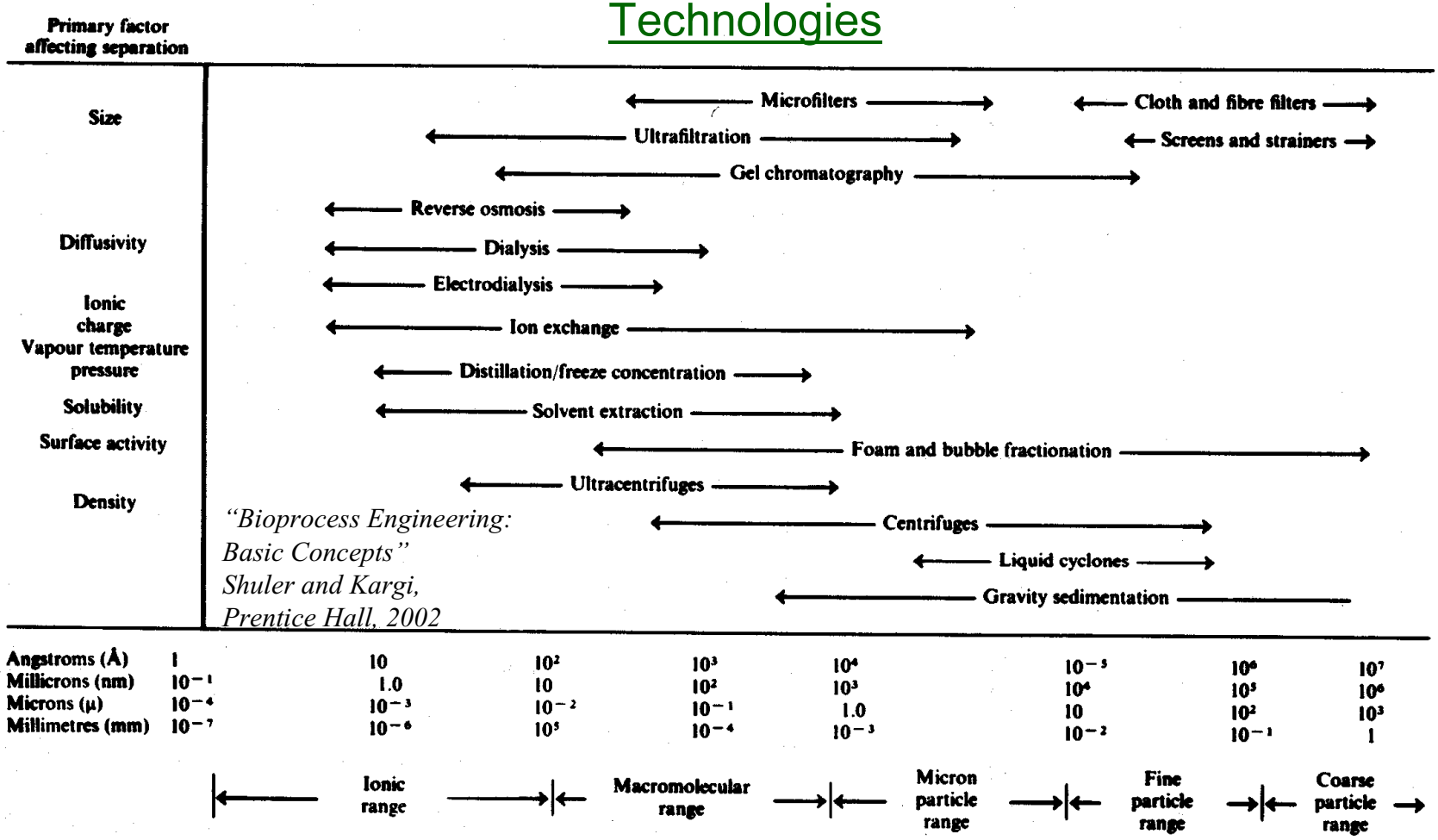

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Figure 11.2
Major Steps in Separating a Protein Product

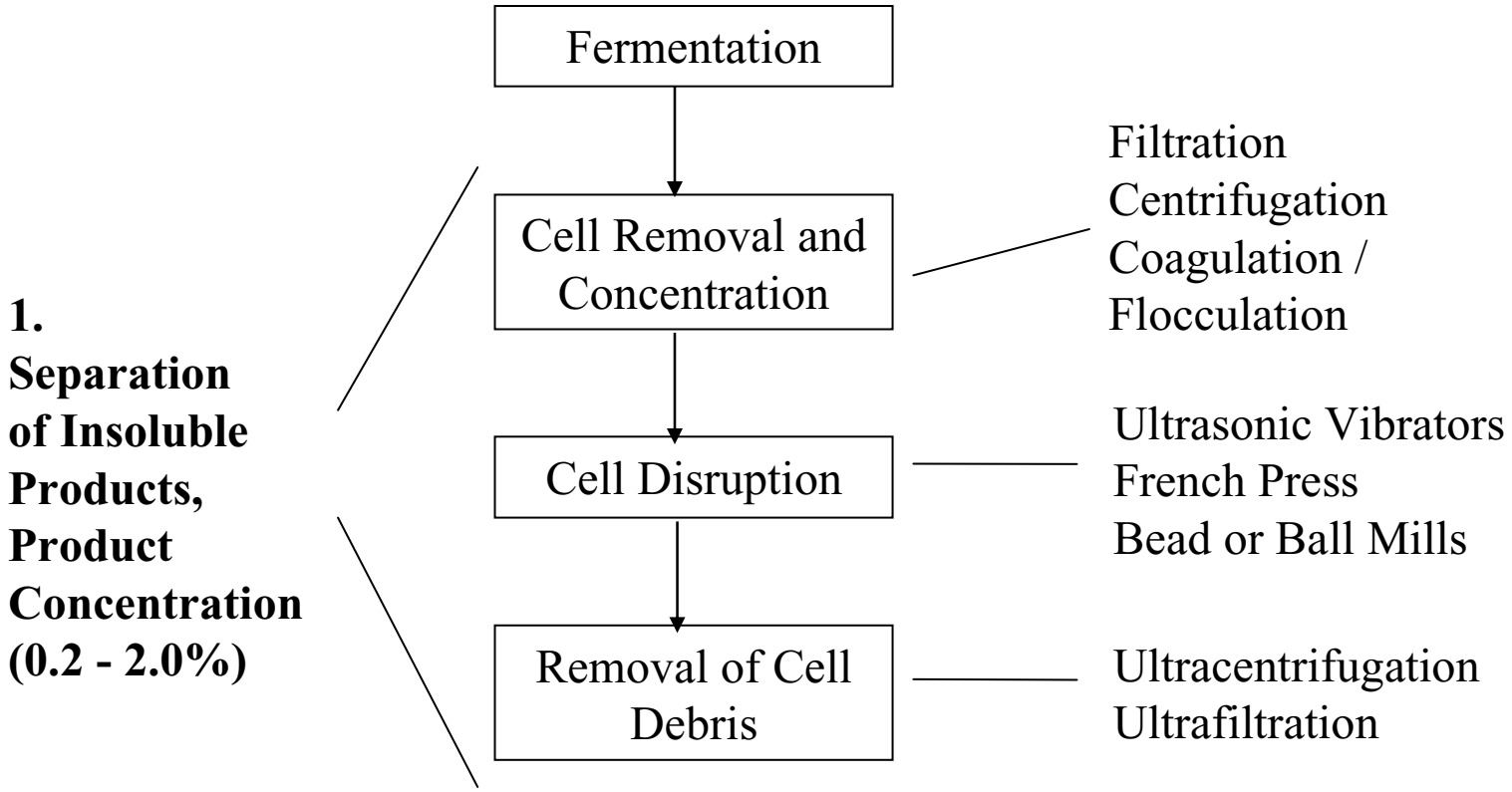


Figure 11.2
Major Steps in Separating a Protein Product

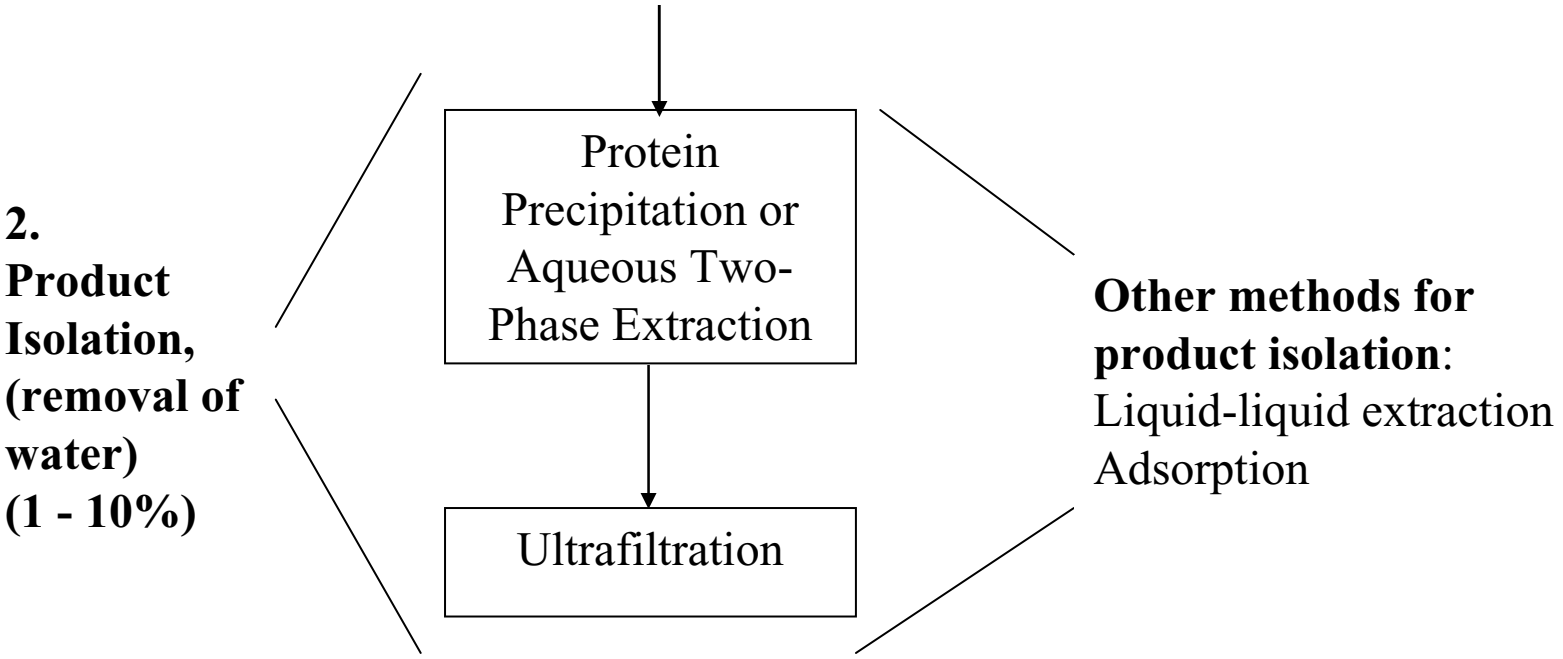
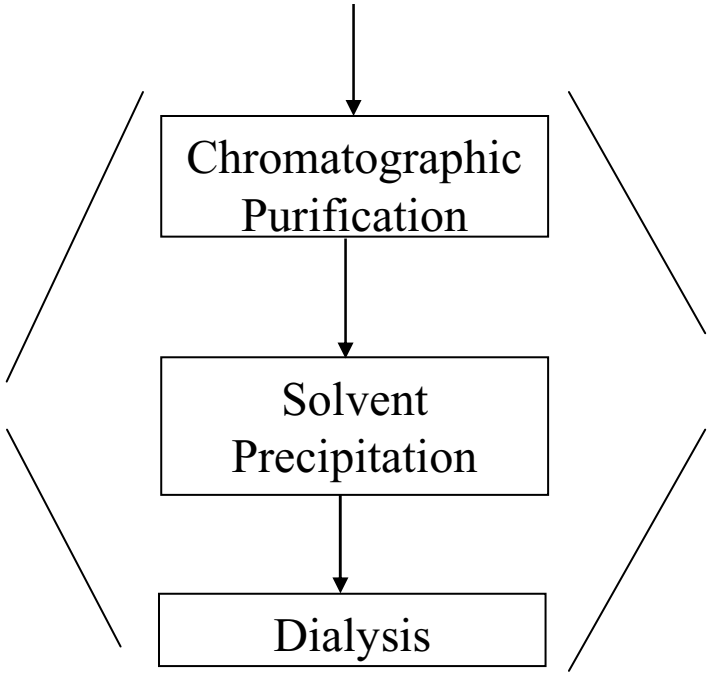


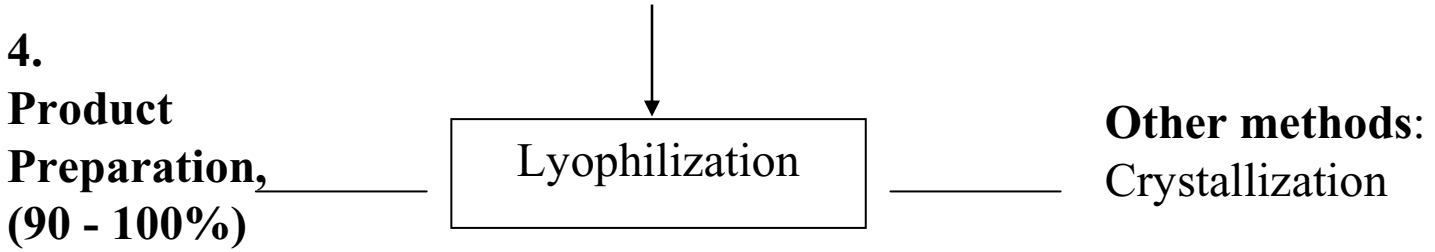
Figure 11.2
Major Steps in Separating a Protein Product

3. Purification / Removal of Contaminant Chemicals, (50 - 80%)

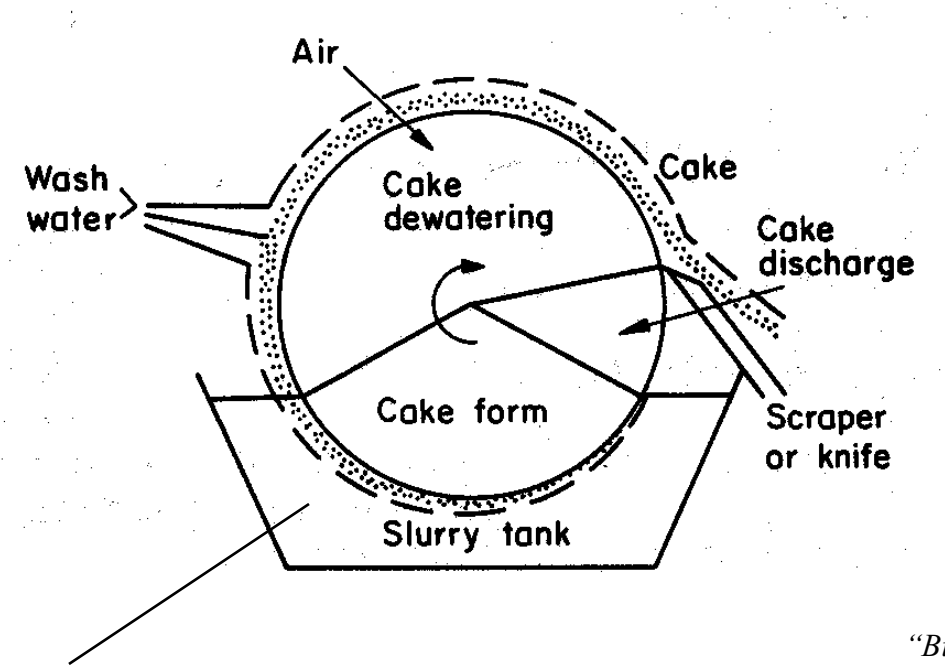


Other methods for product purification:
Reverse Osmosis
Cross-flow Ultrafiltration
Electrophoresis
Electrodialysis

Figure 11.2
Major Steps in Separating a Protein Product



1. Removal of Insoluble Products
Rotary Vacuum Filtration



- coagulation agents/ (filter aids) added
- vacuum applied to rotating drum (ΔP)

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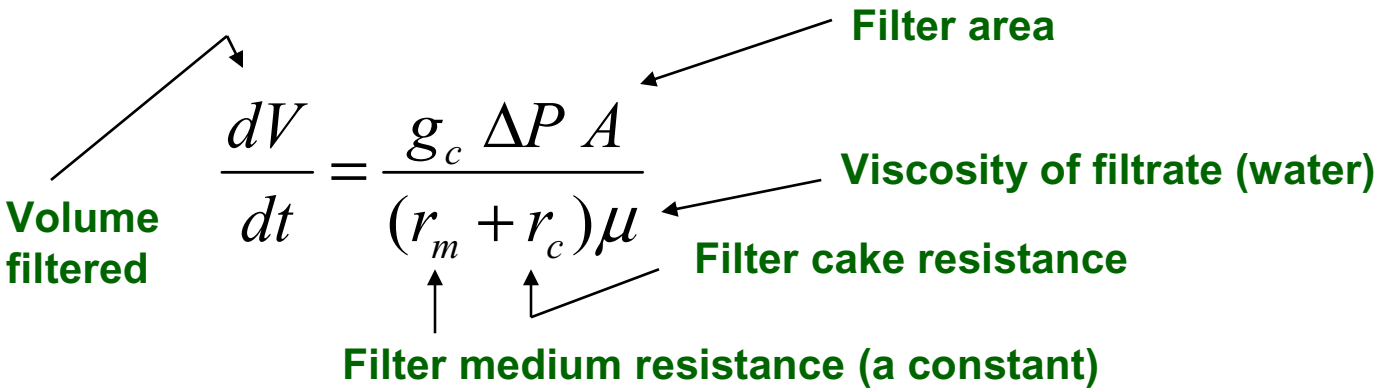
1. Removal of Insoluble Products
Rotary Vacuum Filtration



$$r_c = \alpha \frac{C V}{A}$$

Note: r_c increases with the volume filtered, V

C = wt. of cells per volume filtrate (g cells/L)

α = average specific resistance of filter cake

1. Removal of Insoluble Products
Rotary Vacuum Filtration

Integrate Filter Equation: V=0 at t=0.

$$V^2 + 2VV_o = Kt$$

Ruth Equation

where

$$V_o = \frac{r_m}{\alpha C} A$$

$$K = \left(\frac{2 A^2}{\alpha C \mu} \right) \Delta P \; g_c \dashrightarrow g_c = \left(1 \frac{kg \cdot m}{\frac{s^2}{N}} \right)$$

N = Newton

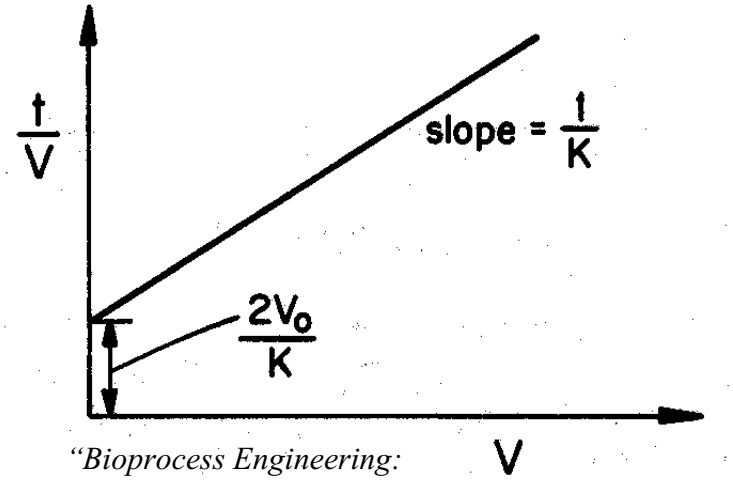
1. Removal of Insoluble Products
Rotary Vacuum Filtration

Rearrange Ruth Equation

$$\frac{t}{V} = \frac{1}{K}(V + 2V_o)$$

$$\alpha = \text{slope} \bullet \left(\frac{2A^2}{C\,\mu}\right)\Delta P\, g_c$$

$$= \frac{1}{K} \bullet \left(\frac{2A^2}{C\,\mu}\right)\Delta P\, g_c$$



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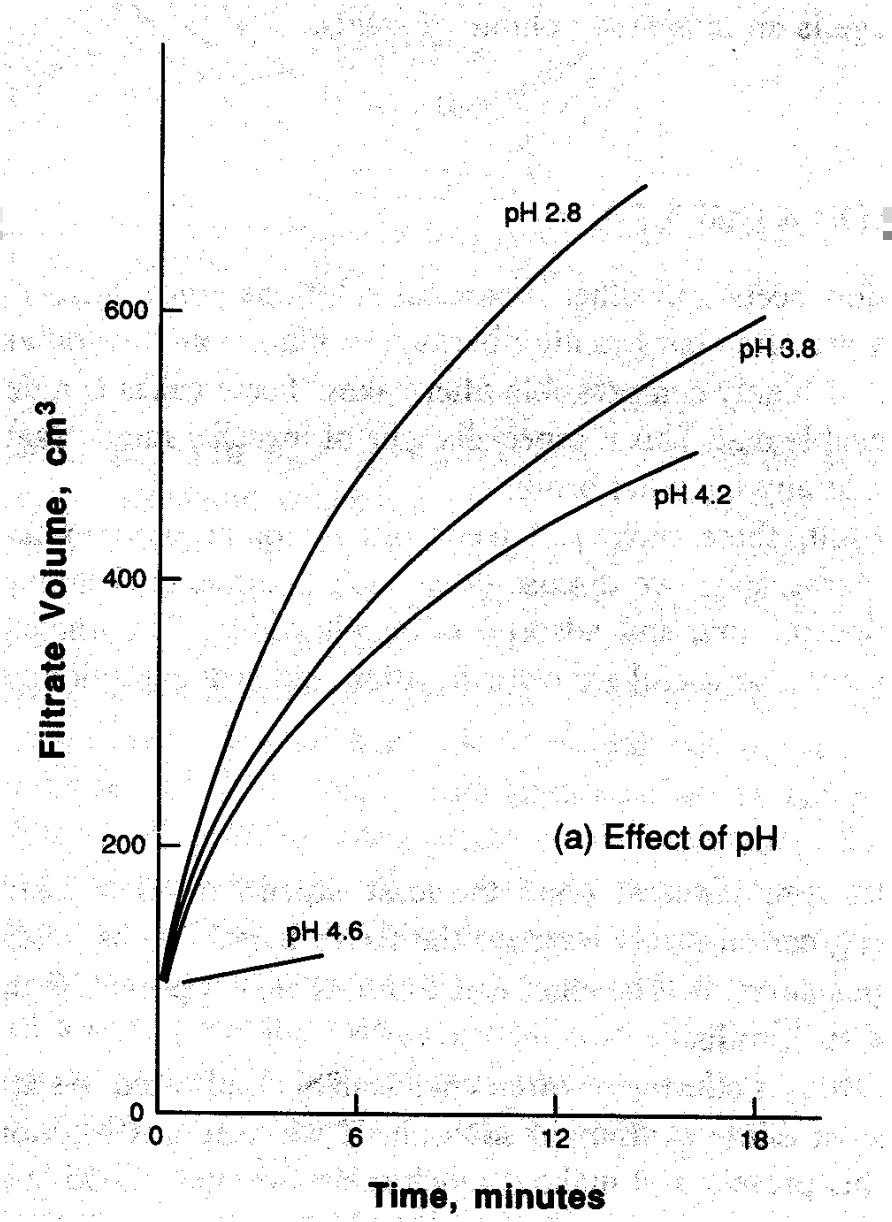
$$V_o = \text{y-intercept} \bullet \frac{K}{2} \quad \Rightarrow \quad r_m = \text{y-intercept} \bullet \frac{K}{2}\frac{\alpha C}{A}$$

Rotary Vacuum Filtration

Effect of pH and time on volume filtered



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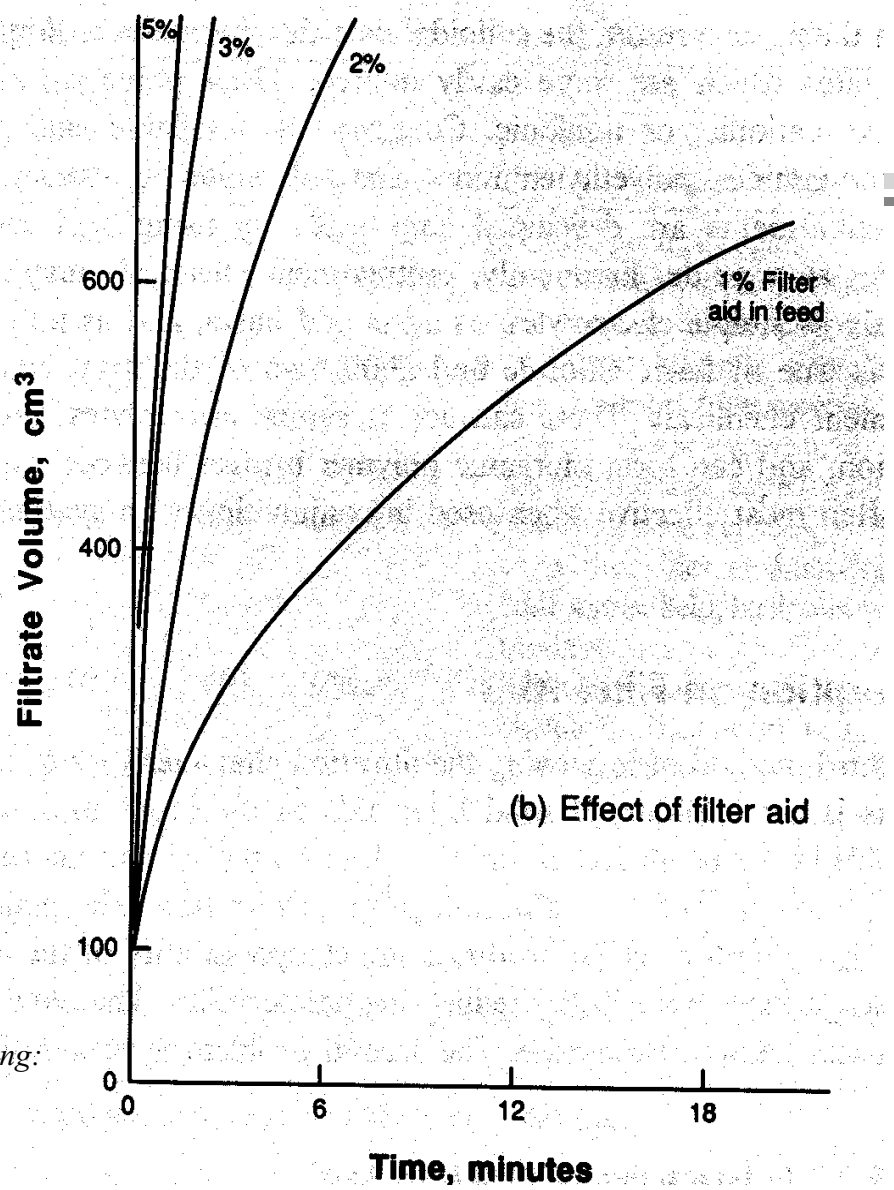
Rotary Vacuum Filtration

Effect of filter aid and time on volume filtered

Typical Filter Conditions

- **pH = 3.6**
- **2% - 3% filter aid**
- **heat treatment, T=80˚C**



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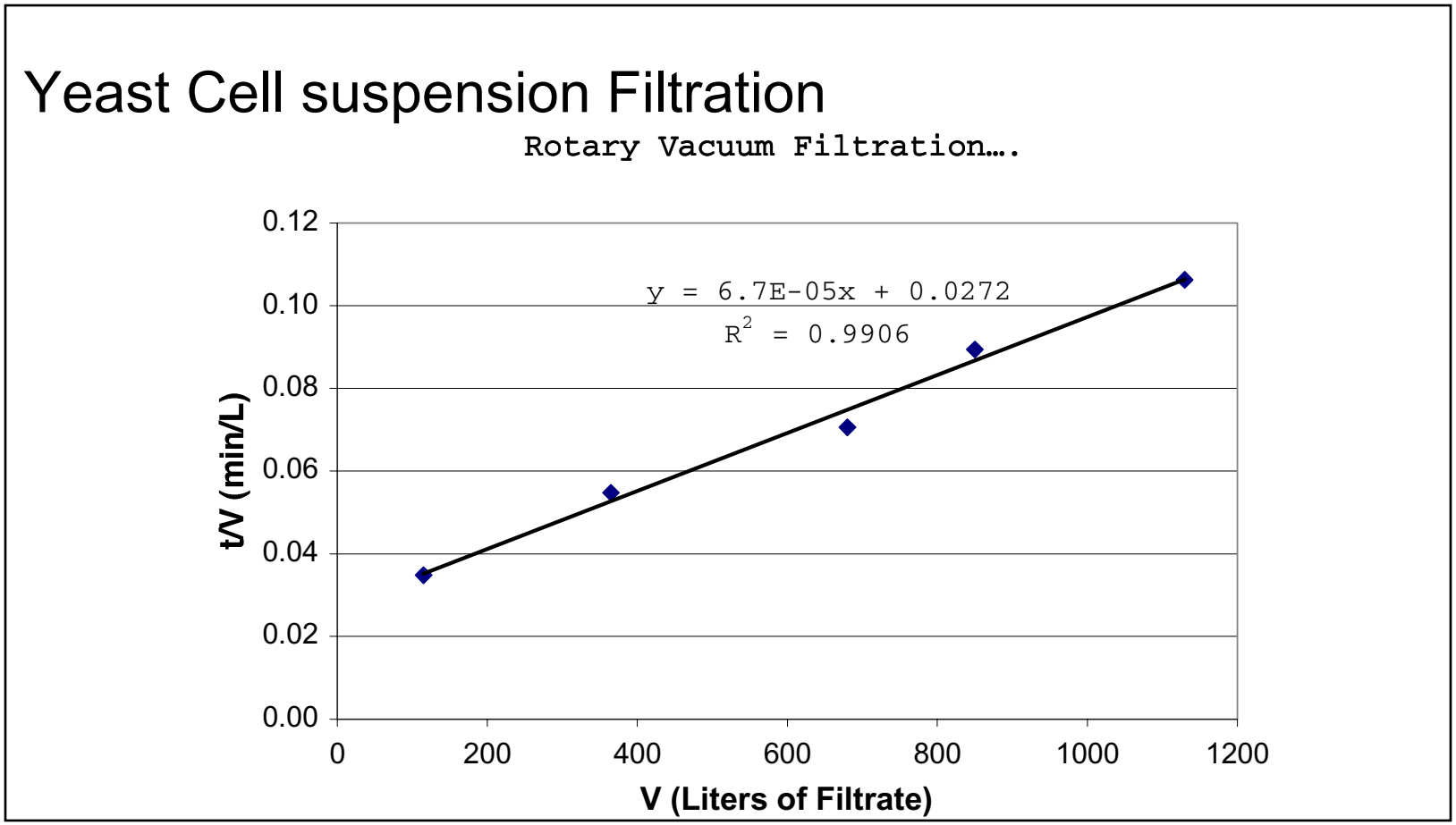
1. Removal of Insoluble Products
Rotary Vacuum Filtration; Example 11.1



1. Removal of Insoluble Products
Rotary Vacuum Filtration; Example 11.1

$$\text{slope} = \frac{1}{K} = 6.7x10^{-5}\ (\text{min/L}^2)$$

$$K = \frac{1}{6.7x10^{-5}} = 1.5x10^{4}\ (\text{L}^2/\text{min}) = \left(\frac{2\,A^2}{\alpha\,C\,\mu}\right)\Delta P\,g_c$$

$$\alpha = \left(\frac{2\,A^2}{K\,C\,\mu}\right)\Delta P\,g_c$$

* not 9.8 $kg_m/(kg_f\ s^2)$ as in the book

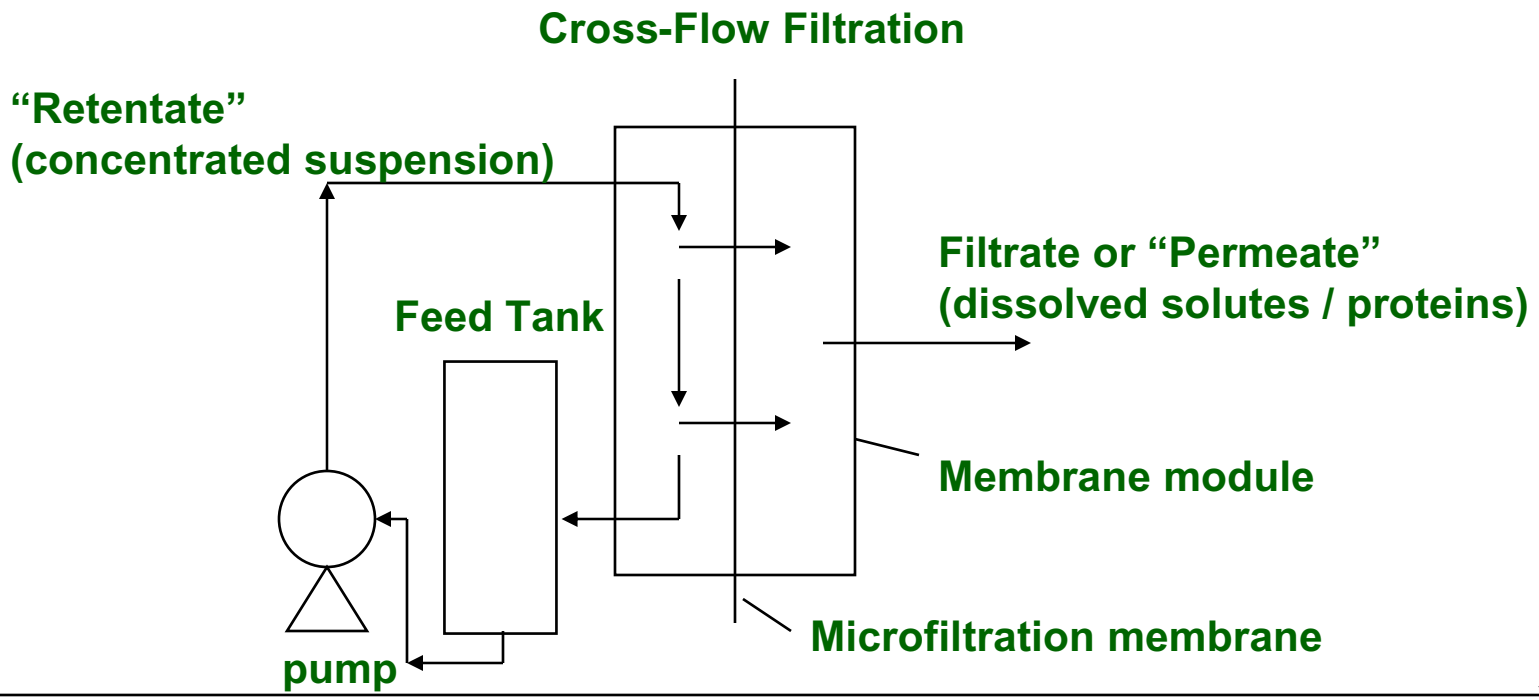
$$= \frac{2(.28\,\text{m}^2)^2\left(2.3x10^{-4}\,\frac{\text{N}}{\text{m}^2}\right)\left(1\,\frac{\text{kg m}}{\text{s}^2 \bullet \text{N}}\right)}{\left(1.5x10^{4}\,\frac{\text{L}^2}{\text{min}}\right)\left(\frac{10^{-3}\,\text{m}^3}{\text{L}}\right)^2\left(19.2\,\frac{\text{kg}}{\text{m}^3}\right)\left(2.9x10^{3}\,\frac{\text{kg}}{\text{m} \bullet \text{s}}\right)\left(\frac{1\,\text{min}}{60\,\text{s}}\right)}$$

* 1,920 as in the book

$$= 2.59\,\frac{\text{m}}{\text{kg}}$$

1. Removal of Insoluble Products Microfiltration/Ultrafiltration

- for particle size range ~ 10^{-9} to 10^{-5} m = d_p
- purpose → to concentrate a cell suspension
  → to recover dissolved solutes / proteins



1. Removal of Insoluble Products
Microfiltration for Removal of Cells

- Mass balance model for separation of cells

(cells are retained in the feed tank)

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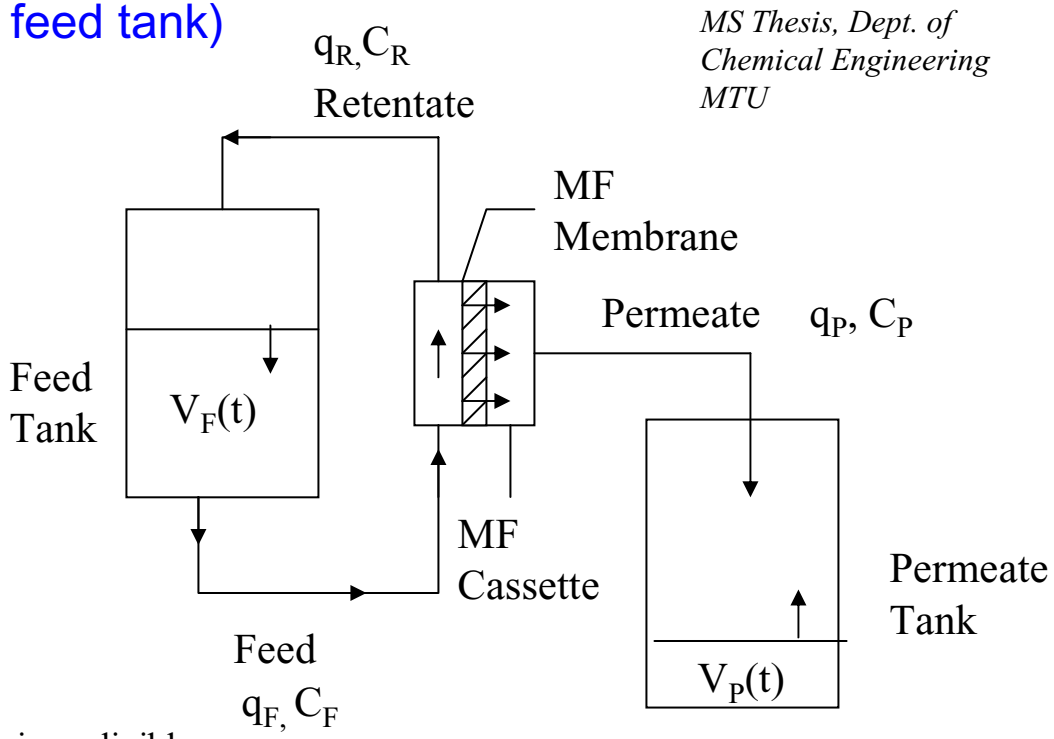

Mass Balance Assumptions

1. Feed tank is well mixed.
2. Permeate tank is well mixed.
3. Volume of fluid in MF cassette is negligible.
4. Densities of each stream are equal.

1. Removal of Insoluble Products Microfiltration for Removal of Cells

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Feed Tank

A total mass balance assuming constant stream densities leads to equation [1] for the change in feed tank volume, $V_F(t)$.

$$\frac{dV_F(t)}{dt} = q_R - q_F = -q_P \quad \text{.....[1]}$$

1. Removal of Insoluble Products Microfiltration for Removal of Cells

And similarly for entering and exit streams for the membrane cassette, where $q_F, q_R,$ and q_P are the volumetric flow rates of the feed, retentate, and permeate streams.

$$q_F = q_R + q_P \quad \text{.....[2]}$$

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1. Removal of Insoluble Products
Microfiltration for Removal of Cells

A cell mass balance on the feed tank results in equation [3], where $C_F, C_R,$ and C_P are the concentrations of the cells in the feed, retentate, and permeate streams.

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$$\frac{d}{dt}(C_F V_F(t)) = q_R C_R - q_F C_F \quad \text{.....[3]}$$

1. Removal of Insoluble Products Microfiltration for Removal of Cells

A cell mass balance on the cassette results in equation [4],

$$q_F C_F = q_R C_R + q_P C_P \quad \text{.....[4]}$$

For a perfectly retained cell: $C_P = 0$, and equation [4] becomes [5]

$$q_R C_R = q_F C_F \quad \text{.....[5]}$$

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1. Removal of Insoluble Products Microfiltration for Removal of Cells

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Substituting [5] into [3] (for a perfectly retained cell)

$$\frac{d}{dt}(C_F V_F(t)) = \frac{d}{dt} m_F = q_R C_R - q_F C_F$$

$$= q_F C_F - q_F C_F = 0$$

$\frac{d}{dt} m_F = 0$ where m_F is mass of cells in feed tank ($m_F = C_F V_F(t)$)….[7]

1. Removal of Insoluble Products
Microfiltration for Removal of Cells

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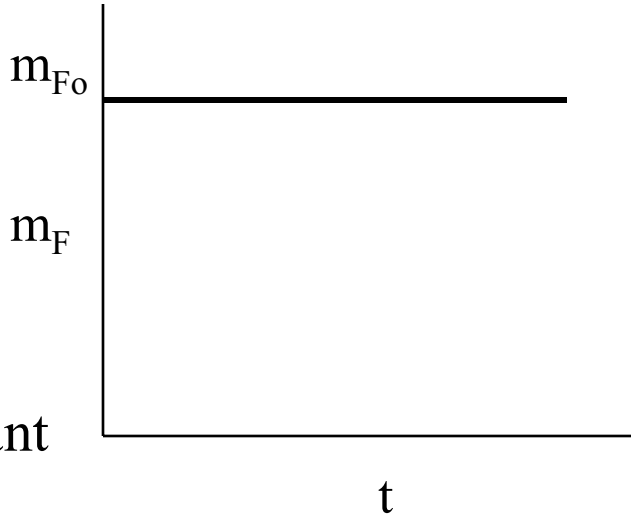

Integrating; $\int dm_F = \int 0 dt \Rightarrow m_F =$ Constant

At $t = 0, m_F = m_{F_0}$ (m_{F_0} is the initial mass of cells in the feed tank)

$\therefore$ $m_F = m_{F_0}$ for all time t "perfectly retained cell"……[8]

1. Removal of Insoluble Products
Microfiltration for Removal of Cells

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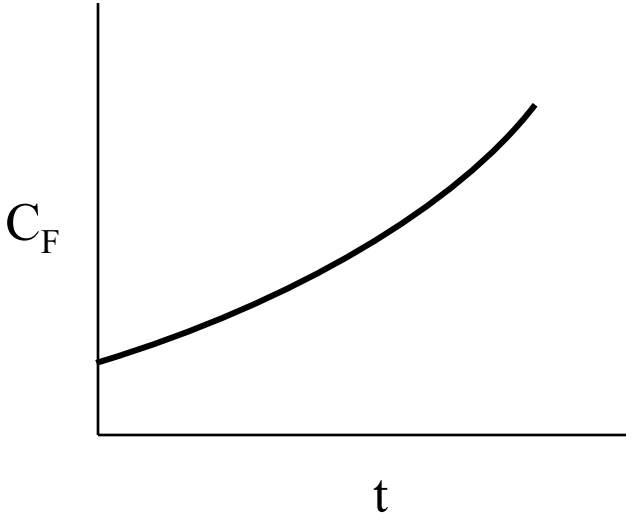
Cell Concentration, $C_F(t)$

$$\int d(C_F V_F(t)) = \int 0\, dt$$

$$C_F V_F(t) = \text{constant} = m_{Fo}$$

$$C_F = \frac{m_{Fo}}{V_F(t)} = \frac{m_{Fo}}{V_{Fo} - q_P t}$$



1. Removal of Insoluble Products
Microfiltration for Removal of Cells

Perfectly Permeating Cell (or Protein)

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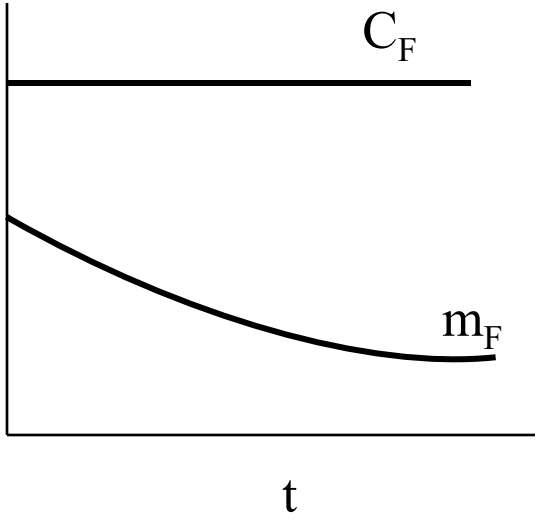
$$m_F = -q_P C_{F_0} t + \text{Constant} \quad \text{.....[11]}$$

At $t = 0, m_F = m_{F_0} \Rightarrow$ Constant $= m_{F_0}$

$$C_F(t) = \frac{m_F(t)}{V(t)} = \frac{m_{Fo} - q_P C_{Fo} t}{V_o - q_P t}$$

note that $C_{Fo} = \frac{m_{Fo}}{V_o}$

$$C_F(t) = \frac{m_{Fo} - q_P \frac{m_{Fo}}{V_o} t}{V_o - q_P t} = \frac{m_{Fo}(1 - \frac{q_P}{V_o} t)}{V_o(1 - \frac{q_P}{V_o} t)} = \frac{m_{Fo}}{V_o} = C_{Fo}$$



1. Removal of Insoluble Products Microfiltration for Removal of Cells

Partially Retained Cell (or Protein)

Some fraction (θ) of the cells (or protein) is of a size that is retained and (1-θ) permeates.

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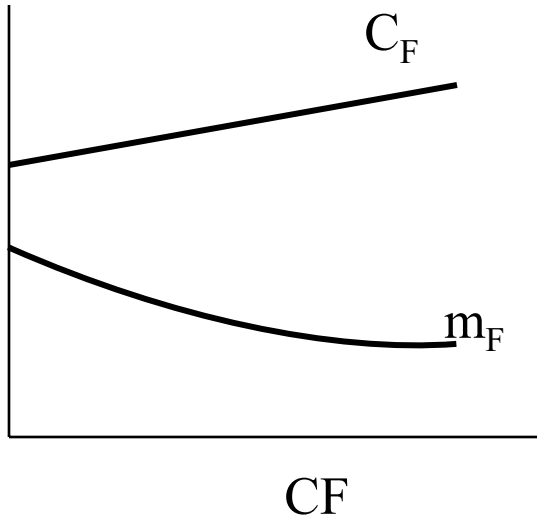
$$m_F = m_{Fo}(\theta + \frac{(1-\theta)}{CF})$$

where CF is Concentration Factor

$$CF = \frac{V_{Fo}}{V_F(t)} = \frac{V_{Fo}}{V_{Fo} - q_P t}$$

$$C_F = C_{Fo}(CF\,\theta + (1-\theta))$$



Microfiltration of Skim Milk to Separate Casein Protein (CP) from Whey Protein (WP)

Protein mass versus concentration factor, CF = V_{Fo}/V_F

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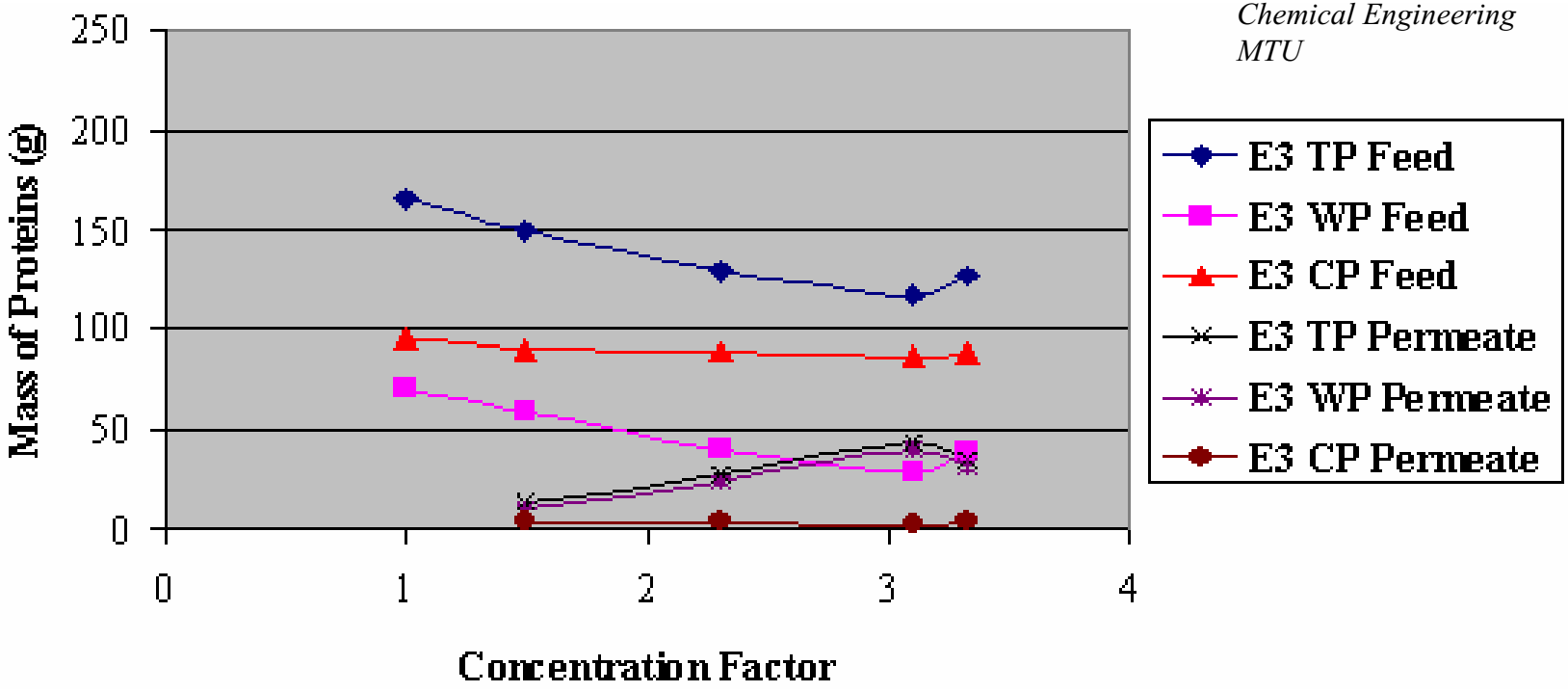

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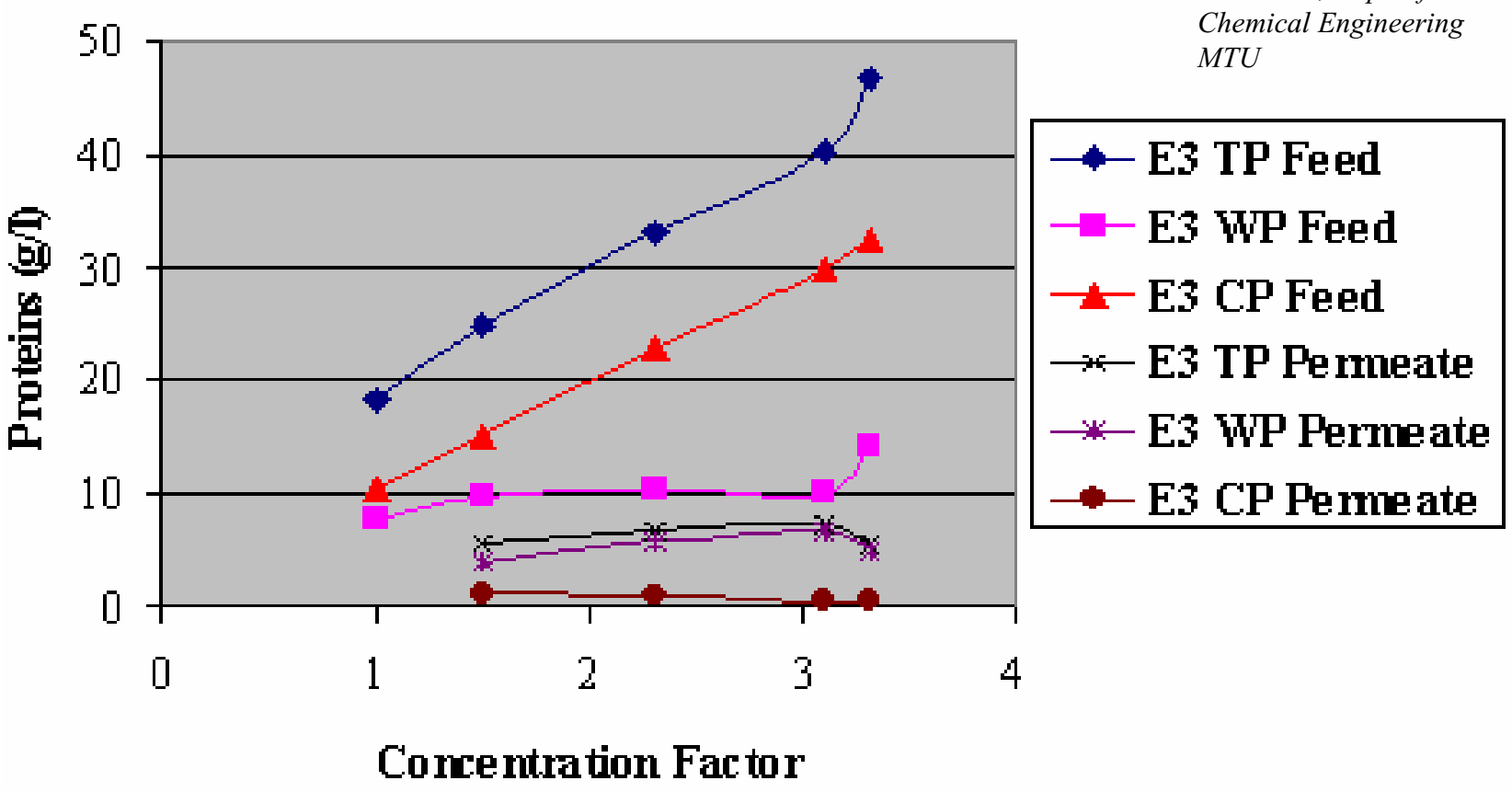
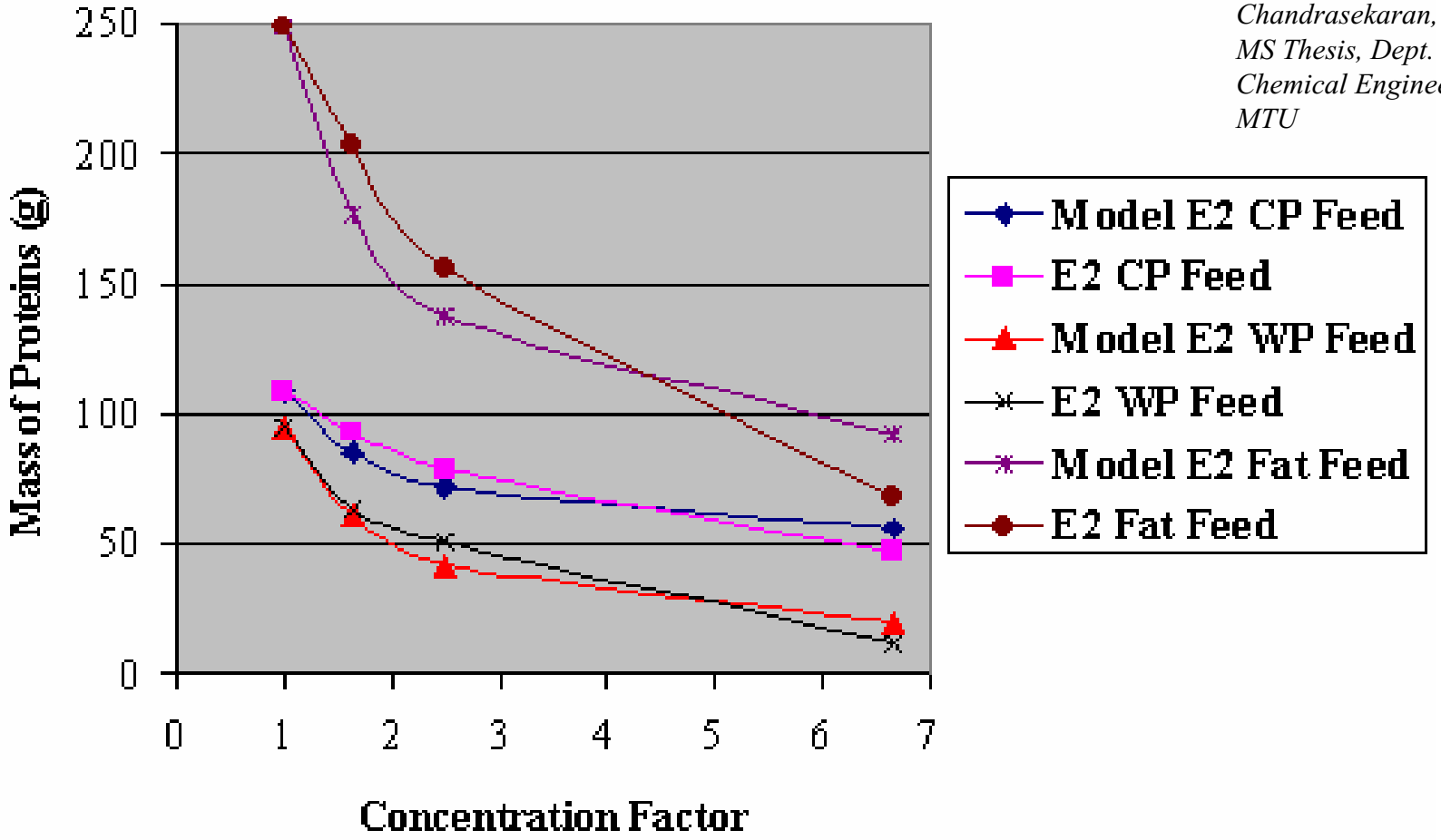


Microfiltration of Skim Milk to Separate Casein Protein (CP) from Whey Protein (WP)

Comparison of model with experimental data



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1. Removal of Insoluble Products Microfiltration/Ultrafiltration

Water (Permeate) Velocity Equation

$$J = K_P (\Delta P_M - \sigma \Delta \pi)$$

where

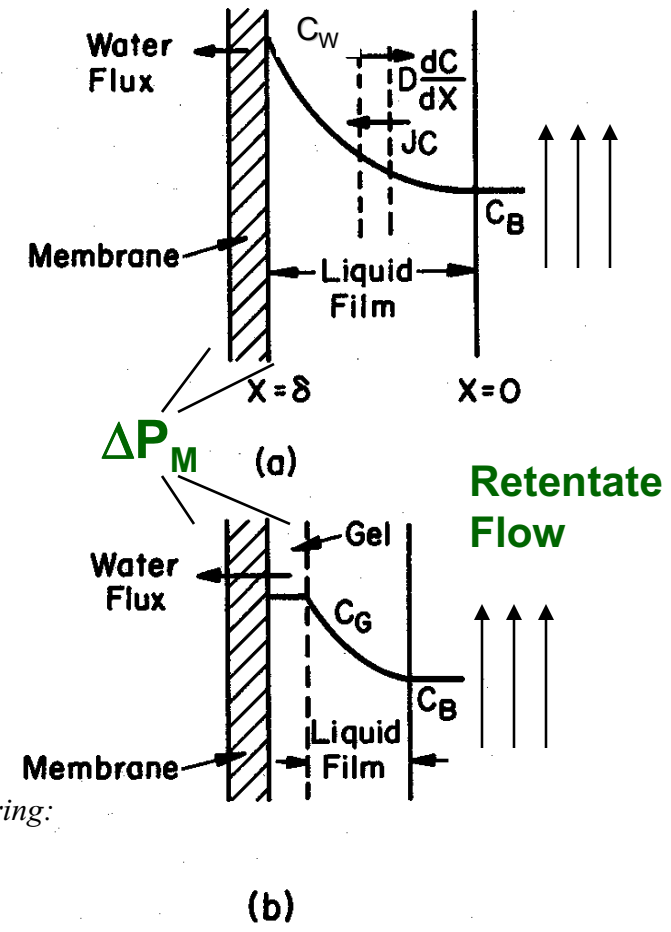
J = water (permeate) velocity

K_P = membrane permeability

ΔP_M = pressure drop across membrane

σ = "reflection coefficient"

$\Delta \pi$ = osmotic pressure (RTC_W)



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1. Removal of Insoluble Products Microfiltration/Ultrafiltration

Concentration Polarization - relating C_W to C_B

In the liquid film;

$$J = D\frac{dC}{dx}$$

$$x = \delta \qquad C = C_W$$

$$x = 0 \qquad C = C_B$$

integrating

$$J = \frac{D}{\delta}\ln\frac{C_W}{C_B}$$

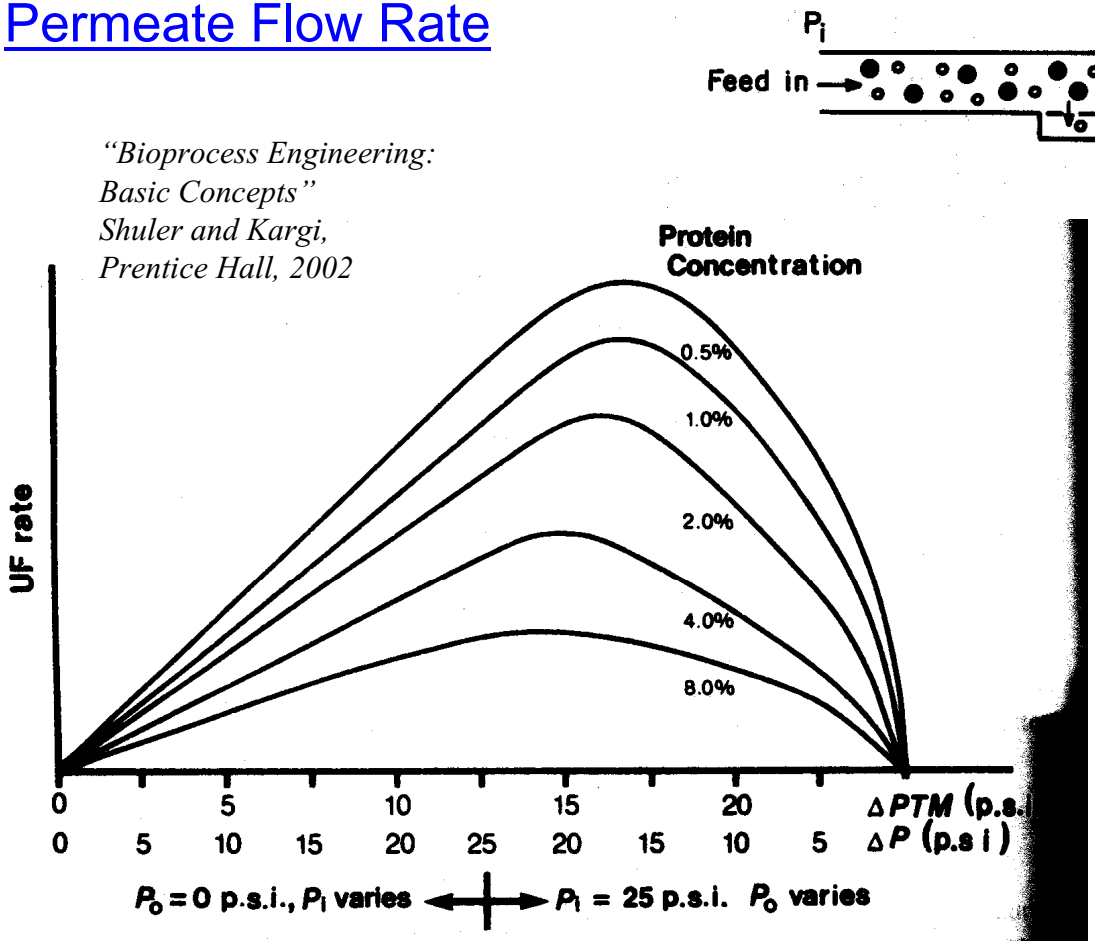
where D is the diffusivity of solute in the film

Gel Formation

When J and/or C_B are high enough, a gel layer will form at the membrane surface, causing an additional resistance (R_G) to solute flux, J.

1. Removal of Insoluble Products Microfiltration/Ultrafiltration

Permeate Flow Rate



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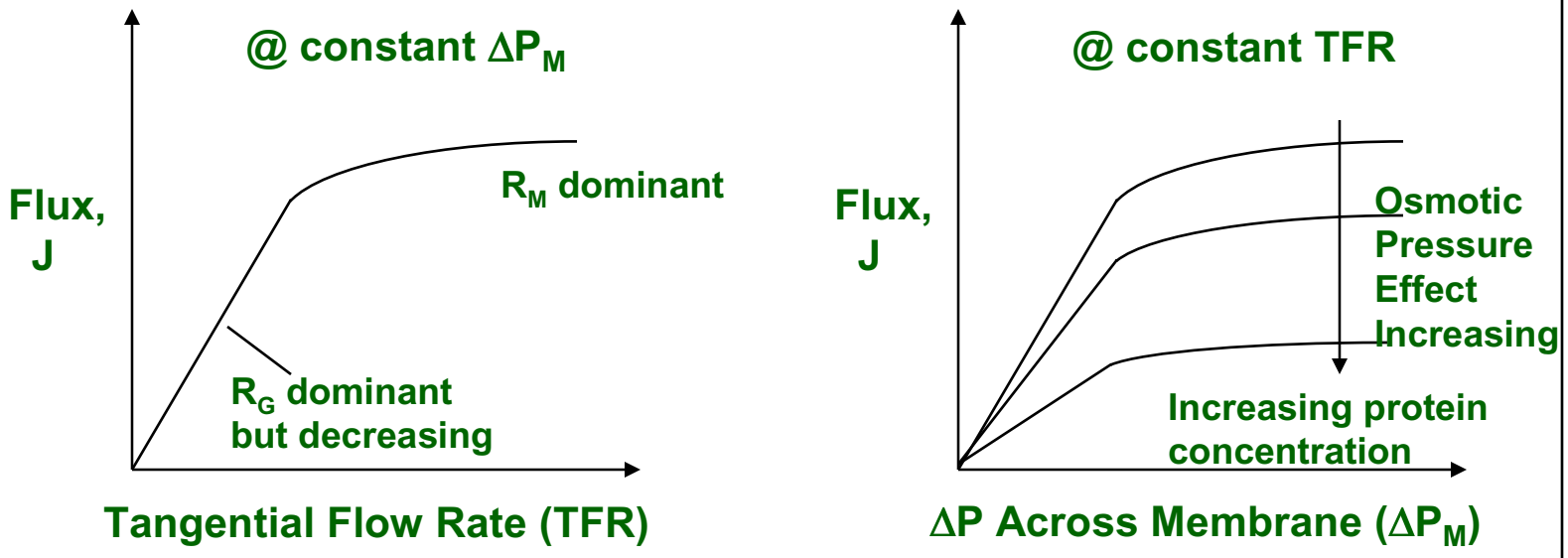
$$\Delta P_M = P_i - \frac{1}{2}(P_i - P_o)$$

$$J = \frac{\Delta P_M}{R_G + R_M}$$

Gel resistance
Membrane resistance

1. Removal of Insoluble Products Microfiltration/Ultrafiltration

Permeate Flow Rate



1. Removal of Insoluble Products Microfiltration/Ultrafiltration

Concentration Polarization - relating C_W to C_B

Example of Protein Ultrafiltration;

$$J = 1.3\text{x}10^{-3}\ \text{cm/sec}$$

$$D = 9.5\text{x}10^{-7}\ \text{cm}^2/\text{sec}\ \ \text{(protein diffusivity)}$$

$$\delta = 180\text{x}10^{-4}\ \text{cm}$$

$$J = \frac{D}{\delta}\ln\frac{C_W}{C_B} \quad \Rightarrow \quad 1.3\text{x}10^{-3}\ \text{cm/sec} = \frac{9.5\text{x}10^{-7}\ \text{cm}^2/\text{sec}}{180\text{x}10^{-4}\ \text{cm}}\ln\frac{C_W}{C_B}$$

$$\frac{C_W}{C_B} = 1.3$$

or C_W is 30% > than C_B

1. Removal of Insoluble Products
Microfiltration/Ultrafiltration

Microfiltration Design - time for filtration

$$\frac{dV}{dt} = -A\,J$$

V = volume of solution remaining to be filtered

A = membrane filter area

if we assume no concentration polarization, $C_W \approx C_B$

$$\frac{dV}{dt} = -A\,K_P\,(\Delta P - \sigma RTC_B)$$

for total reflection of solute, $\sigma = 1$ and $n = C_B V$

and is constant, where n is total solute mass (cells)

$$\frac{dV}{dt} = -A\,K_P\,\Delta P\left(1 - \frac{[RTn/\Delta P}{V}\right)$$

1. Removal of Insoluble Products
Microfiltration/Ultrafiltration

Microfiltration Design - time for filtration (cont.)

$$\frac{dV}{dt} = -A K_P \Delta P \left(1 - \frac{[RTn/\Delta P}{V}\right)$$

at $t = 0$ $\quad V = V_o$ (initial volume of solution)

integrating

$$t = \left[\frac{1}{A K_P \Delta P}\right]\left\{(V_o - V) + \left(\frac{R T n}{\Delta P}\right) \ln\left(\frac{V_o - RTn/\Delta P}{V - RTn/\Delta P}\right)\right\}$$

often $\frac{RTn}{\Delta P} << (V_o - V)$

$$t \approx \left[\frac{1}{A K_P \Delta P}\right](V_o - V)$$

Time to filter from V_o to V.

1. Removal of Insoluble Products
Microfiltration/Ultrafiltration

Microfiltration Design - Example, Cell Microfiltration

$V_o = 1000$ liters, $A = 10\ m^2$

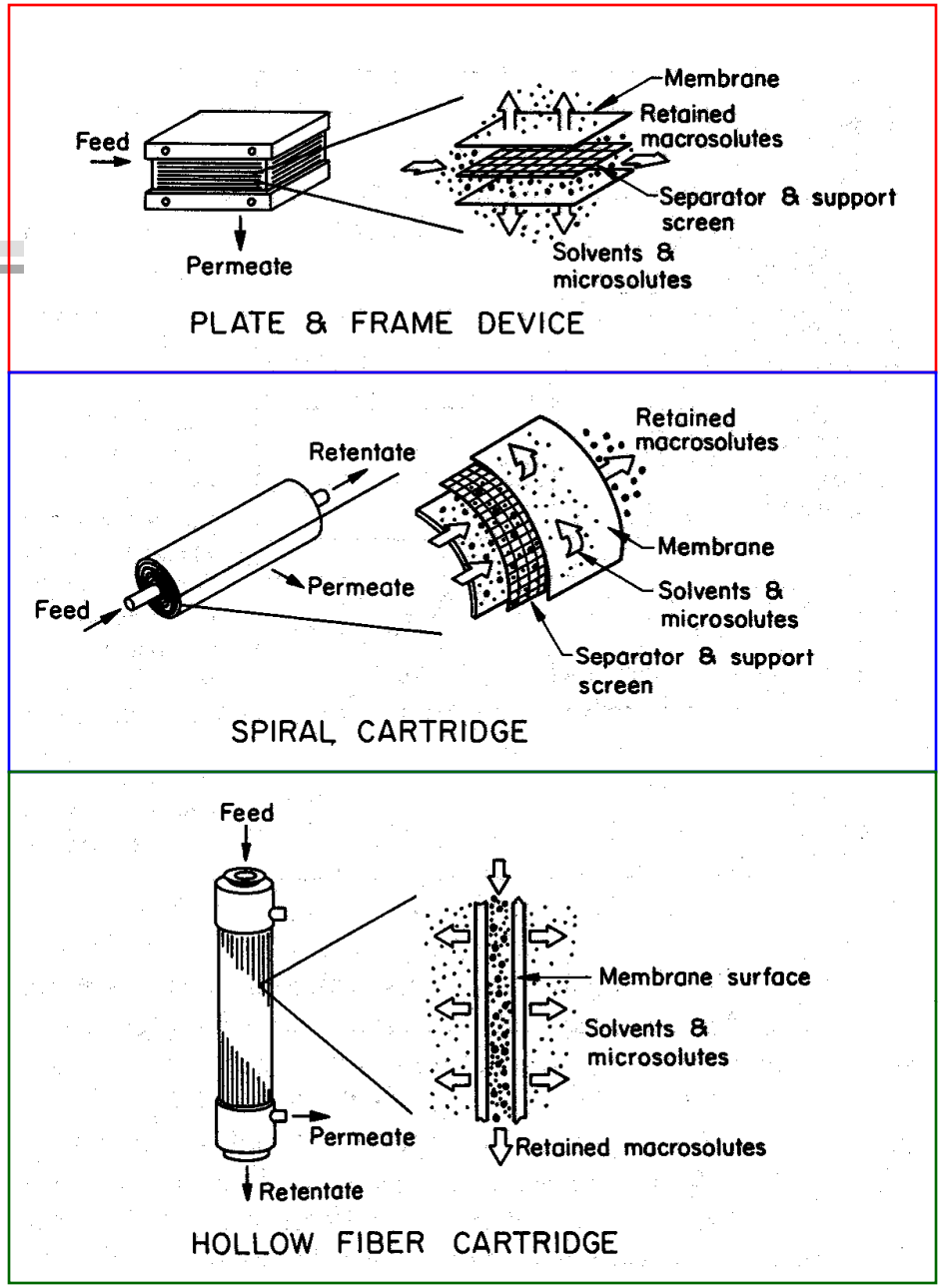
$X_o = 1$ g dcw / L concentration to X = 10 g dcw / L

$K_P \Delta P$ = initial water flux = 5.7×10^{-4} cm / sec

$$V = V_o \frac{X_o}{X} = 1000\ L \left(\frac{1\ g\ dcw / L}{10\ g\ dcw / L} \right) = 100\ L$$

$$t = \left[\frac{1}{(10\ m^2)(100\ cm^2 / m^2)\ (5.7 \times 10^{-4}\ cm / sec)} \right] (1000 - 100) L \left(\frac{10^3\ cm^3}{L} \right)$$

$$= 1.58 \times 10^4\ sec = 4.4\ hours$$



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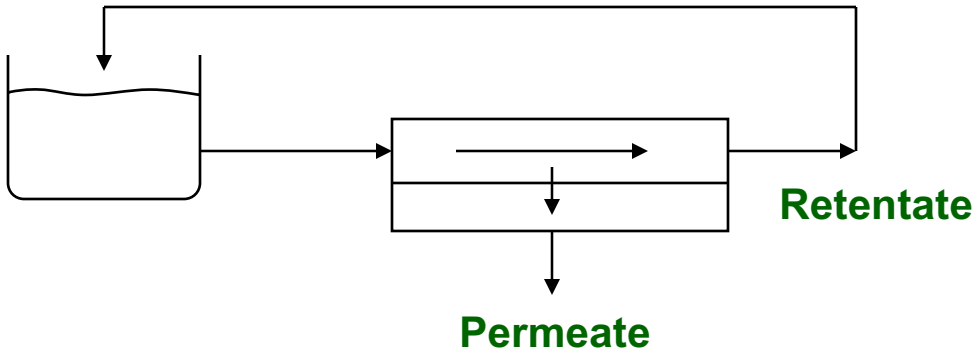
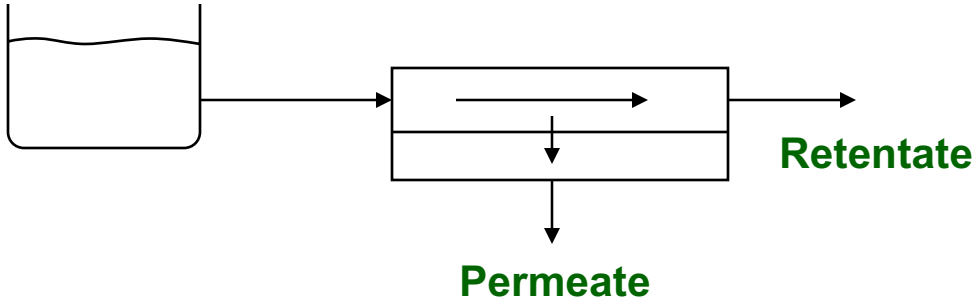
1. Removal of Insoluble Products Microfiltration/Ultrafiltration

Modes of Operation

1. Concentration



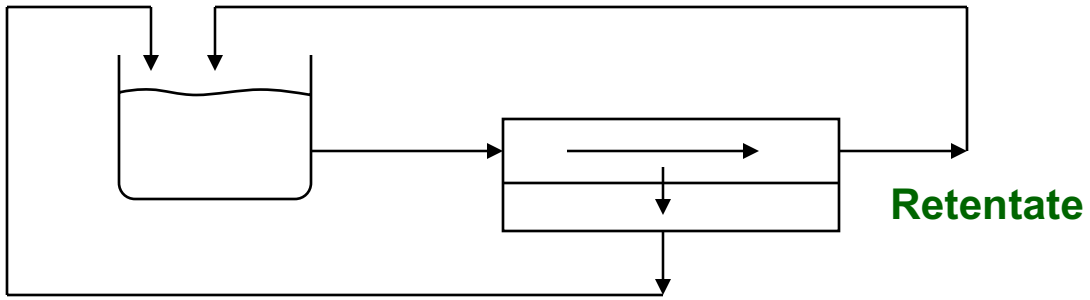
2. One-Pass



1. Removal of Insoluble Products Microfiltration/Ultrafiltration

Modes of Operation

3. Total Recycle Mode - membrane system characterization



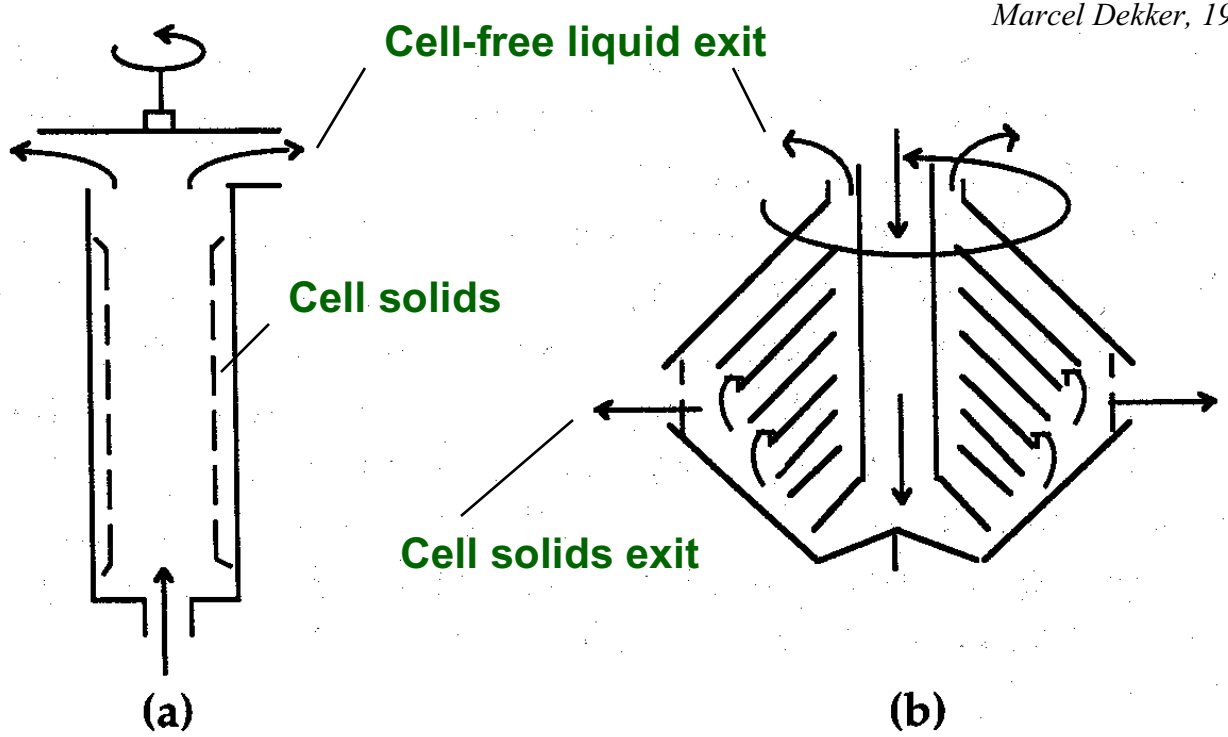
Post-Processing:

Microfiltration of cells is often followed by conventional filtration of retentate or centrifugation. Then, cell disruption for recovery of intracellular proteins occurs.

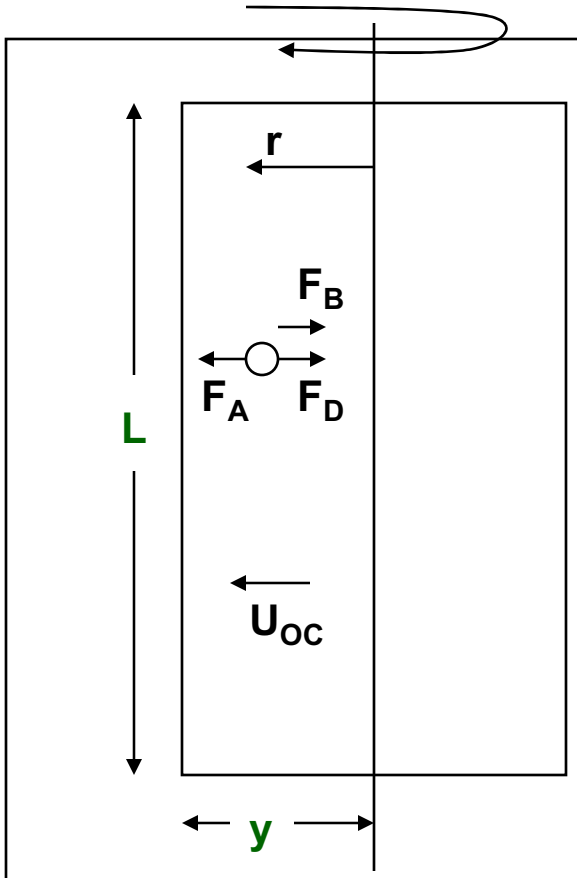
1. Removal of Insoluble Products
Centrifugation

Continuous Centrifuges

"Biochemical Engineering"
Blanch and Clark,
Marcel Dekker, 1997



1. Removal of Insoluble Products
Tubular Centrifugation



$$F_D = \text{drag force on cell} = 3\pi\mu D_P U_{OC} \frac{1}{g_c}$$

$$F_A = \text{centrifugal force on cell} = \frac{\pi}{6} D_P^3 \rho_P r\omega^2 \frac{1}{g_c}$$

$$F_B = \text{bouyancy force on cell} = \frac{\pi}{6} D_P^3 \rho_f r\omega^2 \frac{1}{g_c}$$

$$F_D = F_A - F_B$$

$$3\pi\mu D_P U_{OC} = \frac{\pi}{6} D_P^3 (\rho_P - \rho_f) r\omega^2$$

$$U_{OC} = \frac{r\omega^2 D_P^2 (\rho_P - \rho_f)}{18\mu}$$

1. Removal of Insoluble Products
Tubular Centrifugation

Design Equations

Radial Travel Time = Axial Travel Time

$$\frac{y}{U_{oc}} = \frac{V_c}{F_c}$$

V_c = centrifuge liquid volume; F_c = volumetric flow rate

Solving for F_c; $\boxed{F_c = 2\,U_o\,\Sigma}$

where $U_o = \dfrac{gD_P^2(\rho_P - \rho_f)}{18\mu}$ settling velocity under gravity

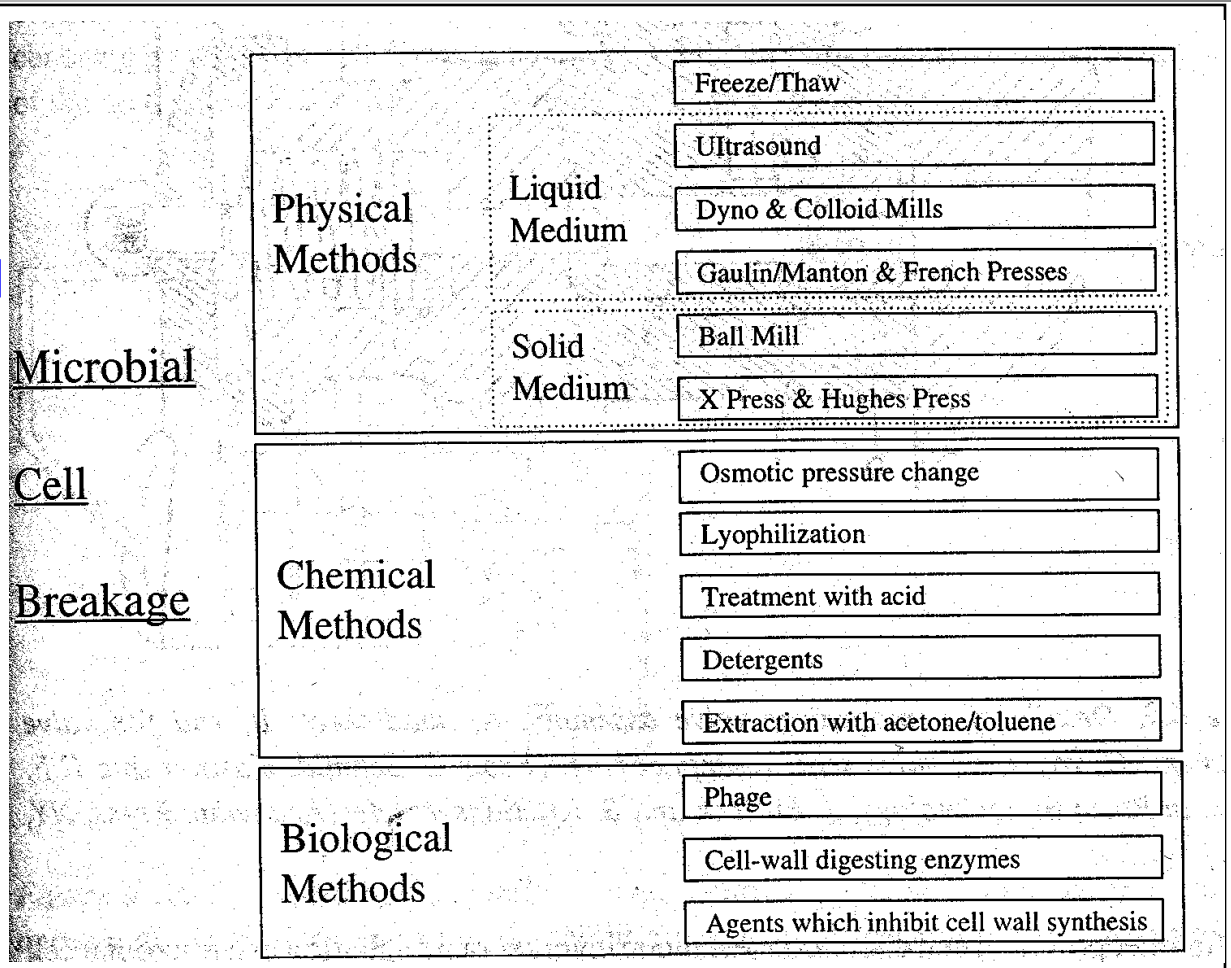
$$\Sigma = \frac{2\pi L\,\omega^2}{g}\left(\frac{3}{4}r_2^2 + \frac{1}{4}r_1^2\right)$$

F_c is proportional to L and r_2^2

1. Removal of Insoluble Products
Cell Disruption ; 11.3

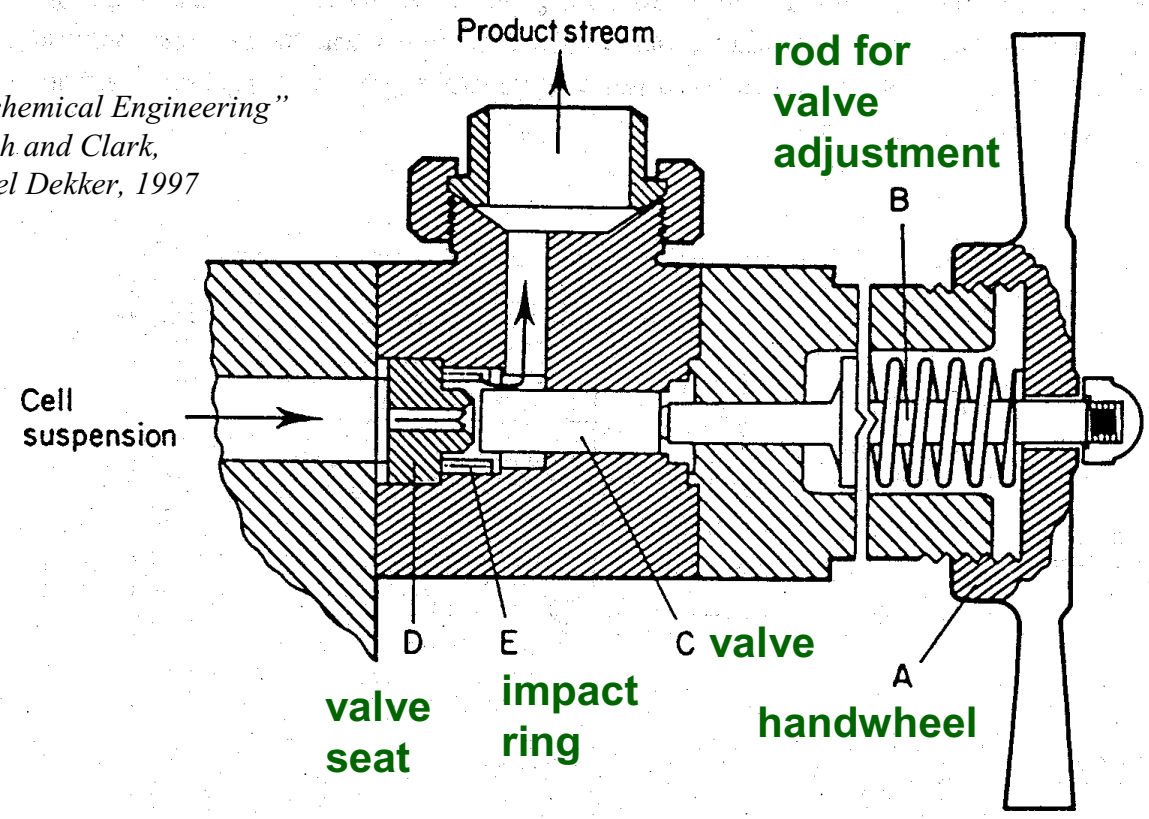
"Biochemical Engineering"
Blanch and Clark,
Marcel Dekker, 1997

If the desired product is intra-cellular, an effective method to break open the cell wall is needed in order to release the products.



1. Removal of Insoluble Products
Cell Disruption Equipment

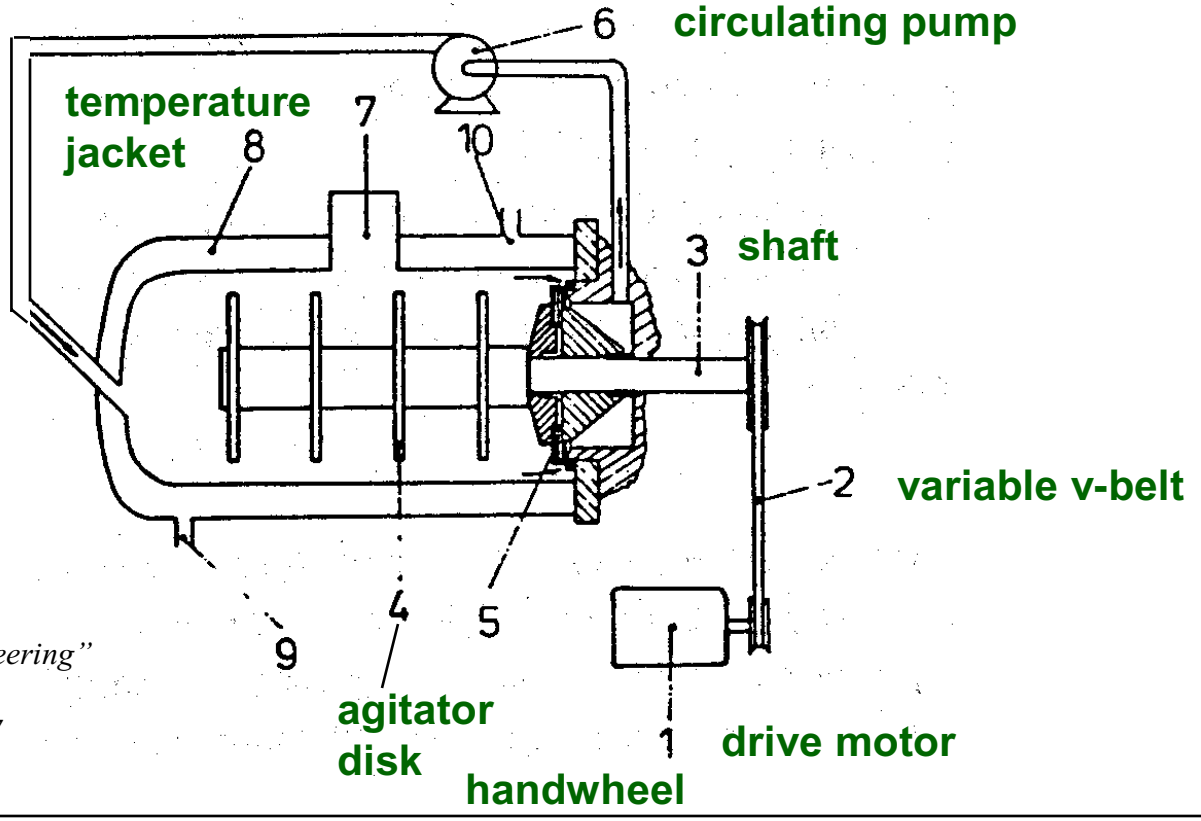
Exposure of cells to high liquid shear rates by passing cells through a restricted orifice under high pressure



"Biochemical Engineering"
Blanch and Clark,
Marcel Dekker, 1997

1. Removal of Insoluble Products
Cell Disruption Equipment

Rapid agitation of a microbial cell suspension with glass beads or similar abrasives



"Biochemical Engineering"
Blanch and Clark,
Marcel Dekker, 1997

1. Removal of Insoluble Products
Cell Disruption Equipment

"Biochemical Engineering"
Blanch and Clark,
Marcel Dekker, 1997

Problem 6.1 Blanch and Clark textbook

Protein release from yeast using disruption by an industrial homogenizer

Protein release depends upon the pressure, P, and number of recycle passes, N

Design Equation

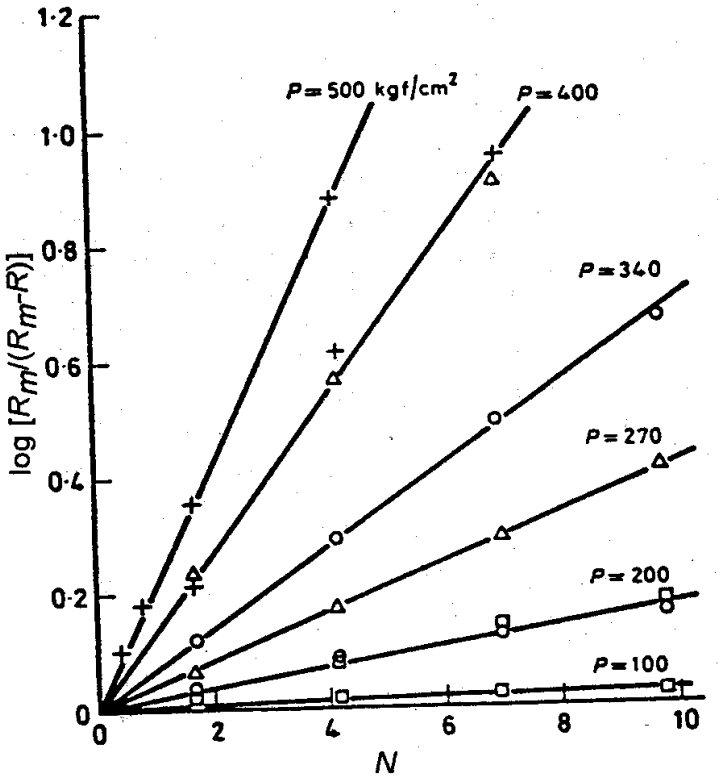
$$\log\left(\frac{R_m}{R_m - R}\right) = KNP^c$$

R_m = maximum protein conc. (mg / L)

R = protein conc. (mg / L)

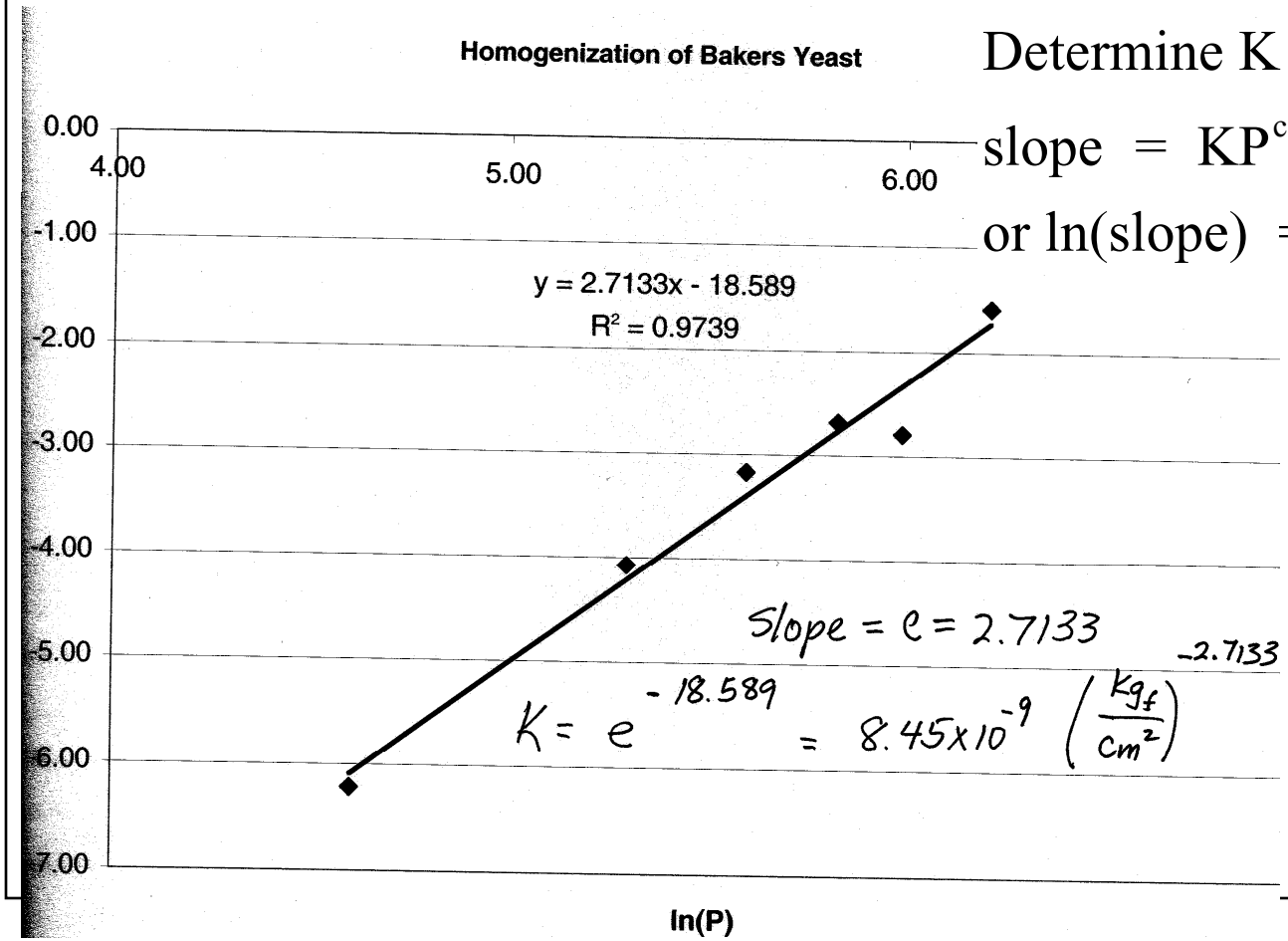
K = constant

C = constant



1. Removal of Insoluble Products
Cell Disruption Equipment

Problem 6.1 Blanch and Clark textbook (cont.)



Determine K and C

$$\text{slope} = KP^c$$

$$\text{or } \ln(\text{slope}) = \ln(K) + C \ln(P)$$

2. Primary Isolation/Concentration of Product: 11.4

Separation Objectives

- Remove water from fermentation broth
- Dilute solute (product) $\rightarrow$ more concentrated solute
- Often these steps concentrate chemically similar byproducts (other proteins / biomolecules)

Separation Methods

A. Extraction (liquid-liquid)
B. Adsorption
C. Precipitation

} not very selective for desired product

None the less, these methods are often applied prior to purification

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction

Liquid-liquid extraction is commonly used, especially in antibiotic fermentations to recover product from broth.

Features of liquid extractant

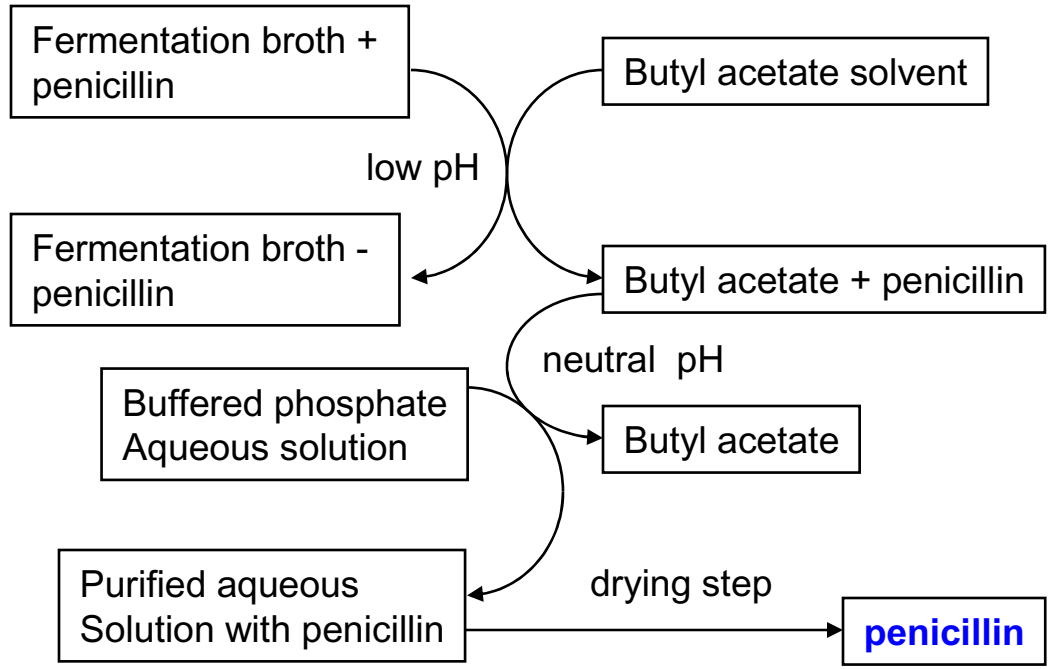
1. nontoxic
2. inexpensive
3. highly selective toward the product
4. immiscible with the fermentation broth

Other Applications

1. removal of inhibitory fermentation products (ethanol and acetone - butanol).

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction

Liquid-liquid extraction is commonly used, especially in antibiotic fermentations to recover products from fermentation broth



2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction - Equilibrium

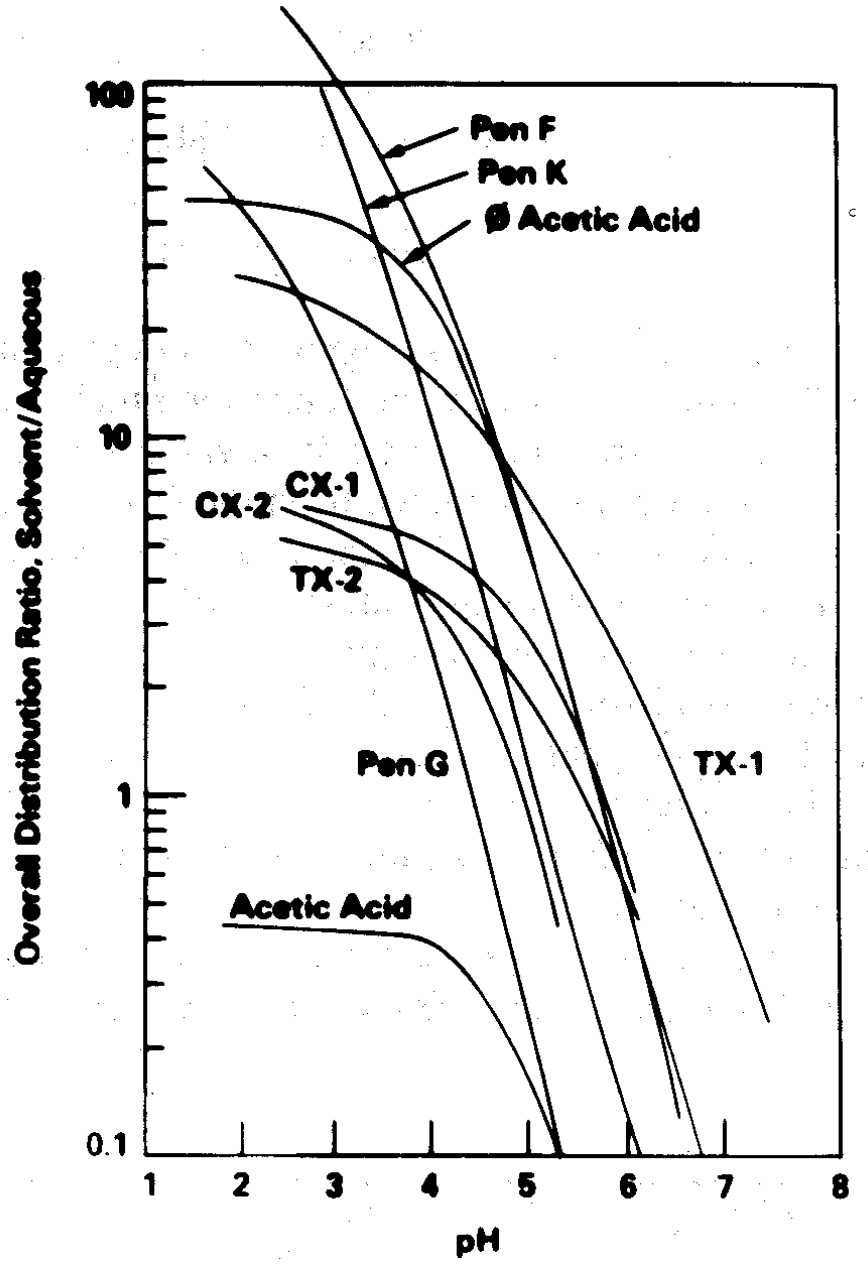
Liquid-liquid extraction takes advantage of solute equilibrium partitioning between the fermentation broth (heavy, H) phase and a light (L) extractant phase.

$$K_D = \frac{Y}{X}, \text{ where } K_D \text{ is a distribution coefficient}$$

Y is the concentration, mass or mole fraction of solute in the light phase

X is the concentration, mass or mole fraction of solute in the heavy phase

$$K_D = \frac{Y}{X}$$



Partitioning is a function of pH for many solutes

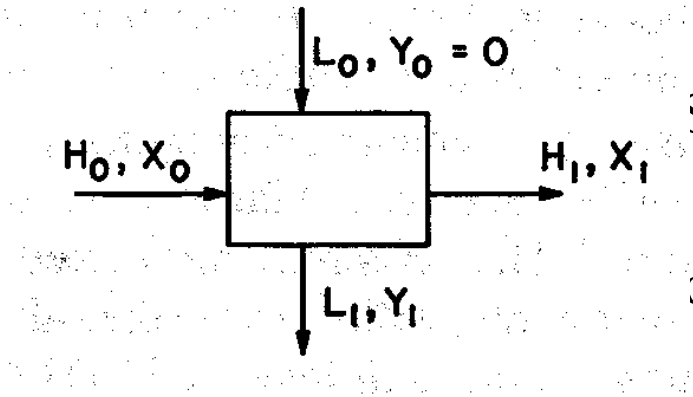
Solvent is Amylacetate

"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi,
Prentice Hall, 2002

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction

Mass balance on a single equilibrium stage

Assumptions: dilute solute and immiscible phases (negligible change in H and L) and constant K_D.



"Bioprocess Engineering: Basic Concepts" Shuler and Kargi, Prentice Hall, 2002

$$H(X_o - X_1) = LY_1 \quad \text{or} \quad X_1 = X_o - \frac{L}{H}Y_1$$

$$\text{Since } K_D = \frac{Y_1}{X_1}, \qquad X_1 = X_o - \frac{LK_D}{H}X_1$$

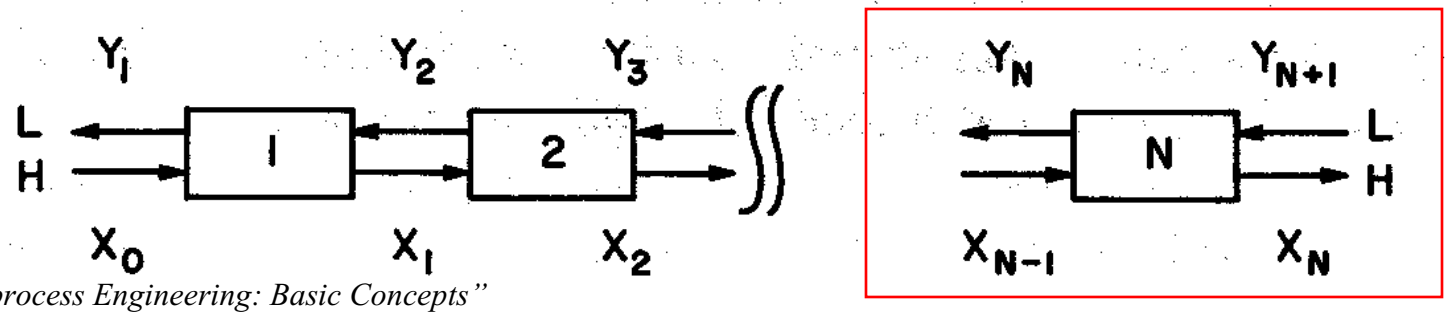
$$\text{or} \quad \boxed{\frac{X_1}{X_o} = \frac{1}{1+(LK_D/H)} = \frac{1}{1+E}}$$

$$\text{where} \quad E = \frac{LK_D}{H_o} \quad \text{is the extraction factor}$$

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction

Mass balance on a multiple equilibrium stages

Assumptions: dilute solute and immiscible phases (negligible change in H and L) and constant K_D.



"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi, Prentice Hall, 2002

$$\text{Stage N, } H(X_{N-1} - X_N) = L(Y_N - Y_{N+1}) \text{ or } X_{N-1} = X_N + \frac{L}{H} Y_N$$

$$\text{Since } K_D = \frac{Y_N}{X_N}, \quad X_{N-1} = X_N + \frac{LK_D}{H} X_N, \quad \text{or } \boxed{X_{N-1} = (1+E)X_N}$$

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction

Stage N-1, $H(X_{N-2} - X_{N-1}) = L(Y_{N-1} - Y_N)$ or

$$X_{N-2} = X_{N-1} + \frac{L}{H}(Y_{N-1} - Y_N), \quad \text{Since } K_D = \frac{Y_{N-1}}{X_{N-1}} = \frac{Y_N}{X_N},$$

$$X_{N-2} = X_{N-1} + \frac{LK_D}{H}(X_{N-1} - X_N), \quad \text{with } X_{N-1} = (1+E)X_N$$

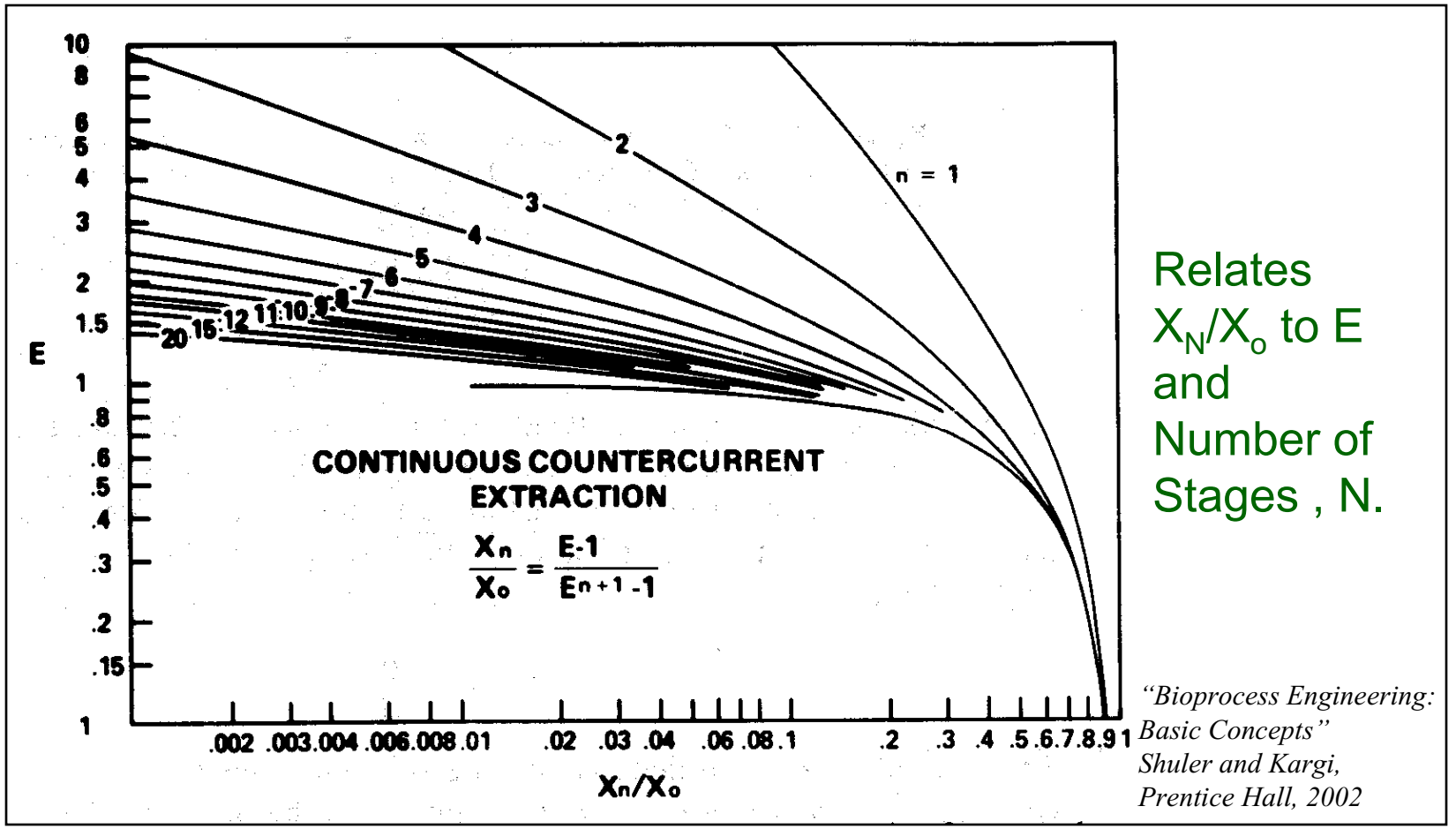
$$X_{N-2} = X_{N-1} + \frac{LK_D}{H}(X_{N-1} - X_N) = (1+E)X_{N-1} - EX_N$$

$$X_{N-2} = (1+E)(1+E)X_N - EX_N = (1+E)^2 X_N - EX_N$$

$$X_{N-2} = (1+E+E^2)X_N$$

All Stags, $\boxed{X_o = \left(\frac{E^{N+!} - 1}{E-1}\right)X_N}$ see Figure 11.9

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction - Figure 11.9



Relates X_N/X_o to E and Number of Stages , N.

"Bioprocess Engineering: Basic Concepts" Shuler and Kargi, Prentice Hall, 2002

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction - Figure 11.9

Example 11.2 Penicillin Extraction using Isoamylacetate

L = isoamylacetate flow rate = 10 L/min

H = aqueous broth flow rate = 100 L/min

$K_D = 50$, $X_o = 20$ g/L, $X_N = .1$ g/L

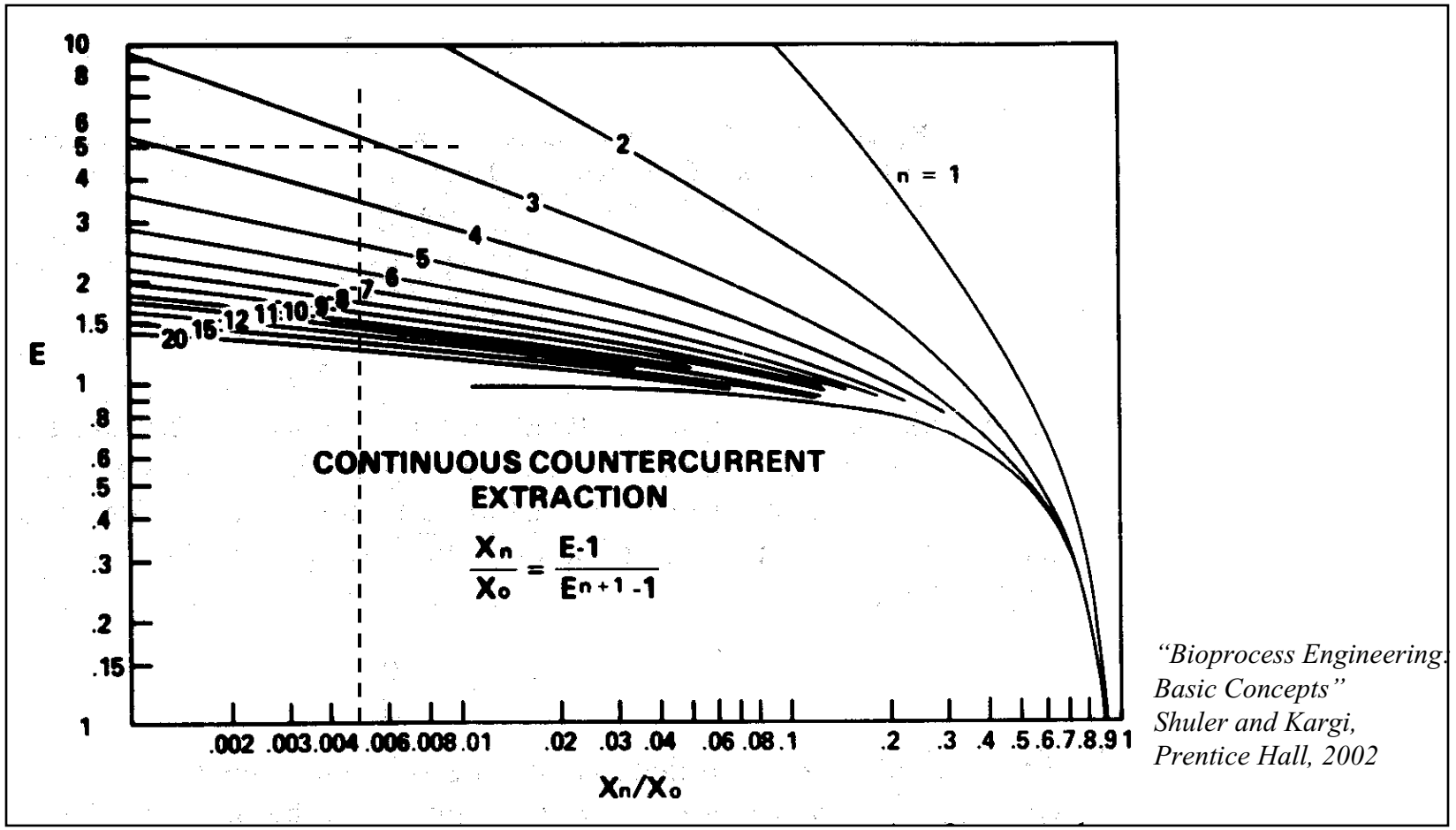
How many stages are required to achieve this separation?

Solution: $X_N / X_o = 0.1/20 = .005$

$E = LK_D/H = (10)(50)/100 = 5$

From Figure 11.9, we see that the required number is stages is between 3 and 4, call it 4 equilibrium stages.

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction - Figure 11.9



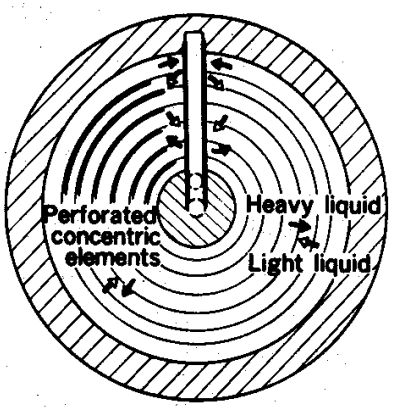
"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi,
Prentice Hall, 2002

2. Primary Isolation/Concentration of Product: Liquid-Liquid Extraction - Equipment

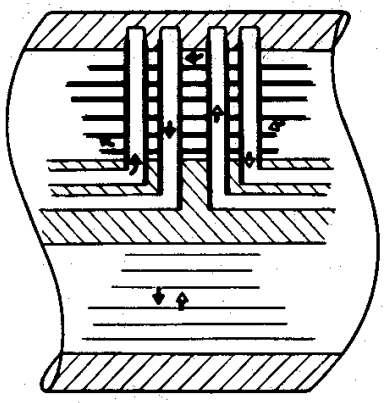
Podbielniak centrifugal extractor

The separation is very rapid, allowing for short residence times which benefits unstable products

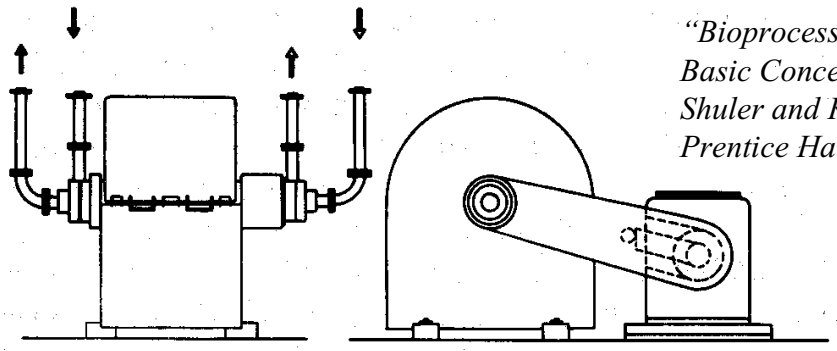
(especially pH-sensitive antibiotics.



(a) Sectional view of rotar.



(b) Sectional view (cont'd).



(c) Side view of Podbielniak.

"Bioprocess Engineering: Basic Concepts" Shuler and Kargi, Prentice Hall, 2002

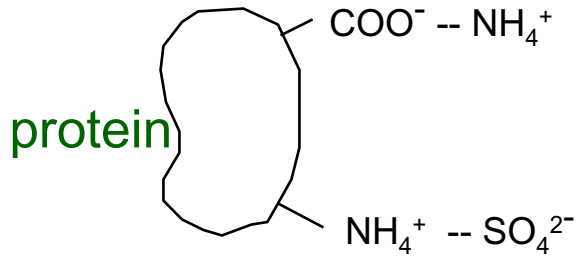
2. Primary Isolation/Concentration of Product: Precipitation

A very common first step after cell disruption for recovery of intracellular proteins.

Water-protein interactions are key to understanding protein precipitation / solubility in water.

Salting-Out

addition of $(NH_4)_2SO_4$ or Na_2SO_4 up to high concentrations $\rightarrow$ 1 to 3 Molar!



salts exclude water from the surface leading to protein-protein interactions and precipitation

2. Primary Isolation/Concentration of Product: Precipitation

Protein solubility is a function of ionic strength (salt concentration).

$$\log\left(\frac{S}{S_o}\right) = -K_S' I$$

S = protein solubility (g/L)

S_o = protein solubility at 0 ionic sstrength, (g/L)

K_S' = a salting out constant (moles/L)

(a function of pH and temperature)

$$I = \text{ionic stsrength} = \frac{1}{2}\sum C_i Z_i^2 \text{ (mole/L)}$$

C_i = molar concentration of salt ion (mole/L)

Z_i = charge on salt ion

2. Primary Isolation/Concentration of Product: Precipitation

Organic Solvent Addition

can also reduce protein-water interactions and promote protein-protein interactions leading to precipitation.

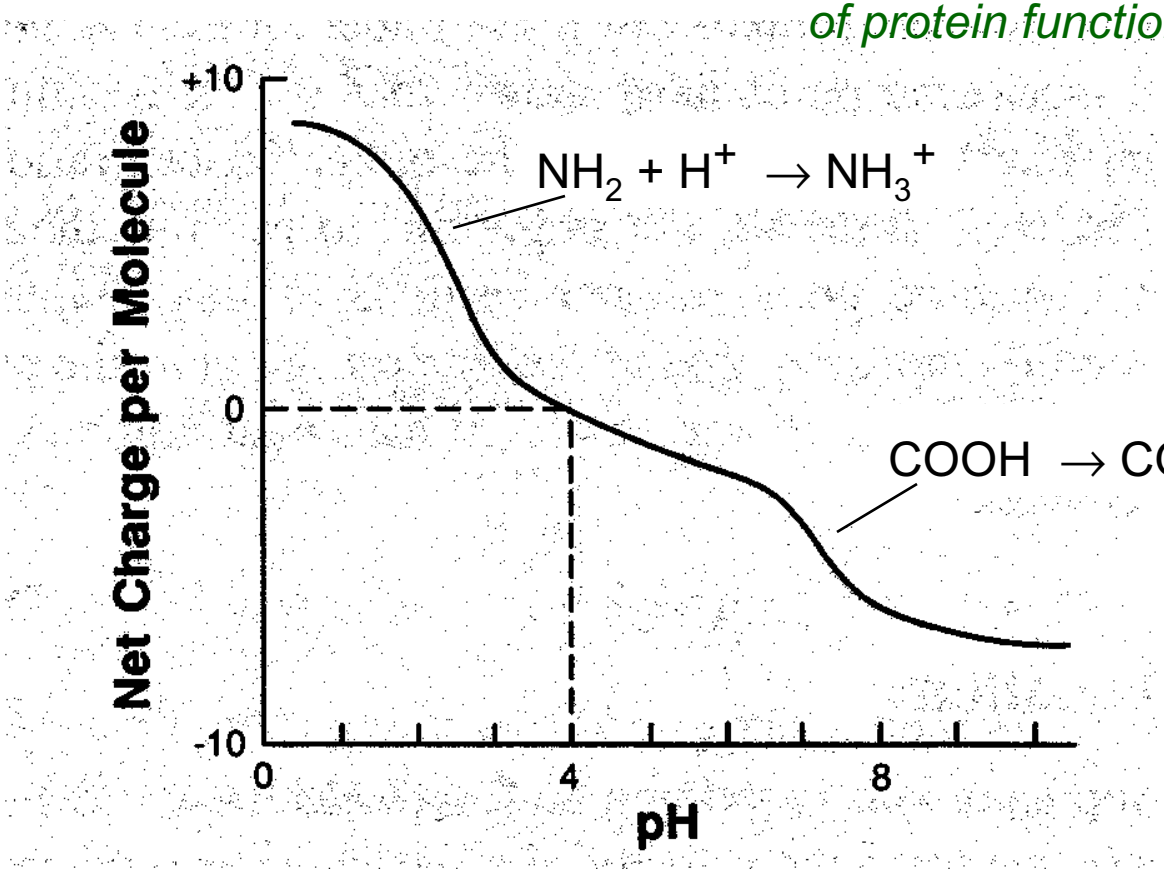
Isoelectric Precipitation

at the pH of the isoelectric point, a protein is uncharged, reducing protein-water interactions which leads to precipitation. Warning: extremes in pH may denature the protein product.

2. Primary Isolation/Concentration of Product: Isoelectric Precipitation

Belter, Cussler & Hu, 1988

Effects of pH on the charge of protein functional groups



3. Product Purification /Contaminant Removal:

Contaminants often remain with product after primary isolation.

Chromatography: is the most important separation method for biochemical products.

Basic Concepts:

1. Separation is based on differential affinities of solutes toward a solid adsorbent material.

3. Product Purification /Contaminant Removal: (cont.)

2. Different kinds of affinity

* → **electric charge ⋯ ion exchange chromatography**
  → **van der Waals force ⋯ adsorption chromatography**
  → **solubility in liquid ⋯ liquid-liquid partitioning chromatog.**
  → **solute size/diffusion ⋯ gel filtration chromatography**

* → **receptor - ligand ⋯ affinity chromatography**
  → **hydrophobic interactions ⋯ hydrophobic chromatography**

* **most common usage**

3. Product Purification /Contaminant Removal: Adsorption - 11.4.4

Definition: the removal of selected chemicals from a mobile fluid phase into an immobile solid phase.

Adsorbents: solid materials to which the chemicals (solutes, adsorbates) adhere. These are the immobile phase.

Examples:
- **activated carbon**
- **ion exchange resins**
- **alumina**
- **silica gel**
- **other gels: dextran or agarose**

3. Product Purification /Contaminant Removal: Adsorption - 11.4.4 (cont.)

"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi,
Prentice Hall, 2002

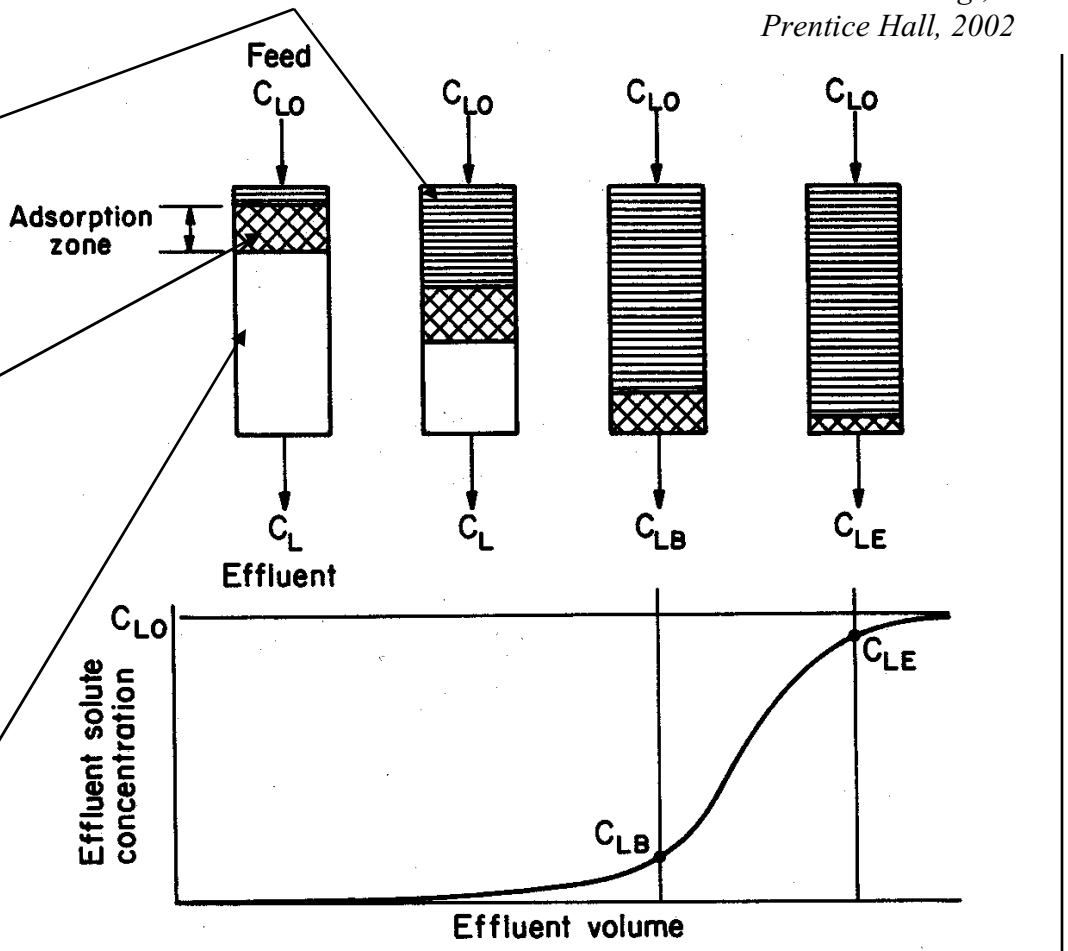
Fixed-Bed Adsorptior

3 Zones

Saturated zone, solute is present in both fluid and solid at maximum concentration.

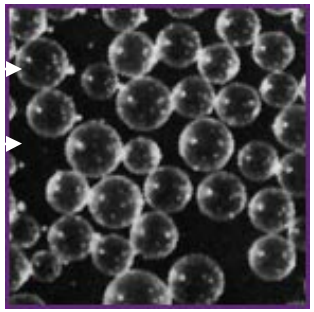
Adsorption zone, concentrations of solute in fluid & solid are in transition.

Virgin zone, concentrations near zero.



3. Product Purification /Contaminant Removal: Adsorption Equilibrium

Freundlich Isotherm: an isotherm describes the partitioning of a solute between the solid and liquid phases at equilibrium.



Ion exchange resin
Graver Technologies
www.gravertech.com

$$C_S^* = K_F \, C_L^{*(1/n)}$$

C_S^* = equilibrium conc. of solute on adsorbent $\left(\frac{\text{mass solute}}{\text{mass dry adsorbent}}\right)$

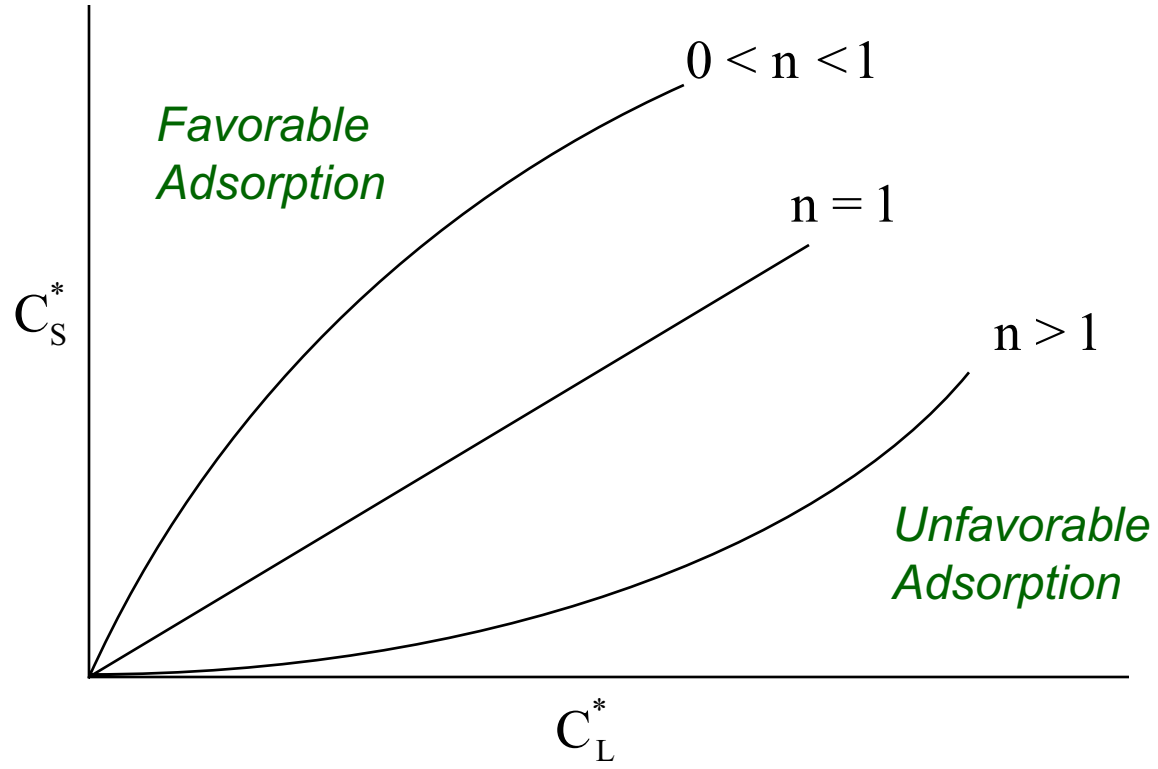
C_L^* = equilibrium conc. of solute in fluid $\left(\frac{\text{mass solute}}{\text{volume of fluid}}\right)$

K_F = equilibrium constant (units depent on exponent)

n = a constant

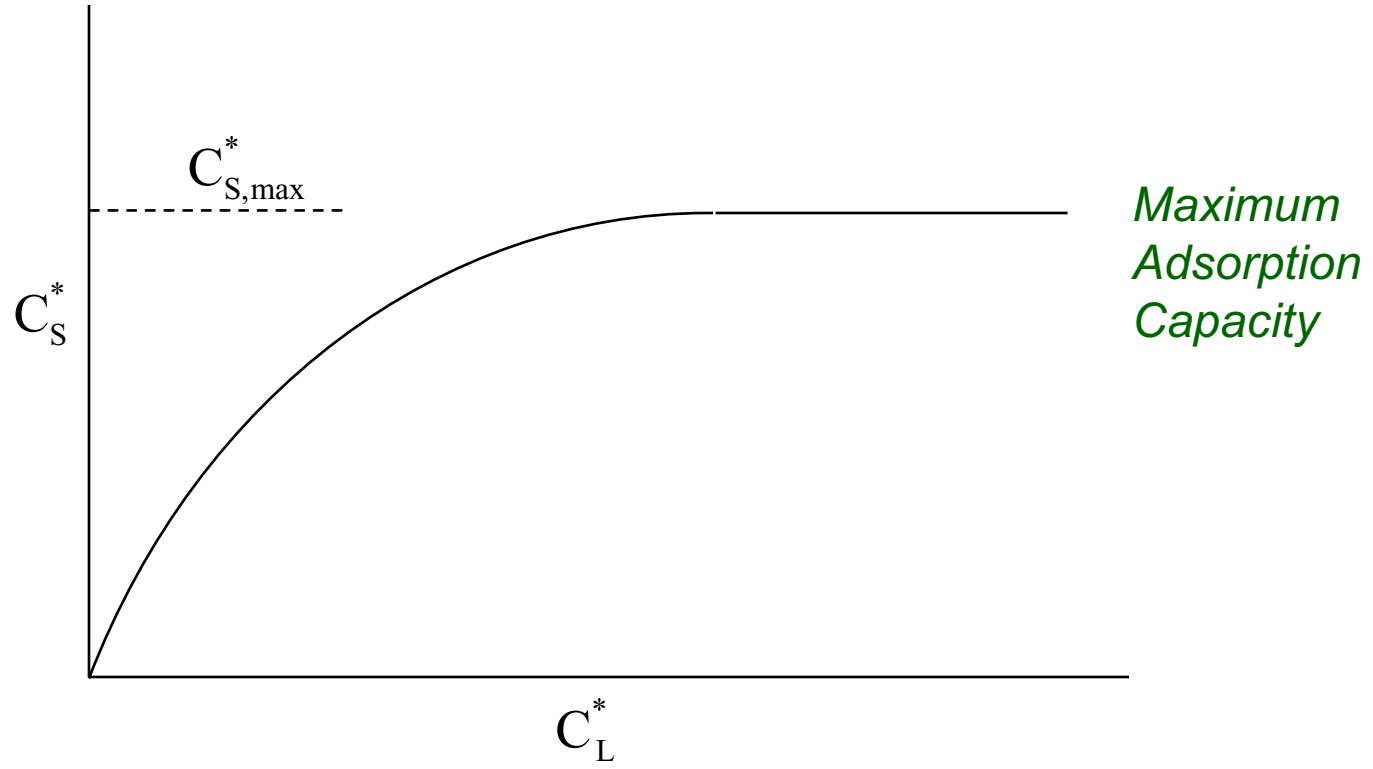
3. Product Purification /Contaminant Removal: Adsorption Equilibrium- Freundlich Isotherm

Freundlich Isotherm:



3. Product Purification /Contaminant Removal: Adsorption Equilibrium- Langmuir Isotherm

Langmuir Isotherm: $$C_S^* = \frac{C_{S,max}^* \, C_L^*}{K_L + C_L^*}$$



3. Product Purification /Contaminant Removal: Adsorption Equilibrium - Adsorbent Capacity

Example Problem:

Calculate the capacity of ion exchange resin to adsorb protein given that:

- m = mass of dry resin in a column = 1 kg
- ε = porosity of the fixed-bed = 0.40 cm^3 fluid/cm^3 bed volume
- ρ_r = resin density = 1.2 g dry resin/cm^3 resin
- n = 1 in the Freundlich Isotherm
- per unit bed volume, there is 100 times more protein adsorbed as there is in the fluid at equilibrium.

C^*_L = 1 mg protein/cm^3 fluid at equilibrium

3. Product Purification /Contaminant Removal: Adsorption Equilibrium - Adsorbent Capacity

Problem Solution:

1. First, calculate K_F in the Freundlich Isotherm.

$C_S^* = K_F C_L^*$ is the Freundlich isotherm for n = 1

Basis of $1\,\text{cm}^3$ bed volume $\Rightarrow$ perform a solute mass balance

"mass absorbed to resin in $1\,\text{cm}^3$ bed volume =

100 times the mass of protein in the fluid in the $1\,\text{cm}^3$ bed volume"

$$C_S^* \rho_r (1-\varepsilon)(1\,\text{cm}^3) = 100\, C_L^* \varepsilon$$

$$\text{or } C_S^* = \frac{100 C_L^* \varepsilon}{\rho_r (1-\varepsilon)} = K_F C_L^* \quad \Rightarrow \quad K_F = \frac{100\varepsilon}{\rho_r (1-\varepsilon)}$$

$$K_F = \frac{100(0.4)}{(1.2)(1-0.4)} = 55.6 \frac{\text{cm}^3 \text{ fluid}}{\text{g dry resin}}$$

3. Product Purification /Contaminant Removal: Adsorption Equilibrium - Adsorbent Capacity

Problem Solution:

2. Use the Freundlich Isotherm plus m= 1 kg resin to calculate capacity.

$$\text{Capacity} = m\,C_S^* = m\,K_F\,C_L^*$$

$$= (1{,}000 \text{ g dry resin})\left(55.6\ \frac{\text{cm}^3 \text{ fluid}}{\text{g dry resin}}\right)\left(1\ \frac{\text{mg protein}}{\text{cm}^3 \text{ fluid}}\right)$$

$$= 55{,}555.6 \text{ mg Protein}$$

For each kg dry resin

3. Product Purification /Contaminant Removal: Adsorption Equilibrium - Batch Adsorption

Example Problem 2: Batch Adsorption

An aqueous solution of protein (10 mg/cm^3) of volume 1000 cm^3 is contacted with 10 g of the resin (from the prior example problem). What is the concentration remaining in the aqueous phase after equilibrium is achieved?

- m = mass of dry resin in a column = 10 g
- n = 1 in the Freundlich Isotherm
- V = volume of aqueous solution = 1,000 cm^3.

K_F = 55.6 cm^3 solution/g dry resin

Mass Balance on Protein

$$C_S^* \, m + C_L^* \, V = \left(10 \frac{\text{mg protein}}{\text{cm}^3}\right) V$$

3. Product Purification /Contaminant Removal: Adsorption Equilibrium - Batch Adsorption

Example Problem 2: Batch Adsorption (cont.)

Equilibrium

$$C_S^* = K_F C_L^{*(1/n)} = K_F C_L^* \text{ for } n = 1$$

Mass Balance Equation becomes:

$$K_F C_L^* (100 \text{ g resin}) + C_L^* (1{,}000 \text{ cm}^3) = 10^4 \text{ mg Protein}$$

$$C_L^* ((100 \text{ g resin}) K_F + 1{,}000 \text{ cm}^3) = 10^4 \text{ mg Protein}$$

$$C_L^* = \frac{10^4 \text{ mg Protein}}{((100 \text{ g resin}) (55.6 \frac{\text{cm}^3 \text{ fluid}}{\text{g resin}}) + 1{,}000 \text{ cm}^3)}$$

$$C_L^* = 1.52 \text{ mg Protein} / \text{cm}^3$$

3. Product Purification /Contaminant Removal: Adsorption Equilibrium - Batch Adsorption

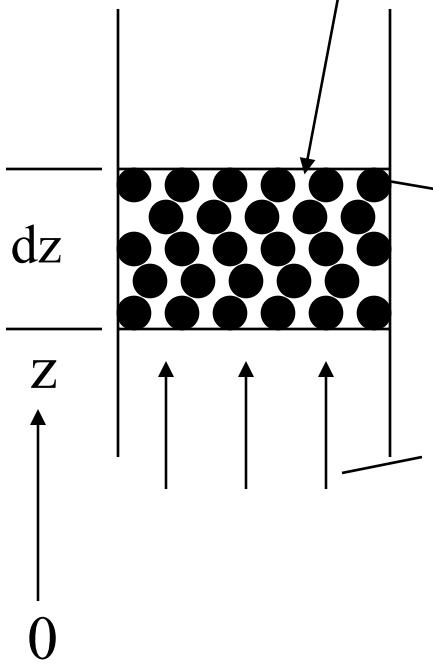
Example Problem 2: Batch Adsorption (cont.)

$$\% \text{ Recovery of Protein} = \left(1 - \frac{C_L^*}{C_{Lo}}\right)100$$

$$= \left(1 - \frac{1.52}{10}\right)100 = 84.76\%$$

3. Product Purification /Contaminant Removal: Fixed Bed Adsorption

Theory of Solute Movement in Fixed-Bed Adsorption Columns:
(Blanch and Clark, "Biochemical Engineering", pg 514-517)



ε = column viod fraction
(between particles)
$= V_L / (V_L + V_S)$

β = particle void fraction
(voids within particles)

u_S = superficial velocity $= \left(\frac{F}{A}\right)$

where F = volumetric flow rate
A = cross sectional area of column

3. Product Purification /Contaminant Removal: Fixed Bed Adsorption

Theory of Solute Movement in Fixed-Bed Adsorption Columns: (cont.)

$$\frac{\partial(V_L C_L)}{\partial t} + \frac{\partial(V_S \bar{s})}{\partial t} + u_S \frac{\partial(VC_L)}{\partial z} = D_L \frac{\partial^2(VC_L)}{\partial z^2}$$

(accumulation in liquid) (accumulation in solid) (convective flow) (axial dispersion)

$\bar{s}(t,z) = \overline{C}_{Li}\,\beta + \rho_P\,\overline{C}_S$ --- avg. concentration inside particle

$$\overline{C}_{Li} = \frac{\int_0^R C_{Li}(t,r,z)4\pi r^2 dr}{\frac{4}{3}\pi R^2} = \frac{3}{R^3}\int_0^R r^2 C_{Li}(t,r,z)dr$$

$$\overline{C}_S = \frac{3}{R^3}\int_0^R r^2 C_{Si}(t,r,z)dr$$

D_L = axial dispersion coefficient (cm^2 / s)

3. Product Purification /Contaminant Removal: Fixed Bed Adsorption

Theory of Solute Movement in Fixed-Bed Adsorption Columns: (cont.)

Assumptions:

$C_{Si} >> C_{Li}$ so $\bar{s} \cong \rho_P \bar{C}_S$

Neglect Dispersion, $D_L \cong 0$

$$\frac{\partial C_L}{\partial t} + u_i \frac{\partial C_L}{\partial z} + \rho_P \left(\frac{1-\varepsilon}{\varepsilon}\right) \frac{\partial \bar{C}_S}{\partial t} = 0$$

Another Assumption: instantaneous equilibrium,

C_{Si} is uniform in the particles

$$\bar{C}_S = C_S = f(C_L)$$

$$\text{so } \frac{\partial \bar{C}_S}{\partial t} = \frac{\partial C_S}{\partial t} = \left(\frac{\partial C_S}{\partial C_L}\right)\left(\frac{\partial C_L}{\partial t}\right) = f'(C_L)\left(\frac{\partial C_L}{\partial t}\right)$$

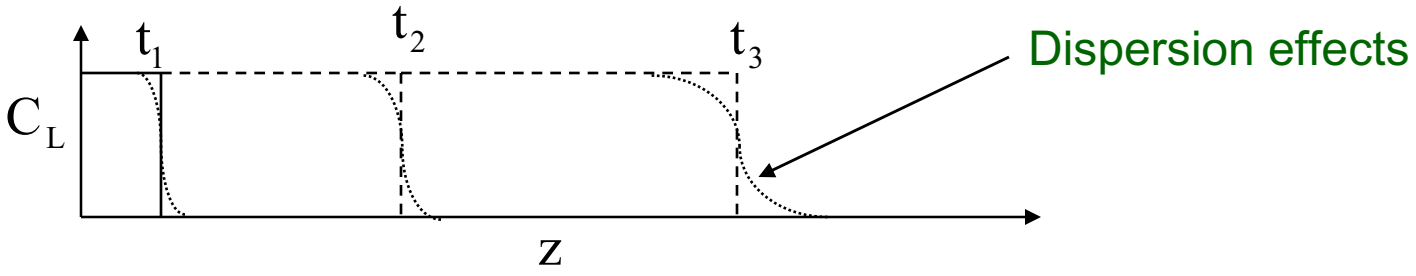
3. Product Purification /Contaminant Removal: Fixed Bed Adsorption

Theory of Solute Movement in Fixed-Bed Adsorption Columns: (cont.)

Therefore

$$\frac{\partial C_L}{\partial t} + \frac{u_i}{\left[1+\rho_P\left(\frac{1-\varepsilon}{\varepsilon}\right)f'(C_L)\right]}\frac{\partial C_L}{\partial z} = 0$$

This is the form of a kinematic wave.



3. Product Purification /Contaminant Removal: Fixed Bed Adsorption

Theory of Solute Movement in Fixed-Bed Adsorption Columns: (cont.)

The velocity of solute propaga

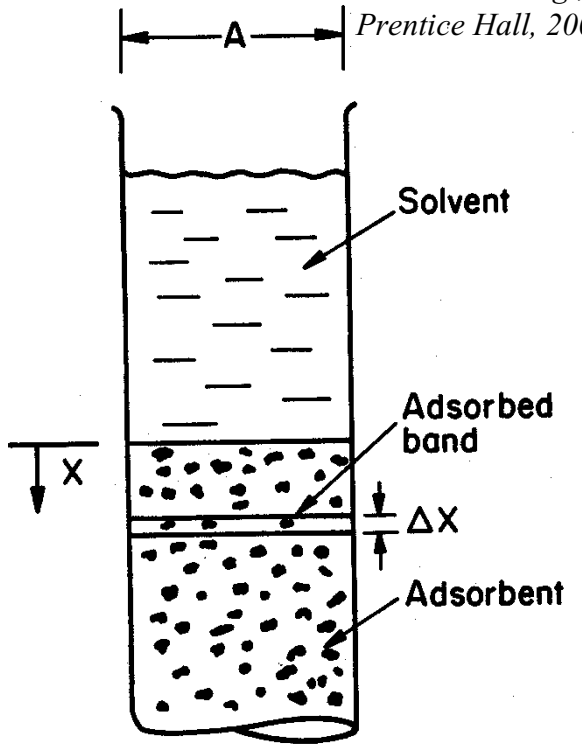
$$-\frac{dz}{dt} = -\frac{\left(\frac{\partial C_L}{\partial t}\right)}{\left(\frac{\partial C_L}{\partial z}\right)} = \frac{u_i}{\left[1+\rho_P\left(\frac{1-\varepsilon}{\varepsilon}\right)f(C_L)\right]}$$

the mean retention time of solute

$$\bar{t} = \frac{L}{u_i}\left[1+\rho_P\left(\frac{1-\varepsilon}{\varepsilon}\right)f(C_L)\right]$$

3. Product Purification /Contaminant Removal: Basics of Chromatography

- a solution containing a mixture of solutes (in a small volume) is added to the top of the column.
- a solvent (volume ΔV) is added to the top of the column.
- the solvent flow carries the solutes toward the bottom of the column.



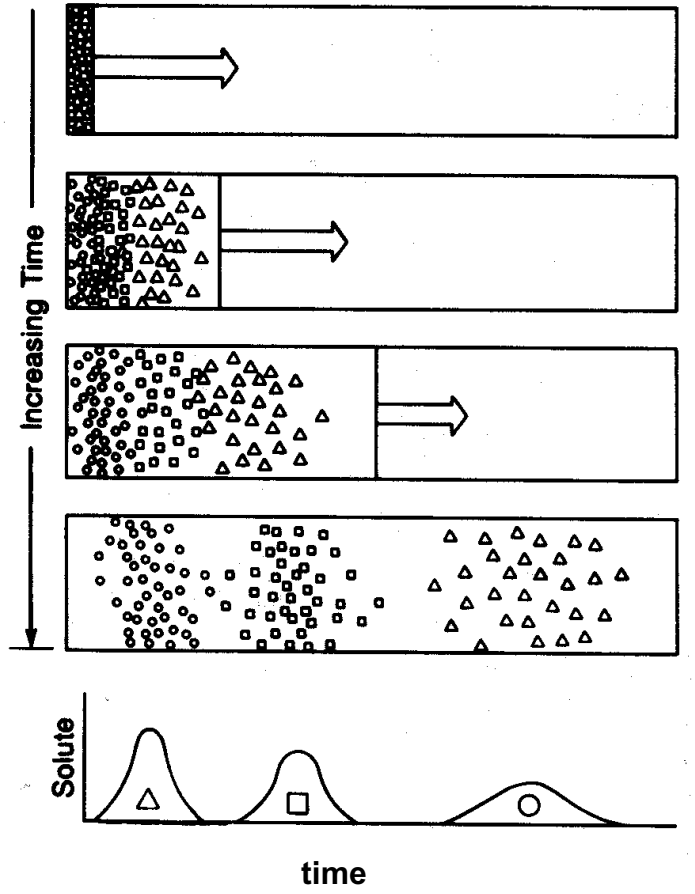
"Bioprocess Engineering: Basic Concepts" Shuler and Kargi, Prentice Hall, 2002

3. Product Purification /Contaminant Removal: Basics of Chromatography

- each solute is carried along at a different apparent velocity, depending upon the strength of interaction with the column packing.

- ideally, each solute exits the column as a discrete band of material.

"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi,
Prentice Hall, 2002



3. Product Purification /Contaminant Removal: Basics of Chromatography

A technique to separate components in a mixture based upon differential affinity for solutes for the adsorbent.

The affinity is quantified by the adsorption isotherm, $C_S^* = f(C_L^*)$, and in particular the derivative, $f'(C_L^*)$.

The affinity could also include size selection as in gel permeation or molecular sieve chromatography.

3. Product Purification /Contaminant Removal: Theory of Chromatography

A Theory of Solute Movement

How much solvent (ΔV) is needed to move a solute a distance Δx?

Solute balance over a differential column height Δx

$$-\left[\left(\frac{\partial C_L}{\partial x}\right)\Delta x\right]\Delta V = \varepsilon A\,\Delta x\left(\frac{\partial C_L}{\partial V}\right)\Delta V + A\,\Delta x\left(\frac{\partial C_S'}{\partial V}\right)\Delta V$$

rate of solute removal by solvent flow	rate of solute removal from void space	rate of solute removal from solid phase

3. Product Purification /Contaminant Removal: Theory of Chromatography (cont.)

Simplifying Yields:

$$\frac{\partial C_L}{\partial x} + A\left(\varepsilon\frac{\partial C_L}{\partial V} + \frac{\partial C_S'}{\partial V}\right) = 0$$

linear adsorption isotherm:

$$C_S' = M\, f(C_L)$$

C_S': amount of adsorbed solute per unit volume of column

M: mass of adsorbent per unit volume of column

$f(C_L)$: a function of C_L

3. Product Purification /Contaminant Removal: Theory of Chromatography (cont.)

Simplifying Yields:

$$-\frac{\partial C_L}{\partial x} = A\left(\varepsilon + M\, f'(C_L)\right)\frac{\partial C_L}{\partial V}$$

rearranging

$$\left(\frac{\partial V}{\partial x}\right) = A\left(\varepsilon + M\, f'(C_L)\right)$$

Integrating from x_o to x and V_o to V

$$\Delta x = \frac{\Delta V}{A\left(\varepsilon + M\, f'(C_L)\right)}$$

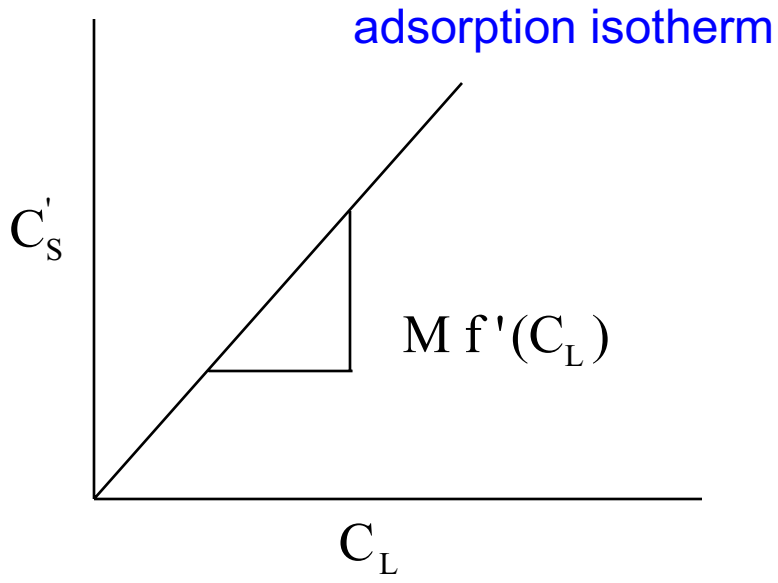
distance that solute band moves

elution volume of solvent

3. Product Purification /Contaminant Removal: Theory of Chromatography (cont.)

- The stronger the adsorption interaction, the shorter the travel distance, Δx, for a given elution volume, ΔV.

- a stronger adsorption interactions means a greater value of M f‘(C_L).



3. Product Purification /Contaminant Removal: Theory of Chromatography (cont.)

Example: Solute A, Adsorbent B

Adsorption isotherm: $C_S = k_1 (C_L)^3$

$$\left[\frac{\text{mg A adsorbed}}{\text{mg B}}\right] \qquad \left[\frac{\text{mg A adsorbed}}{\text{mg B}}\right] \Big/ \left[\left(\frac{\text{mg A}}{\text{mL solution}}\right)^3\right]$$

$$\left[\frac{\text{mg A}}{\text{mL solution}}\right]$$

$k_1 = 0.2$

$C_L = 0.05$ mg A/mL solution

$\varepsilon = 0.35$

M = 5 g adsorbent B/100 mL column volume

ΔV = volume of solvent added = 250 mL [cm^3]

A = column cross-sectional area = 10 cm^2

3. Product Purification /Contaminant Removal: Theory of Chromatography (cont.)

Find Δx

$$f(C_L) = k_1 C_L^3 \quad \text{therefore}$$

$$f'(C_L) = 3k_1 C_L^2$$

$$= (3)(2)(0.05) = .0015 \left[\frac{\left(\frac{\text{mg A ads.}}{\text{mg B}} \right)}{\left(\frac{\text{mg A}}{\text{mL soln.}} \right)} \right]$$

$$M = \frac{5 \text{ g B}}{100 \text{ mL column volume}} = \frac{50 \text{ mg B}}{\text{mL column volume}}$$

3. Product Purification /Contaminant Removal: Theory of Chromatography (cont.)

Find Δx

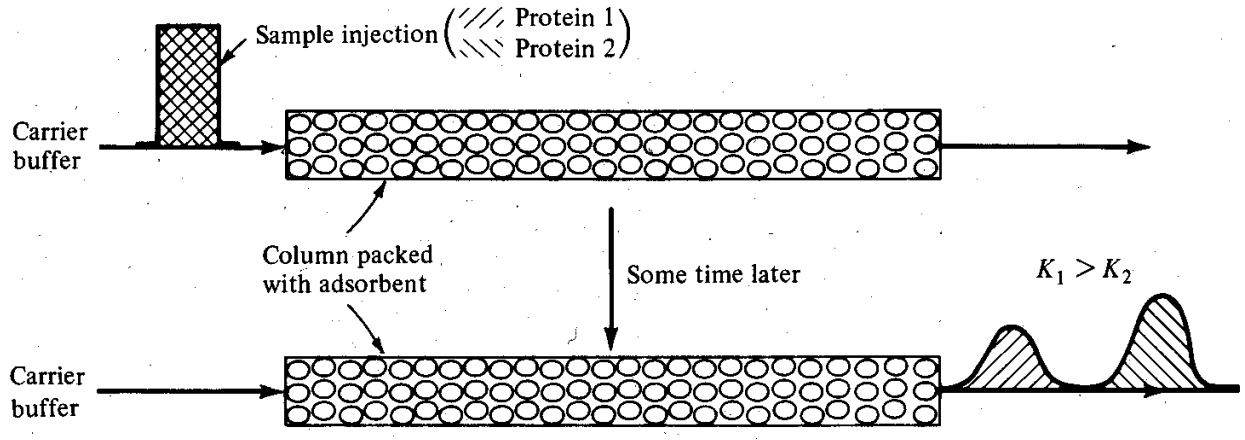
$$\Delta x = = \frac{\Delta V}{A\left(\varepsilon + M\, f'(C_L)\right)}$$

$$\frac{250\ \text{mL}\left(\dfrac{1\ \text{cm}^3\ \text{soln.}}{\text{mL soln.}}\right)}{\left[(10\ \text{cm}^2)\left(0.35\dfrac{\text{cm}^3\ \text{soln.}}{\text{cm}^3\ \text{coln vol}} + 50\dfrac{\text{mg B}}{\text{cm}^3\ \text{coln vol}}\left(.0015\dfrac{\text{mg A / mg B}}{\text{mg A / cm}^3\ \text{soln.}}\right)\right)\right]}$$

$\Delta x = 58.5$ cm

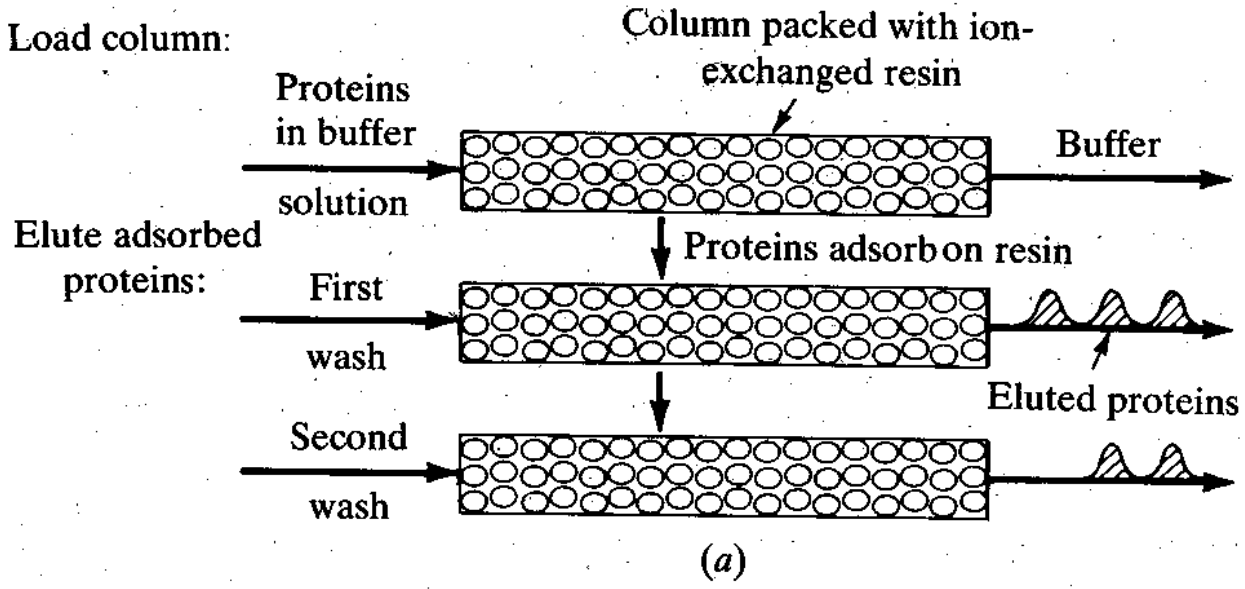
3. Product Purification /Contaminant Removal: Examples of Chromatography

Bailey and Ollis, 1986, Fig. 11.18



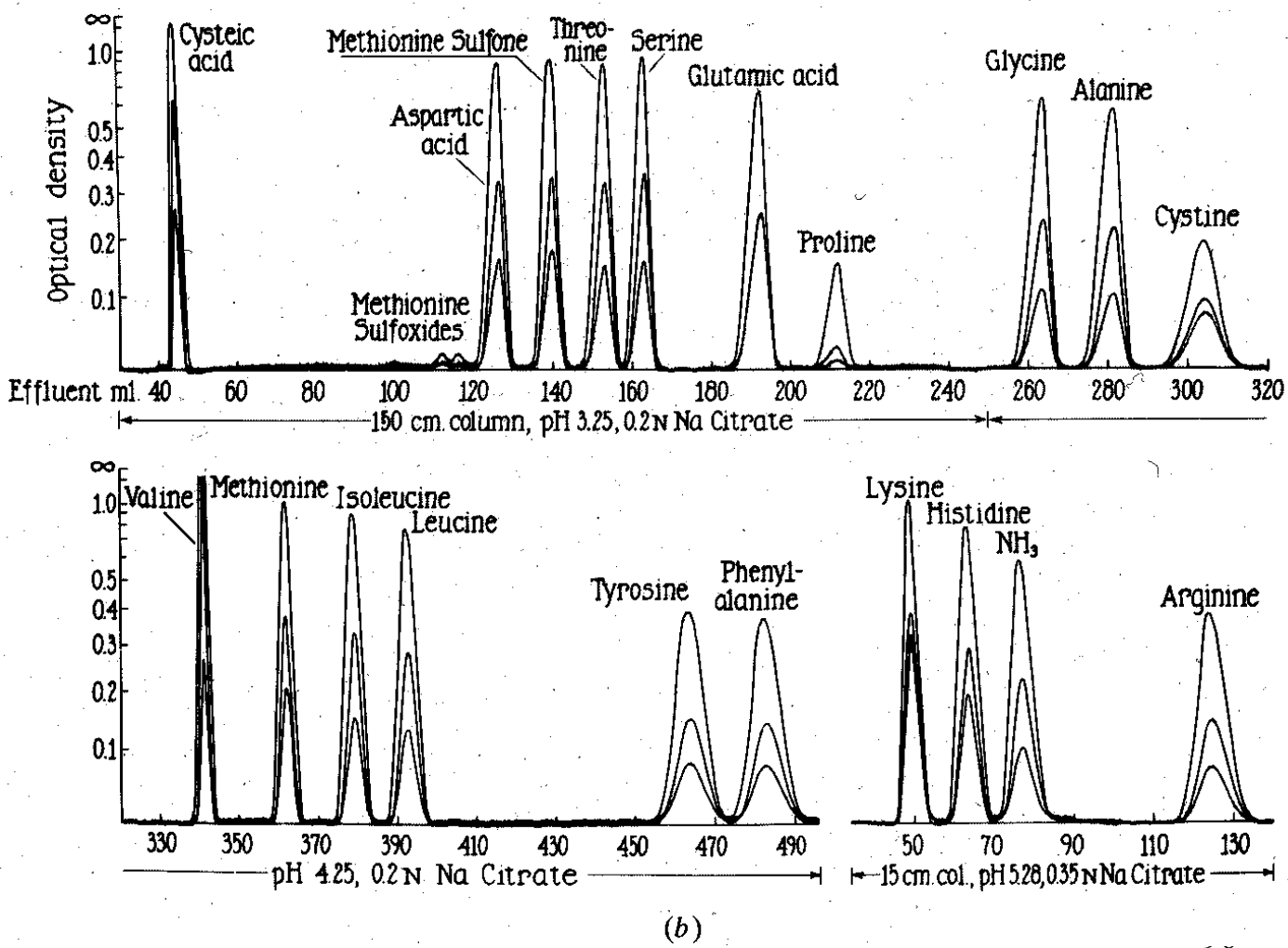
3. Product Purification /Contaminant Removal: Examples of Chromatography

Bailey and Ollis, 1986, Fig. 11.19a,
cation exchange chromatography



3. Product Purification /Contaminant Removal: Examples of Chromatography

Bailey and Ollis, 1986, Fig. 11.19b cation exchange chromato-graphy



(*b*)

3. Product Purification /Contaminant Removal: Examples of Chromatography

Gel Permeation Chromatography: **is based on the penetration of solute molecules into small pores of packing particles on the basis of molecular size and the shape of the solute molecules. It is also known as size exclusion chromatography.**

Equivalent Equilibrium Constant

$$K_{av,i} = \exp(-\pi L(r_g + r_i)^2)$$

where

L = concentration of gel fiber (cm/cm^3)

r_g = radius of a gel fiber (cm)

r_i = radius of a spherical molecule of species, i (cm)

$K_{av,i}$ is equivalent to $f'(C_L)$ in calculating $\bar{t}$ or $\frac{dz}{dt}$

3. Product Purification /Contaminant Removal: Examples of Chromatography

Molecule radii estimated based on protein diffusion coefficients

Bailey and Ollis, 1986,

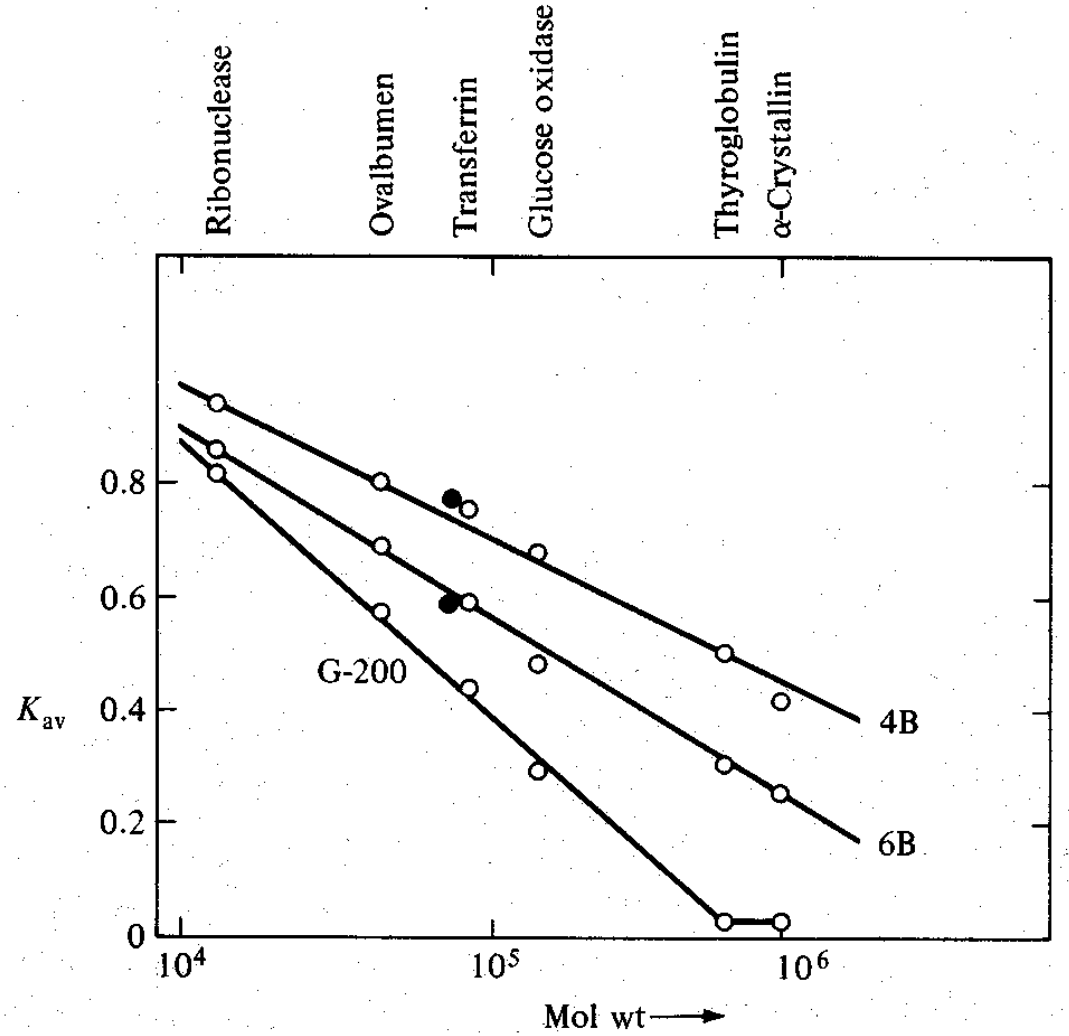
Table 11.6 Some radii estimated from diffusion studies for several molecules†

Protein	Mol wt	Diffusity, $D \times 10^7$, cm^2/s	r_i, Å
Ribonuclease	13,683	11.9	18.0
Lysozyme	14,100	10.4	20.6
Chymotrypsinogen	23,200	9.5	22.5
Serum albumin	65,000	5.94	36.1
Catalase	250,000	4.1	52.2
Urease	480,000	3.46	61.9
Typical fiber radii in gel		r_g, Å	
Sephadex		7	
Agarose		25	

† Selected from summary in C. Tanford, *Physical Chemistry of Macromolecules*, table 21.1, John Wiley & Sons, Inc., New York, 1961.

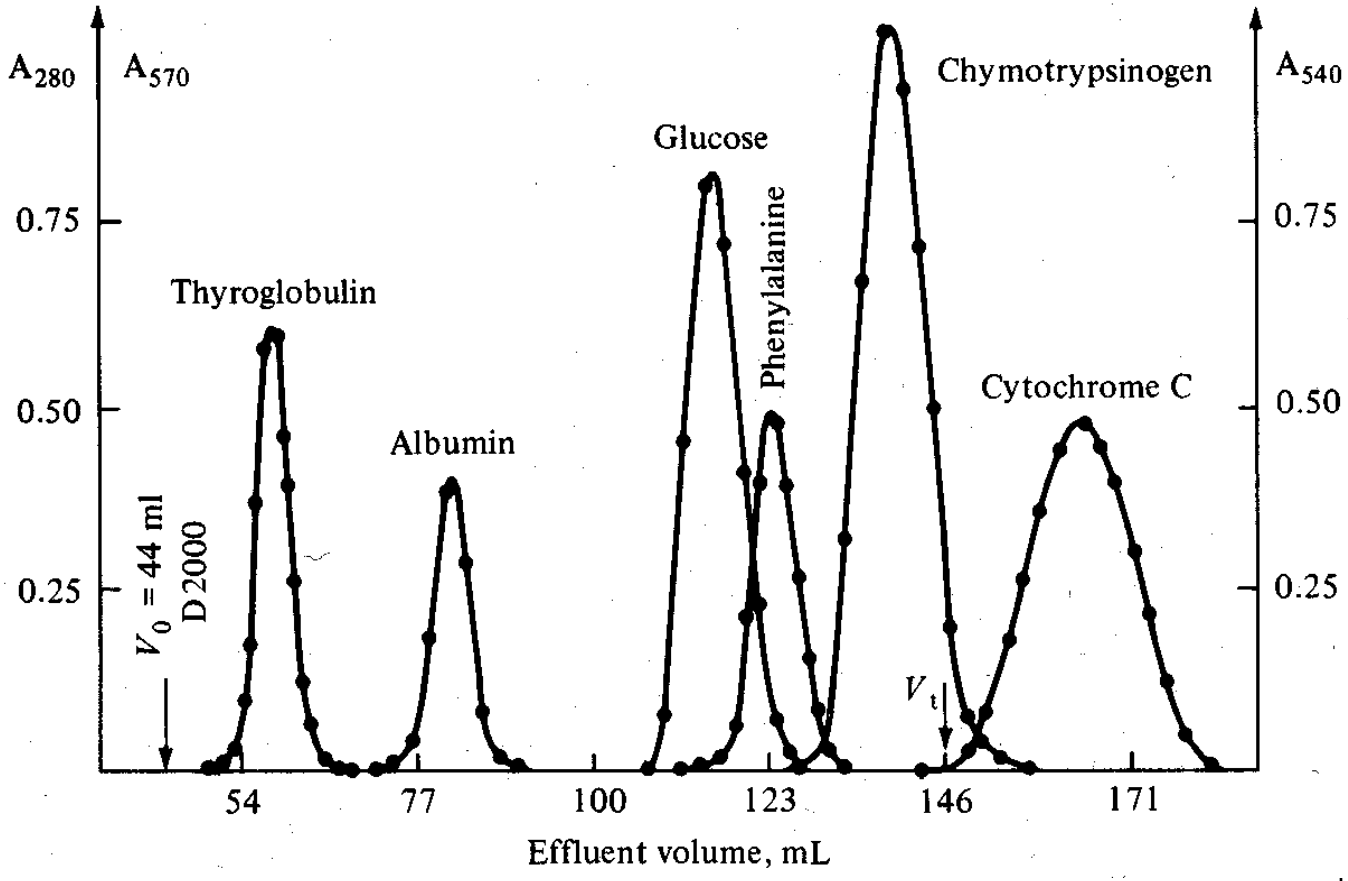
3. Product Purification /Contaminant Removal: Examples of Chromatography

Bailey and Ollis, 1986, Fig. 11.21, gel permeation chromato-graphy



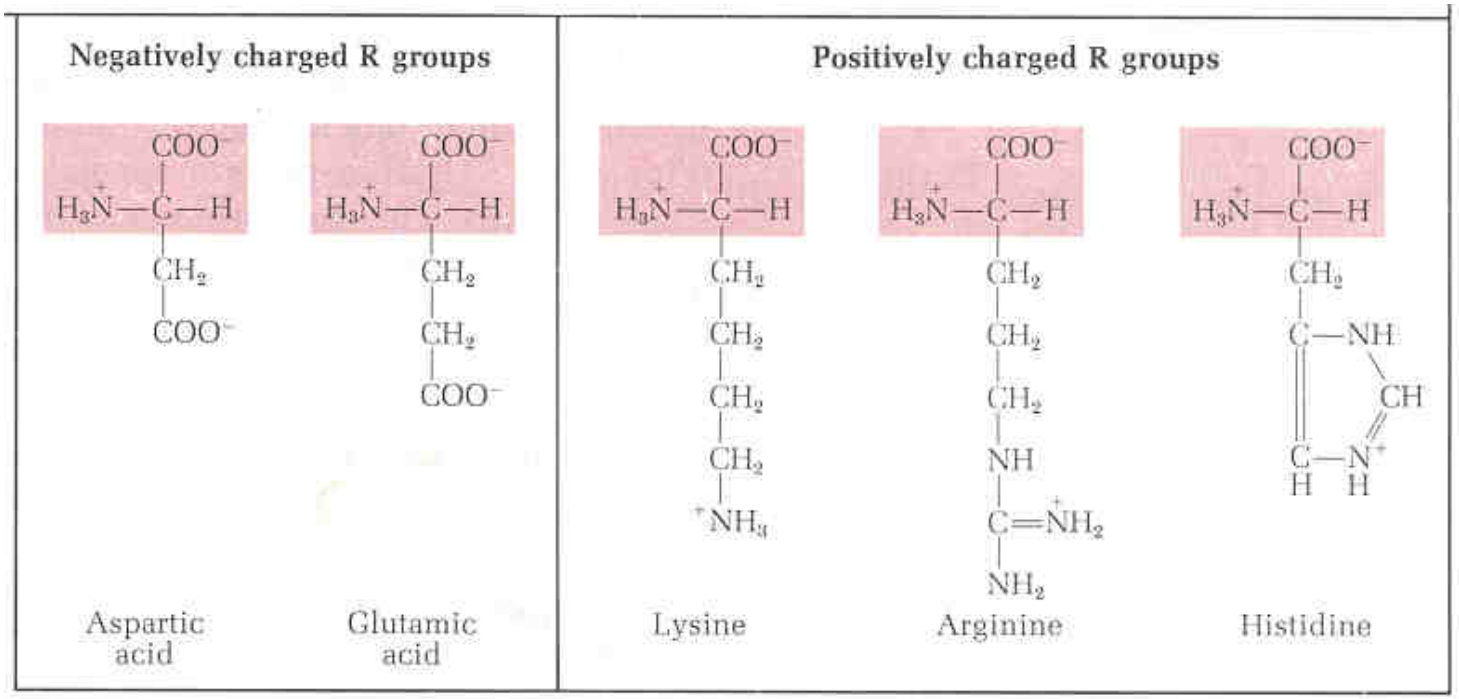
3. Product Purification /Contaminant Removal: Examples of Chromatography

Bailey and Ollis, 1986, Fig. 11.22 molecular sieve chromato-graphy



3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids

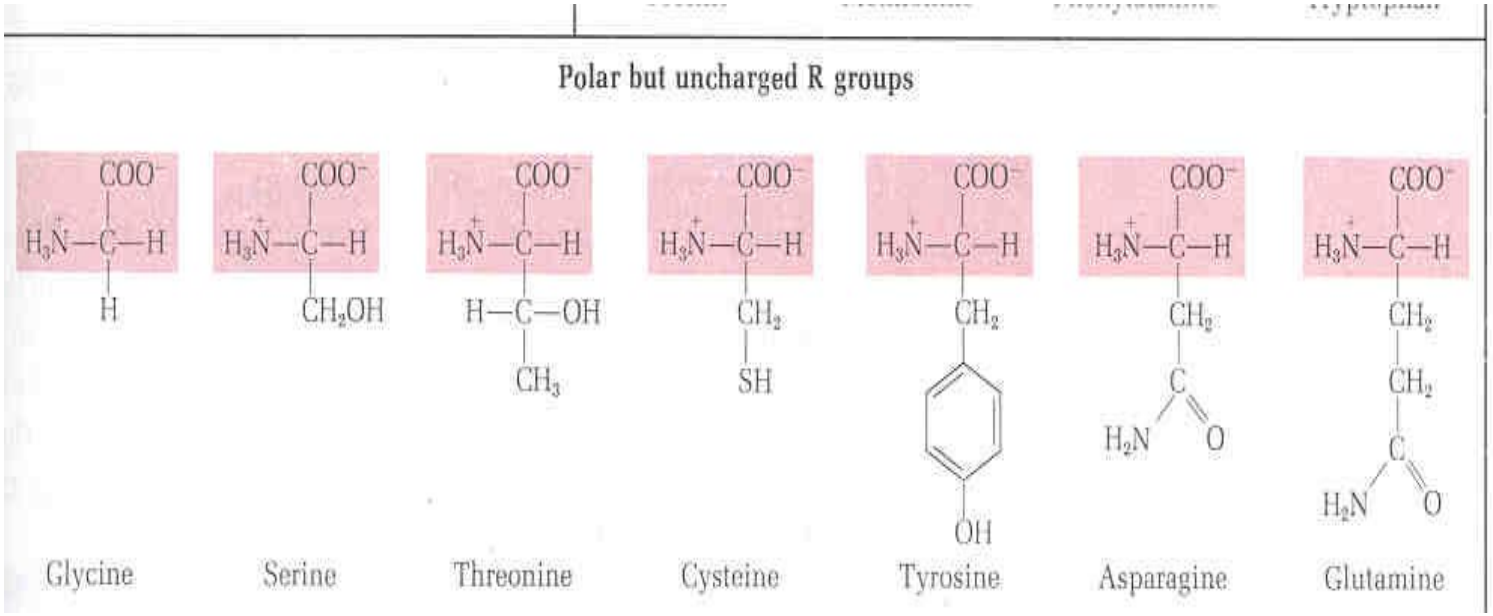
Charged Amino Acid R Groups at Neutral pH



"Principles of Biochemistry"
Lehninger, Worth, 1982

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

Polar Amino Acid R Groups at Neutral pH

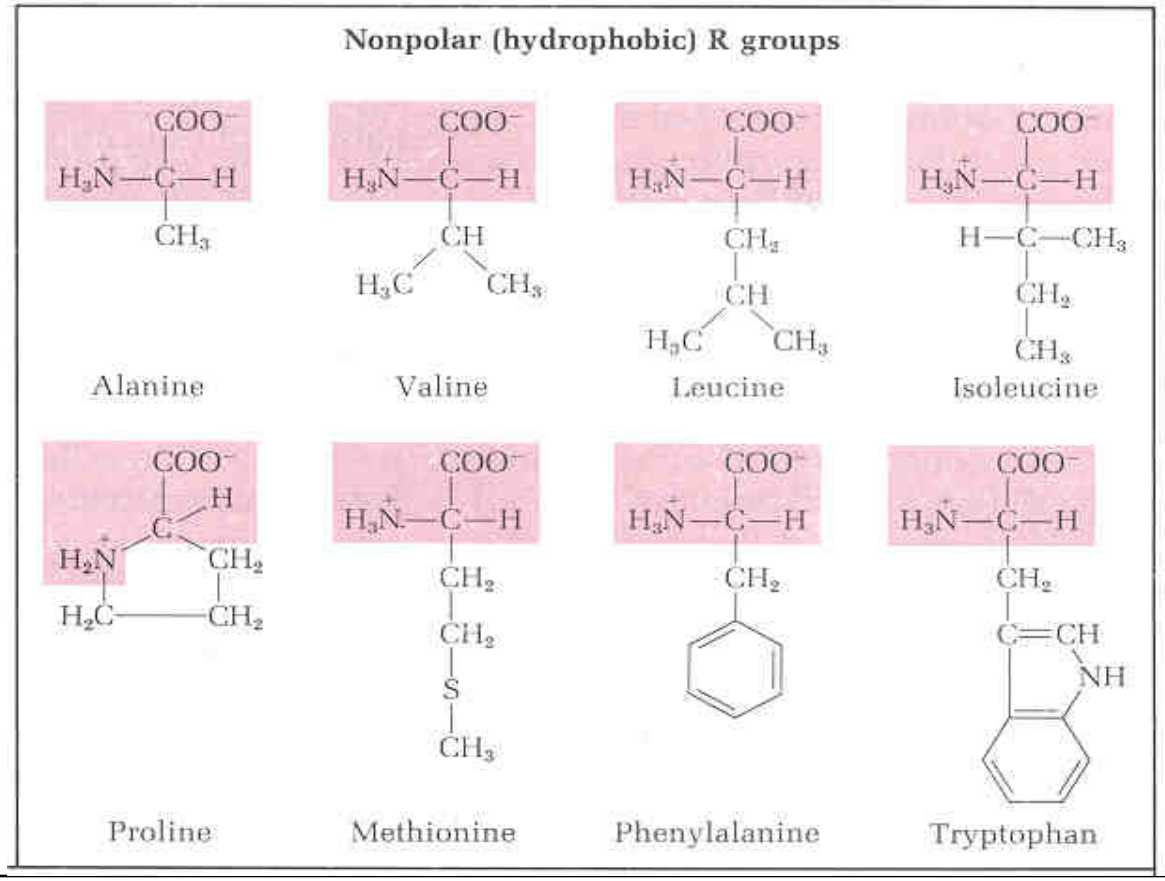


"Principles of Biochemistry"
Lehninger, Worth, 1982

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

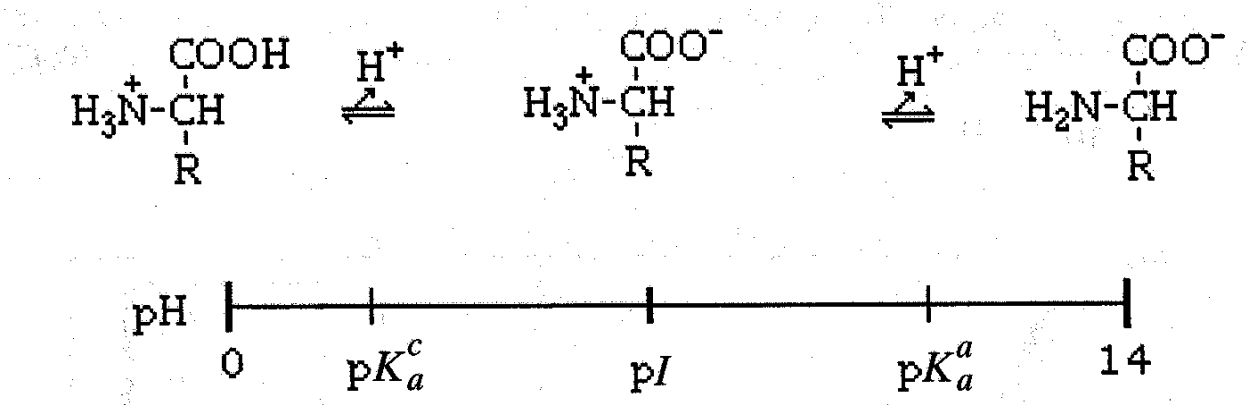
Nonpolar Amino Acid R Groups at Neutral pH

"Principles of Biochemistry"
Lehninger, Worth, 1982



3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

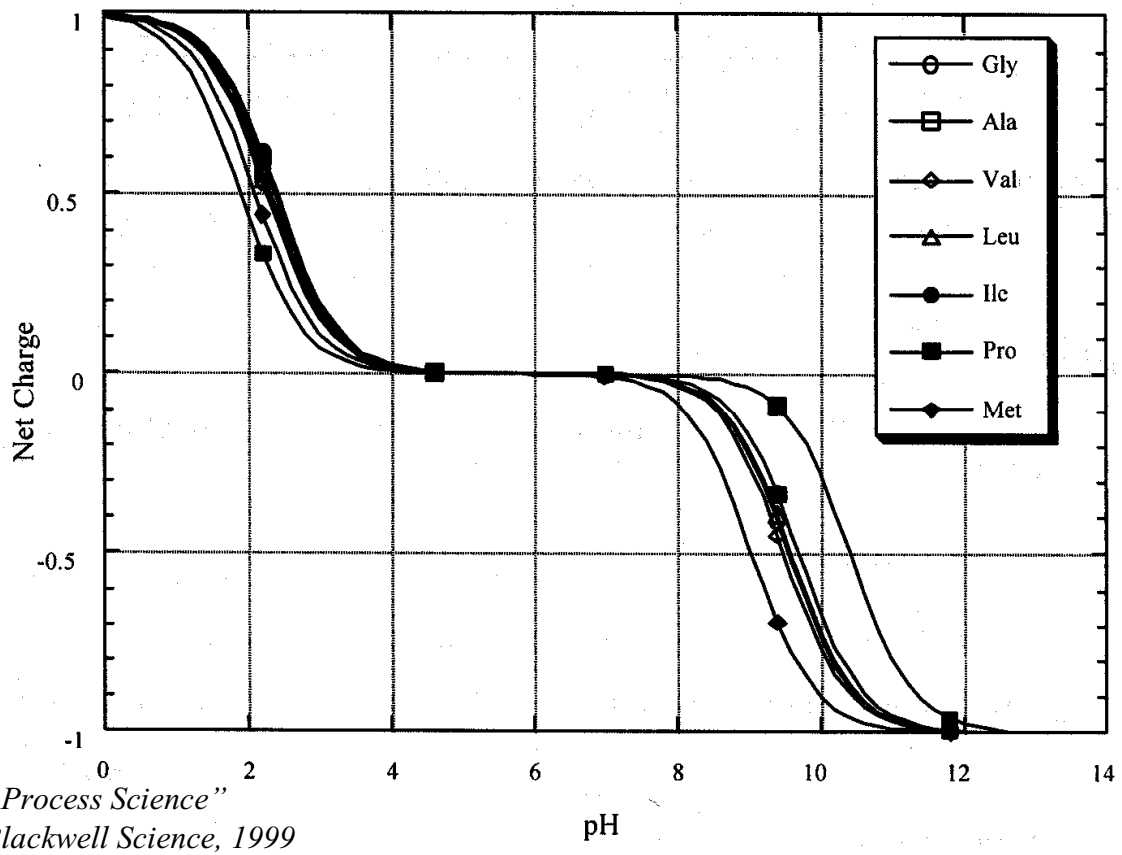
Acid dissociation reactions - Nonpolar and Polar R Groups:



"Bioseparaion Process Science"
Garcia et al., Blackwell Science, 1999

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

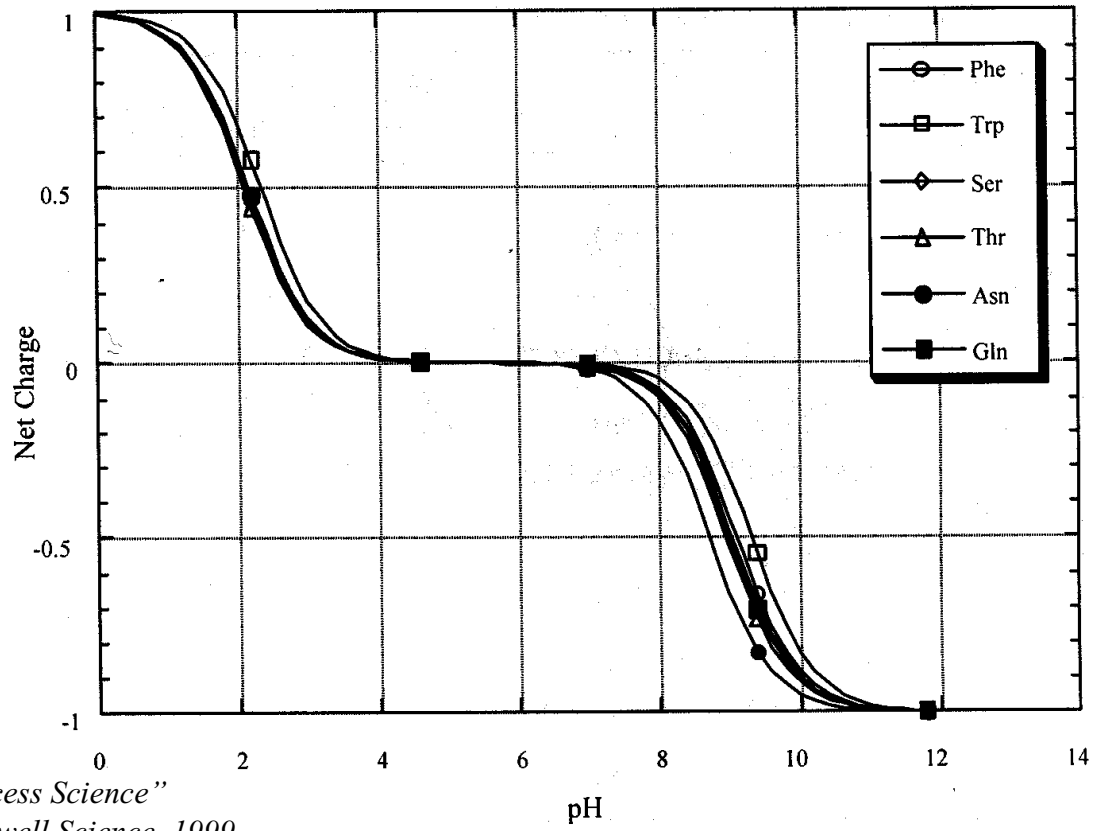
Acid dissociation reactions - Nonpolar and Polar R Groups:



"Bioseparaion Process Science"
Garcia et al., Blackwell Science, 1999

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

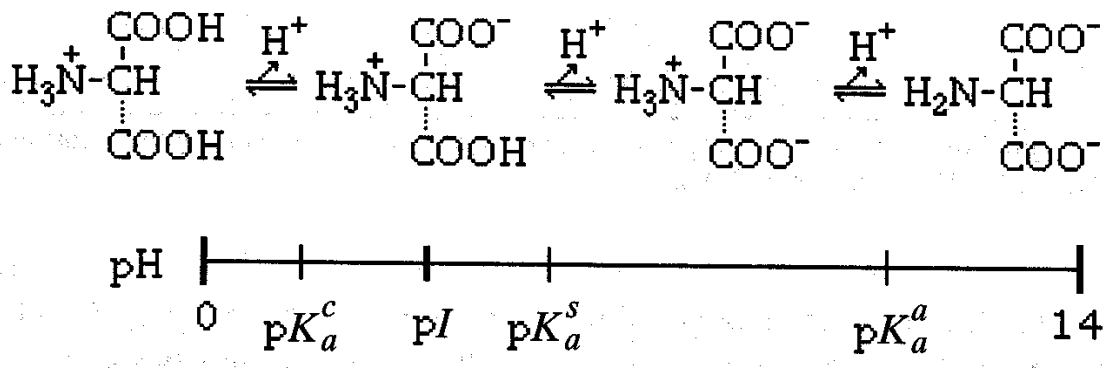
Acid dissociation reactions - Nonpolar and Polar R Groups:



"Bioseparaion Process Science"
Garcia et al., Blackwell Science, 1999

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

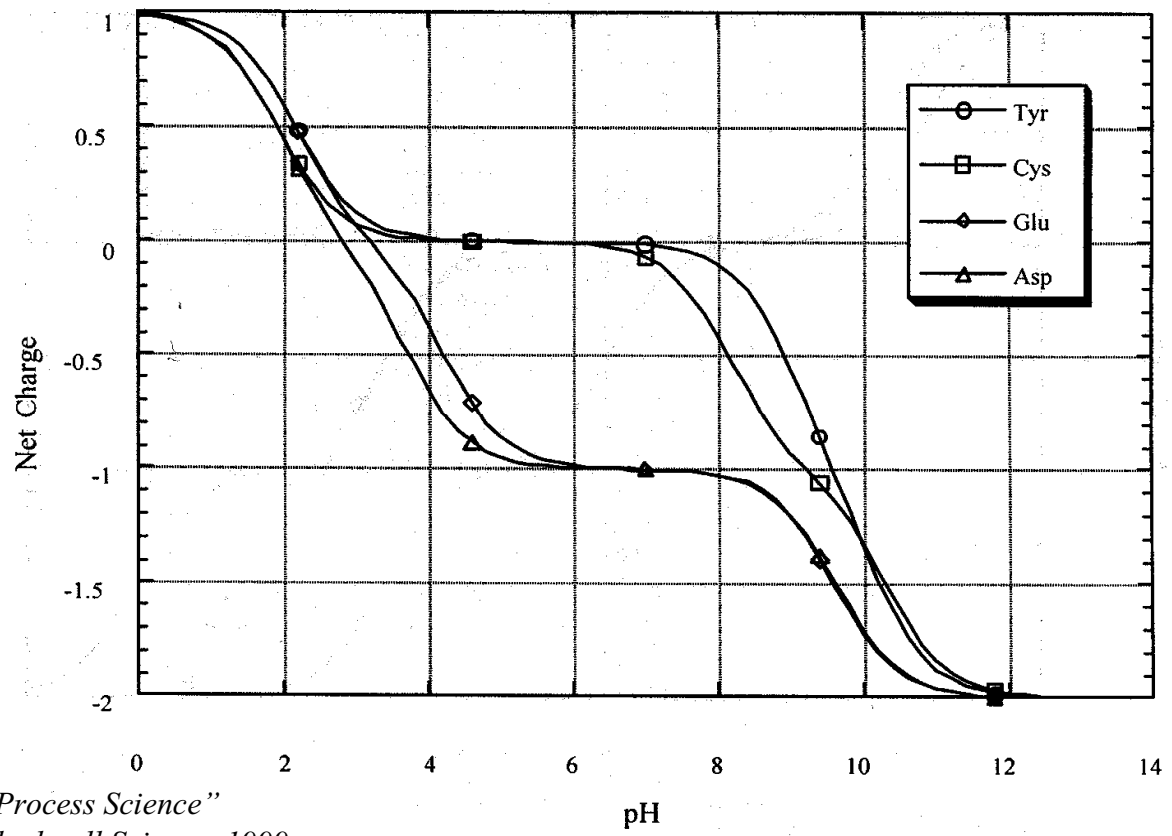
Acid dissociation reactions - Negatively Charged R Groups:



"Bioseparaion Process Science"
Garcia et al., Blackwell Science, 1999

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

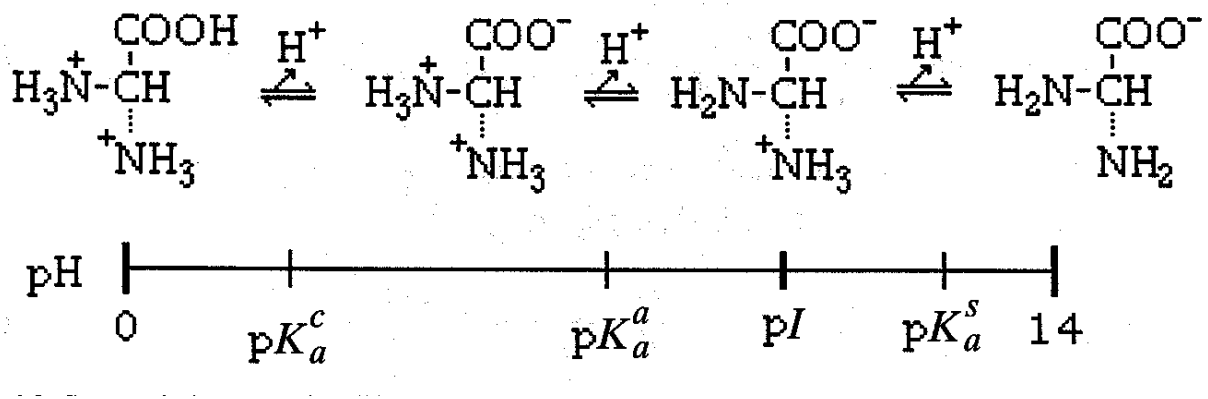
Acid dissociation reactions - Negatively Charged R Groups:



"Bioseparaion Process Science"
Garcia et al., Blackwell Science, 1999

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

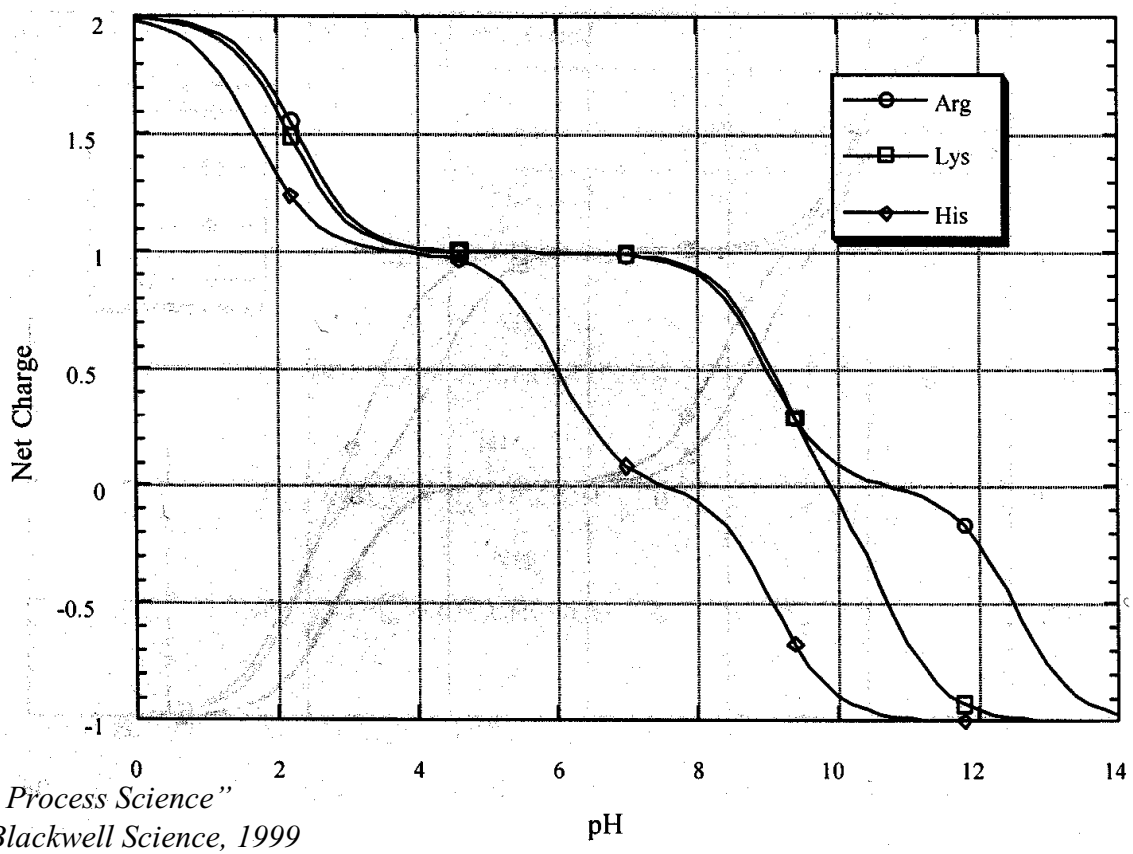
Acid dissociation reactions - Positively Charged R Groups:



"Bioseparaion Process Science"
Garcia et al., Blackwell Science, 1999

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

Acid dissociation reactions - Positively Charged R Groups:



"Bioseparaion Process Science"
Garcia et al., Blackwell Science, 1999

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

Acid dissociation reactions - Stoichiometry of COOH = HA:

$$HA \underset{K_a}{\longleftrightarrow} \bar{A} + H^+ \quad \Rightarrow \quad K_a = \frac{[H^+][\bar{A}]}{[HA]}$$

$$\log K_a = \log [H^+] + \log \frac{[A^-]}{[HA]}$$

$$pH = -\log [H^+] \quad \text{and} \quad pK_a = -\log K_a$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad \text{or} \quad \frac{[A^-]}{[HA]} = 10^{(pH-pK_a)}$$

$$\text{but } [HA]_o = [HA] + [\bar{A}] \quad \text{or} \quad = [HA]_o - [\bar{A}]$$

$$\frac{[A^-]}{[HA]_o - [\bar{A}]} = 10^{(pH-pK_a)} \quad \text{and} \quad \frac{[A^-]}{[HA]_o} = \frac{10^{(pH-pK_a)}}{1+10^{(pH-pK_a)}}$$

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

Acid dissociation reactions - Stoichiometry of $NH_3^+ = HA^+$:

$$HA^+ \underset{K_a}{\longleftrightarrow} A + H^+ \quad \Rightarrow \quad K_a = \frac{[H^+][A]}{[HA^+]}$$

$$\log K_a = \log [H^+] + \log \frac{[A]}{[HA^+]}$$

$$pH = -\log [H^+] \qquad \text{and} \qquad pK_a = -\log K_a$$

$$pH = pK_a + \log \frac{[A]}{[HA^+]} \qquad \text{or} \qquad \frac{[A]}{[HA^+]} = 10^{(pH-pK_a)}$$

$$\text{but } [HA^+]_o = [HA^+] + [A] \qquad \text{or} = [HA^+]_o - [HA^+]$$

$$\frac{[HA^+]_o - [HA^+]}{[HA^+]} = 10^{(pH-pK_a)} \qquad \text{and} \qquad \frac{[HA^+]}{[HA^+]_o} = \frac{1}{1+10^{(pH-pK_a)}}$$

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

Charge on amino acid groups:

$$\text{Charge of } \alpha\text{ - amino group} = \frac{1(+1)}{1+10^{\left(pH-pK_a^c\right)}}$$

$$\text{Charge of } \alpha\text{ - carboxyl group} = \frac{1(-1)}{1+\frac{1}{10^{\left(pH-pK_a^a\right)}}}$$

$$\text{Charge of side chain} = \frac{1(-1)}{1+\frac{1}{10^{\left(pH-pK_a^s\right)}}}$$

$$\text{Charge of side chain} = \frac{1(+1)}{1+10^{\left(pH-pK_a^s\right)}}$$

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

Net Charge is sum of all reactions:

$$\text{Net charge of amino acid} = \frac{1(+1)}{1+10^{\left(pH-pK_a^c\right)}} + \frac{1(-1)}{1+\frac{1}{10^{\left(pH-pK_a^a\right)}}}$$

$$\text{Net charge of amino acid with (-R)} = \frac{1(+1)}{1+10^{\left(pH-pK_a^c\right)}} + \frac{1(-1)}{1+\frac{1}{10^{\left(pH-pK_a^a\right)}}} + \frac{1(-1)}{1+\frac{1}{10^{\left(pH-pK_a^s\right)}}}$$

$$\text{Net charge of amino acid with (+R)} = \frac{1(+1)}{1+10^{\left(pH-pK_a^c\right)}} + \frac{1(-1)}{1+\frac{1}{10^{\left(pH-pK_a^a\right)}}} + \frac{1(+1)}{1+10^{\left(pH-pK_a^s\right)}}$$

3. Product Purification /Contaminant Removal: Ion Exchange of Amino Acids (cont.)

Amino acid	pI	α-COOH (pK_a^c)	pKa α–NH_3^+ (pK_a^a)	Side Chain (pK_a^s)
Glycine	5.97	2.36	9.56	
Alanine	6.02	2.31	9.70	
Valine	5.97	2.26	9.49	
Leucine	5.98	2.27	9.57	
Proline	6.30	1.9	10.41	
Isoleucine	6.02	2.4	9.7	
Methionine	5.06	2.10	9.05	
Phenylalanine	5.48	2.17	9.11	
Tryptophan	5.88	2.34	9.32	
Serine	5.68	2.13	9.05	
Threonine	5.60	2.63	10.48	8.16
Cysteine	5.02	1.96	10.29	10.11
Tyrosine	5.67	2.17	9.04	
Asparagine	5.41	2.15	8.72	
Glutamine	5.70	2.16	9.01	
Aspartic acid	2.98	1.94	9.62	3.70
Glutamic acid	3.22	2.18	9.59	4.20
Lysine	9.74	2.19	9.12	10.68
Arginine	10.76	2.3	9.02	12.48
Histidine	7.59	1.7	9.09	6.02

3. Product Purification /Contaminant Removal: Ion Exchange of Proteins

A computer algorithm for computing charge of proteins
(Genetics Computer Group, Inc. 1993)

$$\begin{bmatrix} Net \\ charge \end{bmatrix} = \begin{bmatrix} \#\text{ of positively} \\ \text{charged residues} \end{bmatrix} - \begin{bmatrix} \#\text{ of negatively} \\ \text{charged residues} \end{bmatrix} + \begin{bmatrix} \#\text{ of protonated} \\ \text{amino termini} \end{bmatrix} - \begin{bmatrix} \#\text{ of deprotonated} \\ \text{carboxy termini} \end{bmatrix}$$

$$N(p) = N(t)\frac{[H^+]}{[H^+] + K(N)}$$

N(p) is the number of protonated residues
N(t) is the total number of residues of a specific type
[H+] is the hydrogen ion concentration
K(N) is the aminoacid dissociation constant

3. Product Purification /Contaminant Removal: Ion Exchange of Proteins

Calculate the net charge of the following peptide NH2-Lys-Pro-Lys-COOH

Information

Lysine positively charged

$pK_a^c = 2.19$

$pK_a^a = 9.12$

$pK_a^s = 10.68$

Information

Proline nonpolar aminoacid

$pK_a^c = 1.9$

$pK_a^a = 10.41$

$$\begin{bmatrix} Net \\ charge \end{bmatrix} = \begin{bmatrix} \#\text{ of positively} \\ \text{charged residues} \end{bmatrix} - \begin{bmatrix} \#\text{ of negatively} \\ \text{charged residues} \end{bmatrix} + \begin{bmatrix} \#\text{ of protonated} \\ \text{amino termini} \end{bmatrix} - \begin{bmatrix} \#\text{ of deprotonated} \\ \text{carboxy termini} \end{bmatrix}$$

$$2 \qquad 0 \qquad 1 \text{ from lys} \qquad 1 \text{ from lys}$$

Information

Lysine positively charged

$pK_a^c = 2.19$

$pK_a^a = 9.12$

$pK_a^s = 10.68$

Information

Proline nonpolar aminoacid

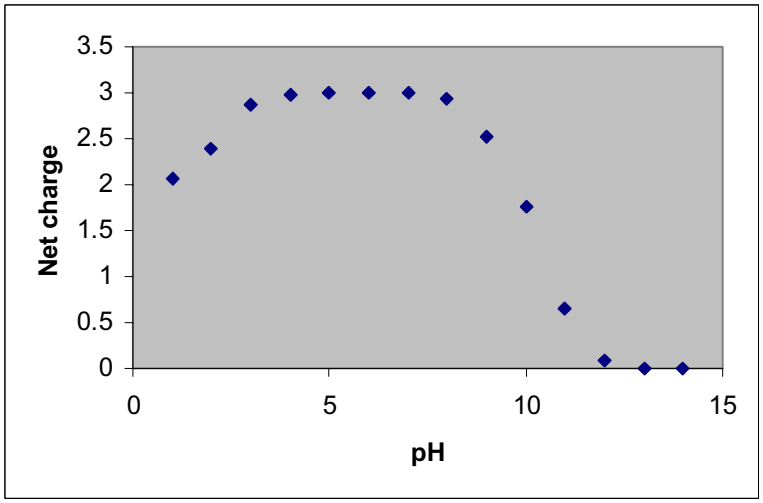
$pK_a^c = 1.9$

$pK_a^a = 10.41$

$$\begin{bmatrix} Net \\ charge \end{bmatrix} = \begin{bmatrix} \#\text{ of positively} \\ \text{charged residues} \end{bmatrix} - \begin{bmatrix} \#\text{ of negatively} \\ \text{charged residues} \end{bmatrix} + \begin{bmatrix} \#\text{ of protonated} \\ \text{amino termini} \end{bmatrix} - \begin{bmatrix} \#\text{ of deprotonated} \\ \text{carboxy termini} \end{bmatrix}$$

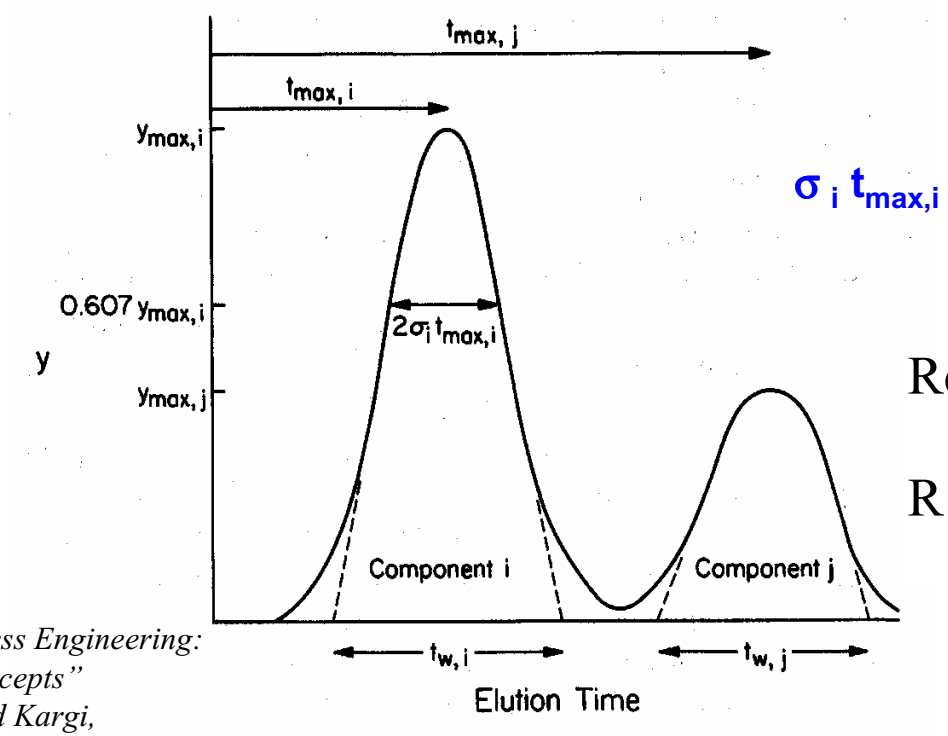
2 0 1 from lys 1 from lys

$$\begin{bmatrix} \text{Net} \\ \text{charge} \end{bmatrix} = 2\frac{[H^+]}{[H^+]+10^{-10.68}} + \frac{[H^+]}{[H^+]+10^{-9.12}} - \frac{[H^+]}{[H^+]+10^{-2.19}}$$



3. Product Purification /Nonideal effects on Chromatographic Separations

Dispersion, Wall Effects, and Nonequilibrium:



"Bioprocess Engineering: Basic Concepts" Shuler and Kargi, Prentice Hall, 2002

Gaussian Peak

σ_i $t_{max,i}$ = standard deviation

Resolution of Peaks

$$R_S = \frac{t_{max,j} - t_{max,i}}{1/2(t_{w,i} + t_{w,j})}$$

3. Product Purification /Nonideal Effects on Chromatographic Separations

Prediction of Peak Width:

$$y_i = y_{\max,i} \exp\left[- \frac{(t - t_{\max,i})^2}{2(\sigma t_{\max,i})^2} \right]$$

σ depends on dispersion and adsorption kinetics

$$\sigma^2 = \frac{v}{kal}$$

v = superficial velocity,

ka = surface adsorption reaction rate

l = column length

3. Product Purification /Nonideal Effects on Chromatographic Separations

Prediction of Peak Height:

$y_{\max,i}$ is inversely proportional to $\sqrt{\sigma t_{\max,i}}$

σ may depend on other processes

$\sigma^2 \propto \frac{vd^2}{l}$ internal diffusion control,

$\sigma^2 \propto \frac{v^{1/2} / d^{3/2}}{l}$ external film control,

$\sigma^2 \propto \frac{vd^2}{Dl}$ Taylor dispersion (laminar flow),

3. Product Purification /Scale Up of Chromatographic Separations

To Handle Increased Amount of Product:

1. Increase solute concentration using same column
   (may saturate column, leading to reduced purity)

2. Increase column cross sectional area, *A*, and particle diameter, *d*
   (maintains flow patterns, but σ increases if *d* increases)

3. Fix *d* but increase *v* and *l*, but maintain ratio of *v* to *l* constant
   (σ will be unchanged, but pressure drop will increase)

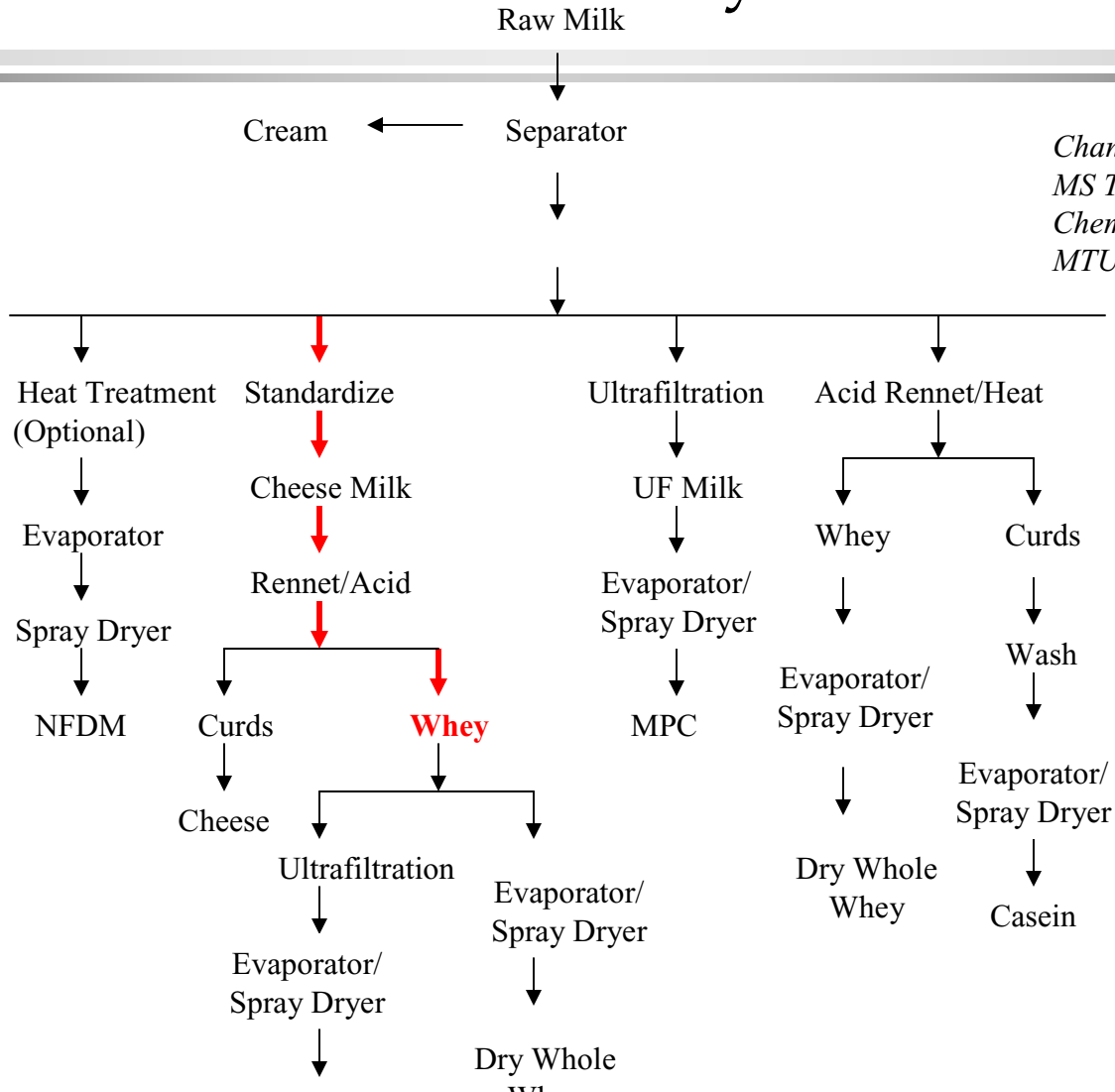
4. Increase *A* and volumetric flow rate, such that *v* is constant
   (σ remains constant, the desired outcome!)

3. Product Purification /Scale Up of Chromatographic Separations

Recent Advances in Chromatographic Packing:

1. Rigid beads with *macropores* inside particles
2. Allows higher flowrates without bead compression
3. Allows higher flowrates without excessive pressure drop
4. Good mass transfer is maintained between *macropores* and *micropore* within particles.

3. Chromatographic Separation of Proteins from Cheese Whey



Chandrasekaran, R., MS Thesis, Dept. of Chemical Engineering MTU

3. Chromatographic Separation of Proteins from Cheese Whey (cont.)

Chandrasekaran, R., MS Thesis, Dept. of Chemical Engineering MTU

Table 1.2 Composition of Whey (Weight %) (Kosikowski et al., 1997)

	Fluid Sweet Whey
Water	93.7
Total Solid	6.35
Fat	0.5
Protein	**0.8**
Lactose	4.85
Ash	0.5
Lactic Acid	0.05

3. Chromatographic Separation of Proteins from Cheese Whey (cont.)

Whey proteins are finding increasing application in the fields of nutrition (protein powder), as an antibiotic, and in other pharmaceutical applications. Individual whey proteins can be separated using cation exchange chromatography, using pH change during elution to recover individual proteins.

Table 1. Isoelectric Points of Major Whey Proteins [1]

Whey Protein	**Isoelectric Point**
β-lactoglobulin	5.35-5.49
α-lactalbumin	4.2-4.5
Bovine Serum Albumin	5.13
Immunoglobulins	5.5-8.3
Lactoferrin	7.8-8.0
Lactoperoxidase	9.2-9.9

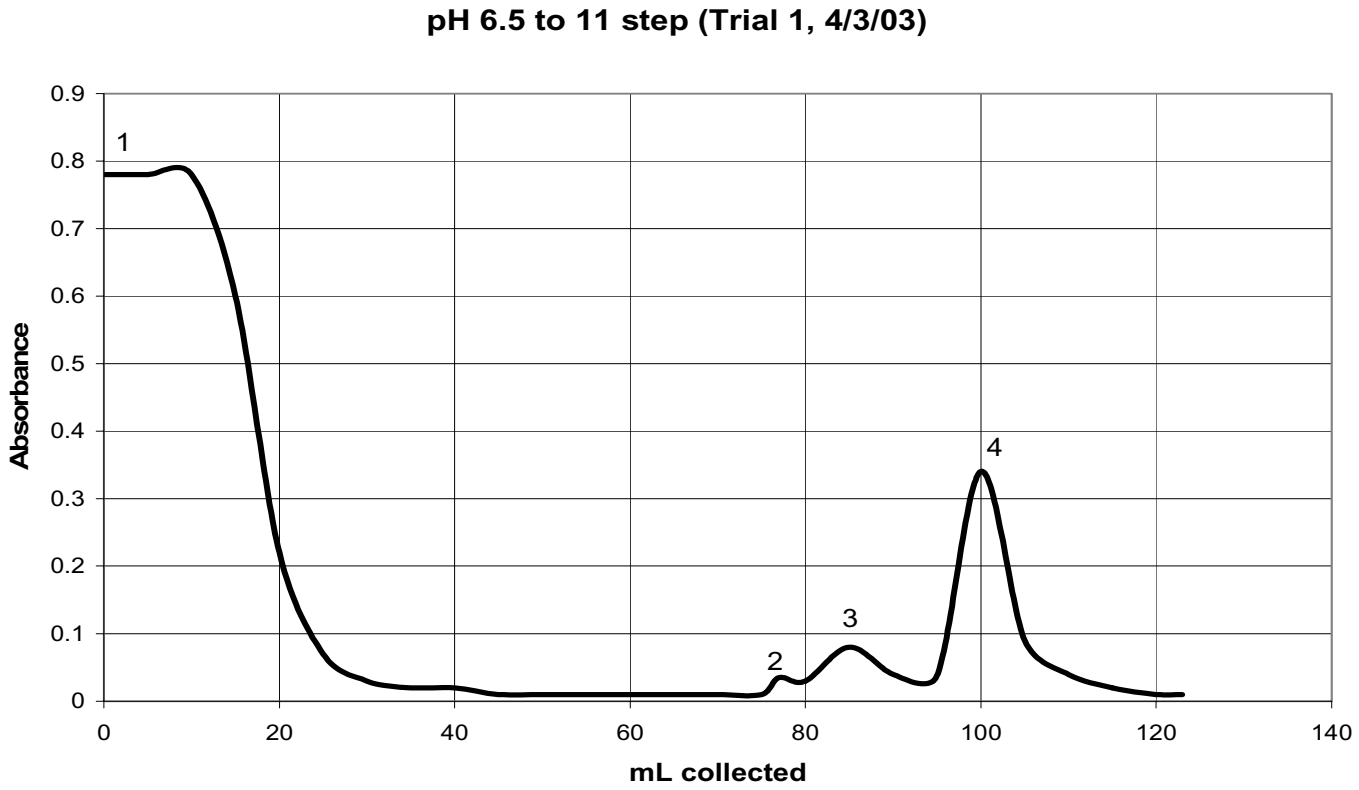
3. Chromatographic Separation of Proteins from Cheese Whey (cont.)

Whey proteins have a range of molecular weights.

Table 2. Major Whey Protein Molecular Weights [1]

Whey Protein	**Molecular Weight**
β-lactoglobulin	18,300
α-lactalbumin	14,000
Bovine Serum Albumin	69,000
Immunoglobulins	150,000
Lactoferrin	**77,000**
Lactoperoxidase	**77,500**

3. Chromatographic Separation of Proteins from Cheese Whey (cont.)



20 mL HiPrep 16/10 SP XL

Flow Rate = 3 mL/min

Temp. = 4 deg. C

300 mL of 5 g/L protein

solution loaded pH 6.5

Elution

60 mL pH 6.5 buffer

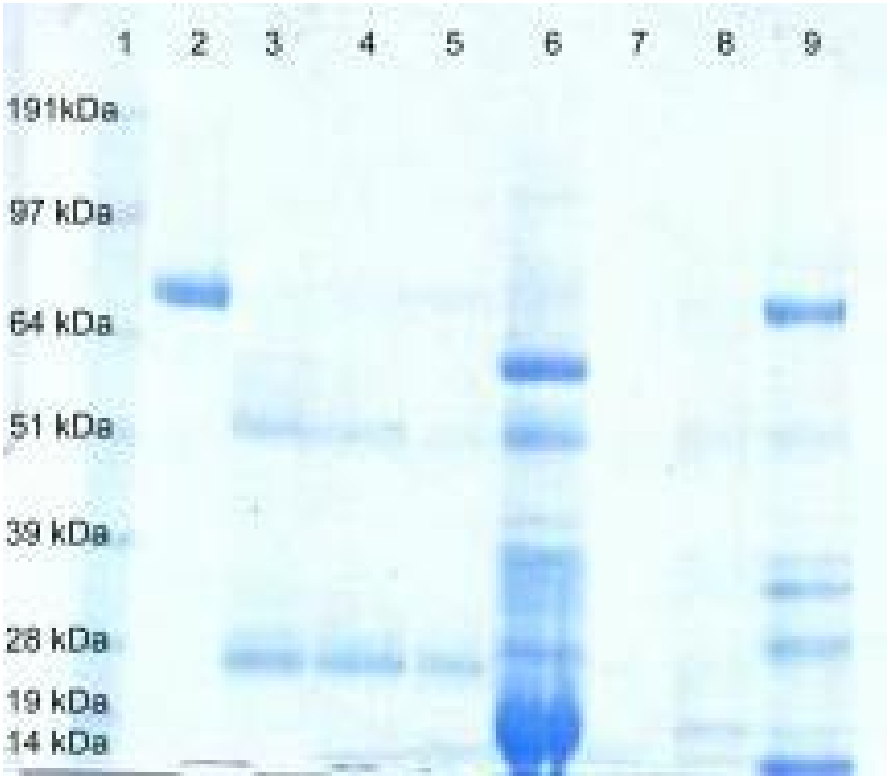
to
1 min. gradient
to

60 mL pH 11 buffer

3. Chromatographic Separation of Proteins from Cheese Whey (cont.)

Well	Sample
1	MW Markers
2	Lactoferrin Standard
3	Peak 2a Colostrum pH 6.5 to 11
4	Peak 2b Colostrum pH 6.5 to 11
5	Peak 2c Colostrum pH 6.5 to 11
6	Peak 1 Trial 1 4/3/03
7	Peak 2 Trial 1 4/3/03
8	Peak 3 Trial 1 4/3/03
9	Peak 4 Trial 1 4/3/03

Lactoperoxidase and/or Lactoferrin appear to be in Lane 9, Peak 4.



3. Effects of pH Gradient on Peak Resolution

500 ml of a solution of 5 g/L whey protein powder were loaded in the column HiPrep 16/10 SP XL and eluted using gradients from 0 to 85% pH11 (+ 15% pH 6.5 yielding pH 8.5 solution) in 2, 4, 6, 8, 10, 12 and 14 min, using program 2.

Program 2

Breakpoint (min)	Conc %B	Flow rate (ml/min)	Fraction volume (ml)	Tube A	Tube B	Valve position
0	0	3	5	pH 6.5	pH 11	Load
20	0	3	5	pH 6.5	pH 11	Load
(20+x)	85	3	5	pH 6.5	pH 11	Load
(40+x)	85	3	5	pH 6.5	pH 11	Load
(43+x)	100	3	5	pH 6.5	pH 11	Load
(58+x)	100	3	5	pH 6.5	pH 11	Load

Where x is the time for the pH gradient from 0% pH 11 to 85% pH 11.

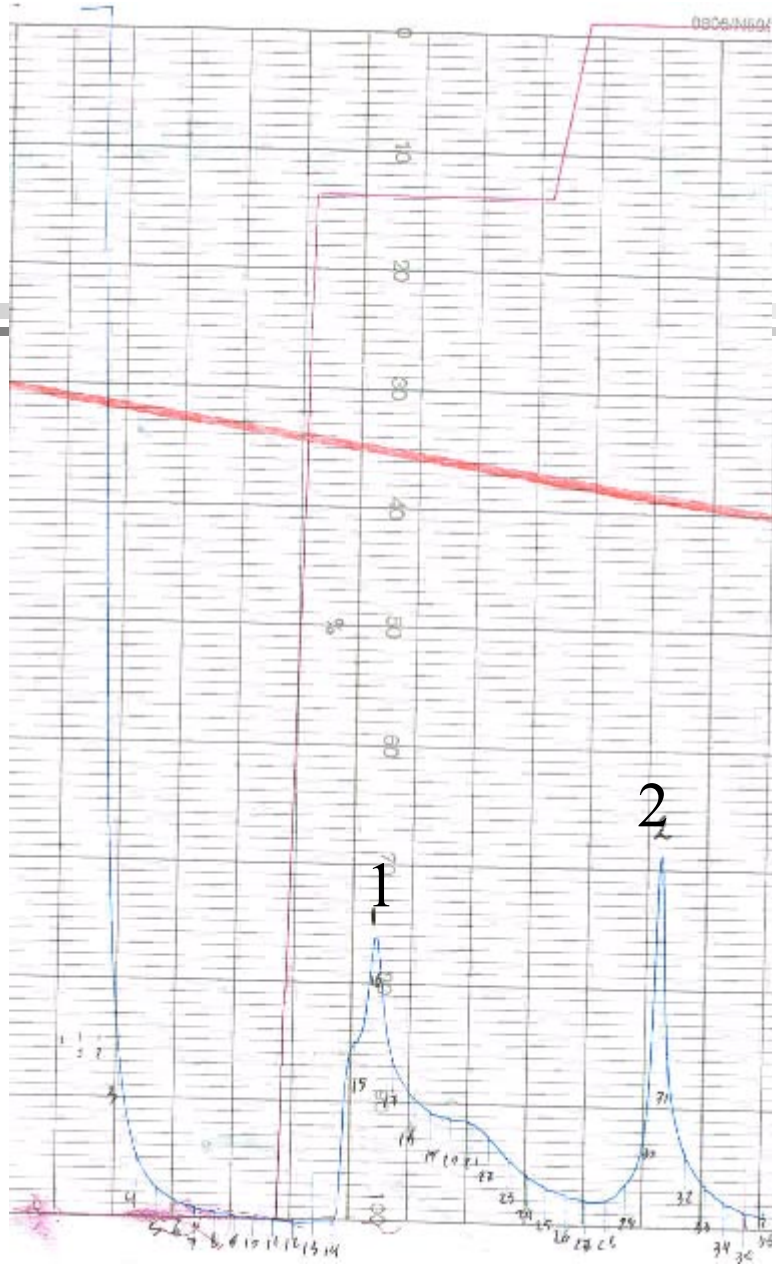


***Figure 2**. Change of 0% pH 11 to 85% pH 11 in 2 min.*

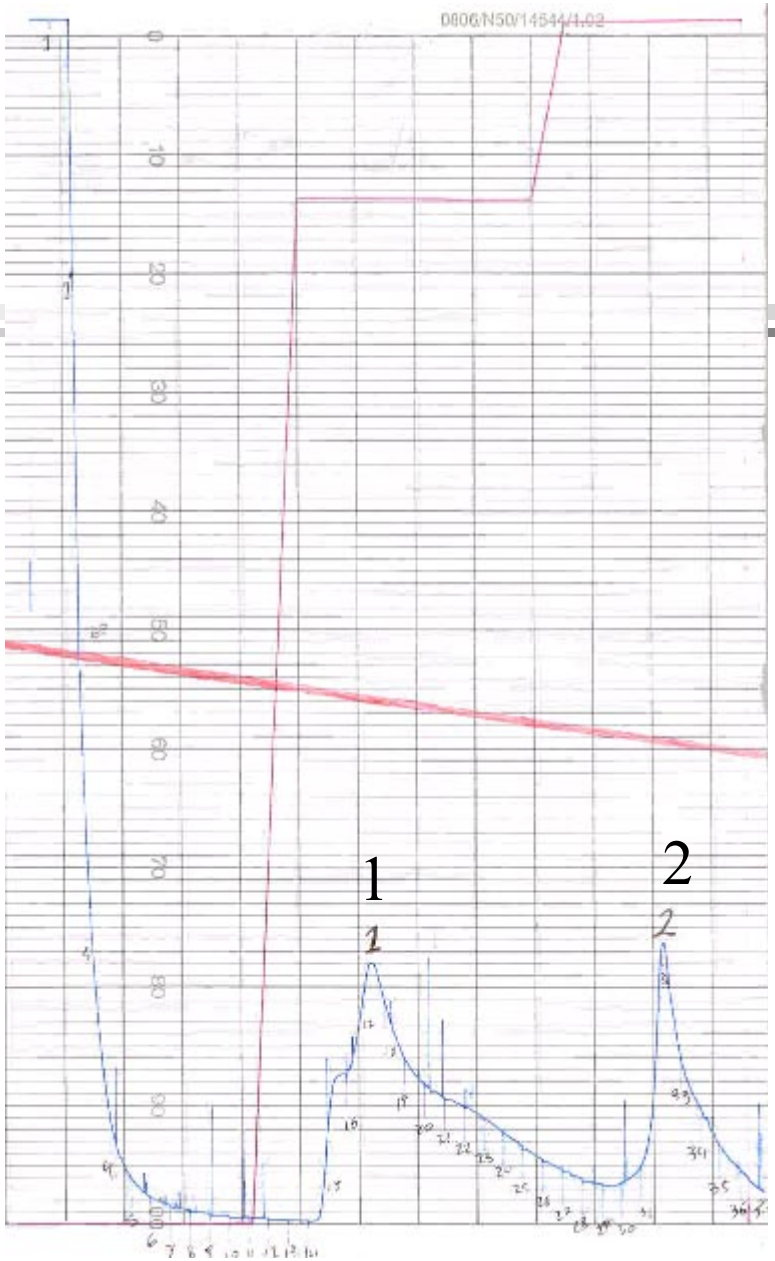


***Figure 3.** Change of 0% pH 11 to 85% pH 11 in 4 min.*

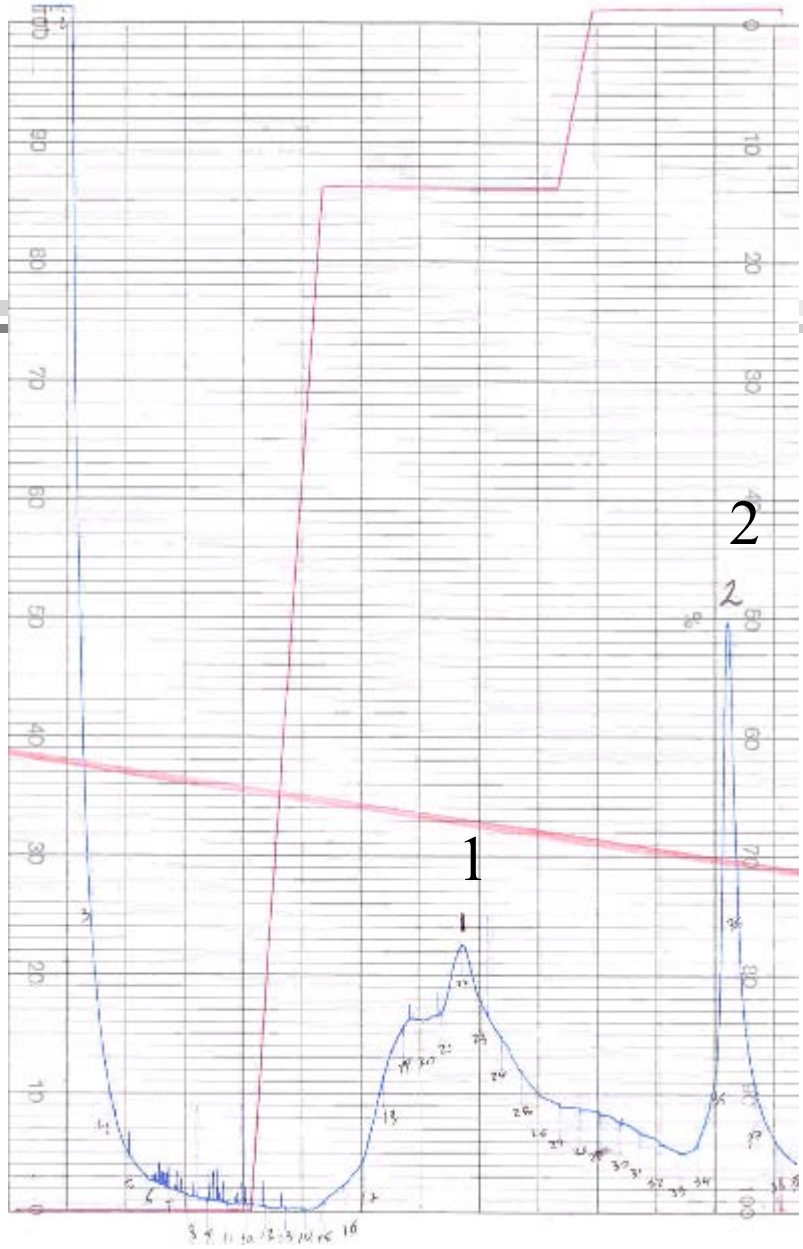


Figure 4. *Change of 0% pH 11 to 85% pH 11 in 6 min.*

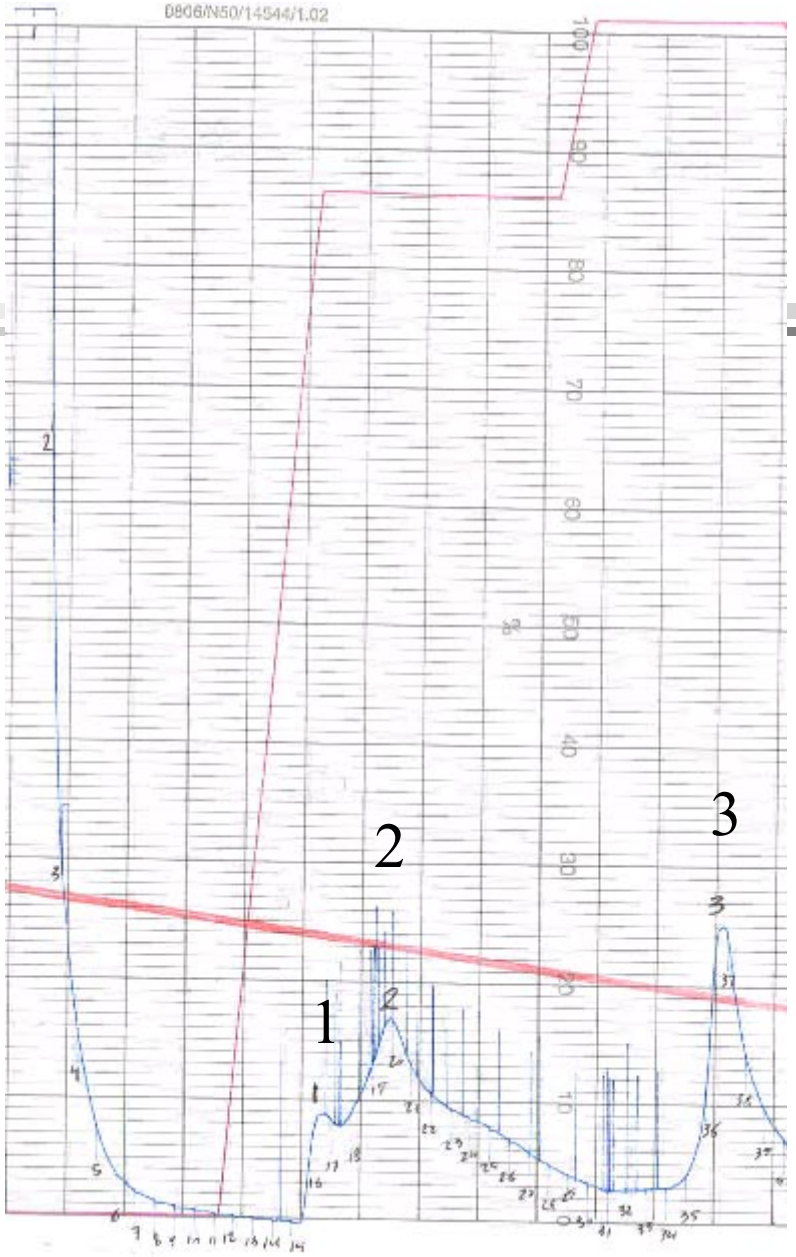


Figure 5. *Change of 0% pH 11 to 85% pH 11 in 8 min*

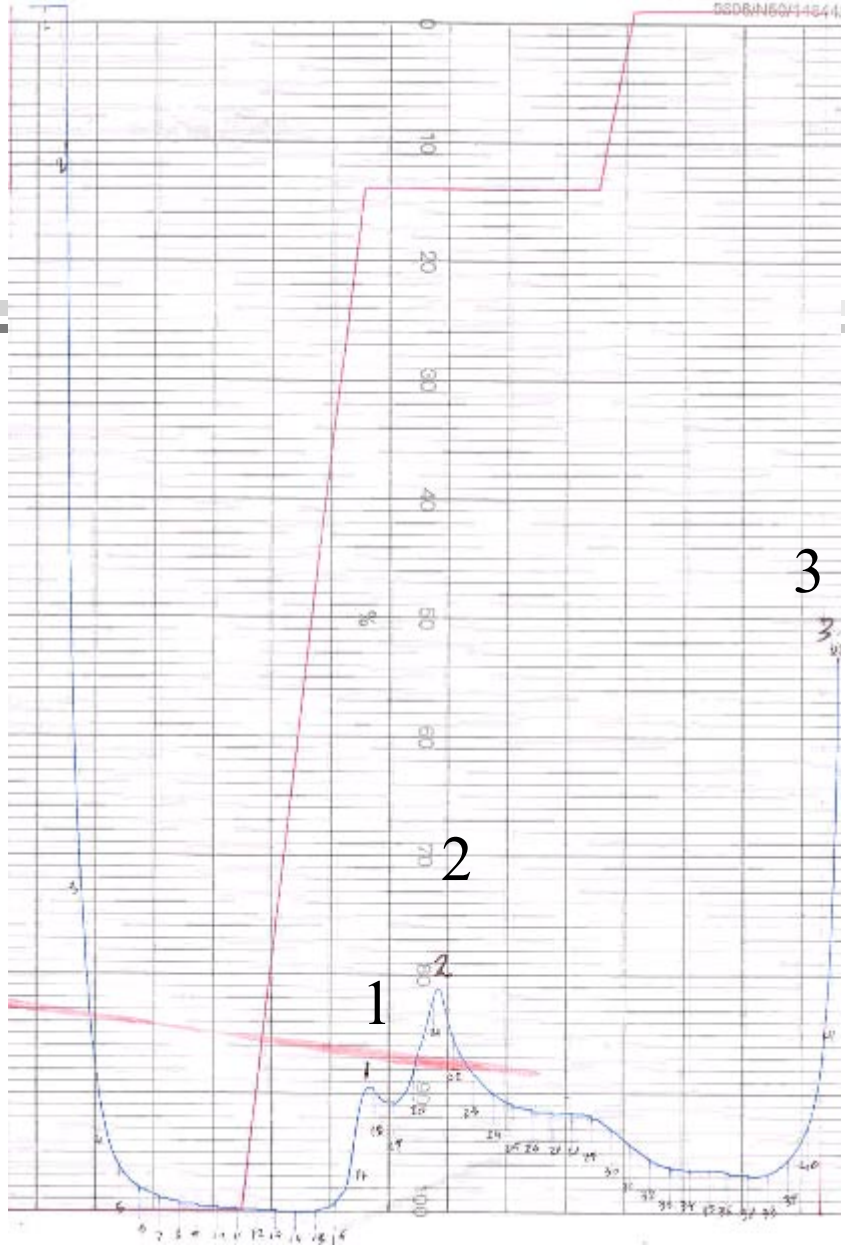


***Figure 6**. Change of 0% pH 11 to 85% pH 11 in 10 min.*

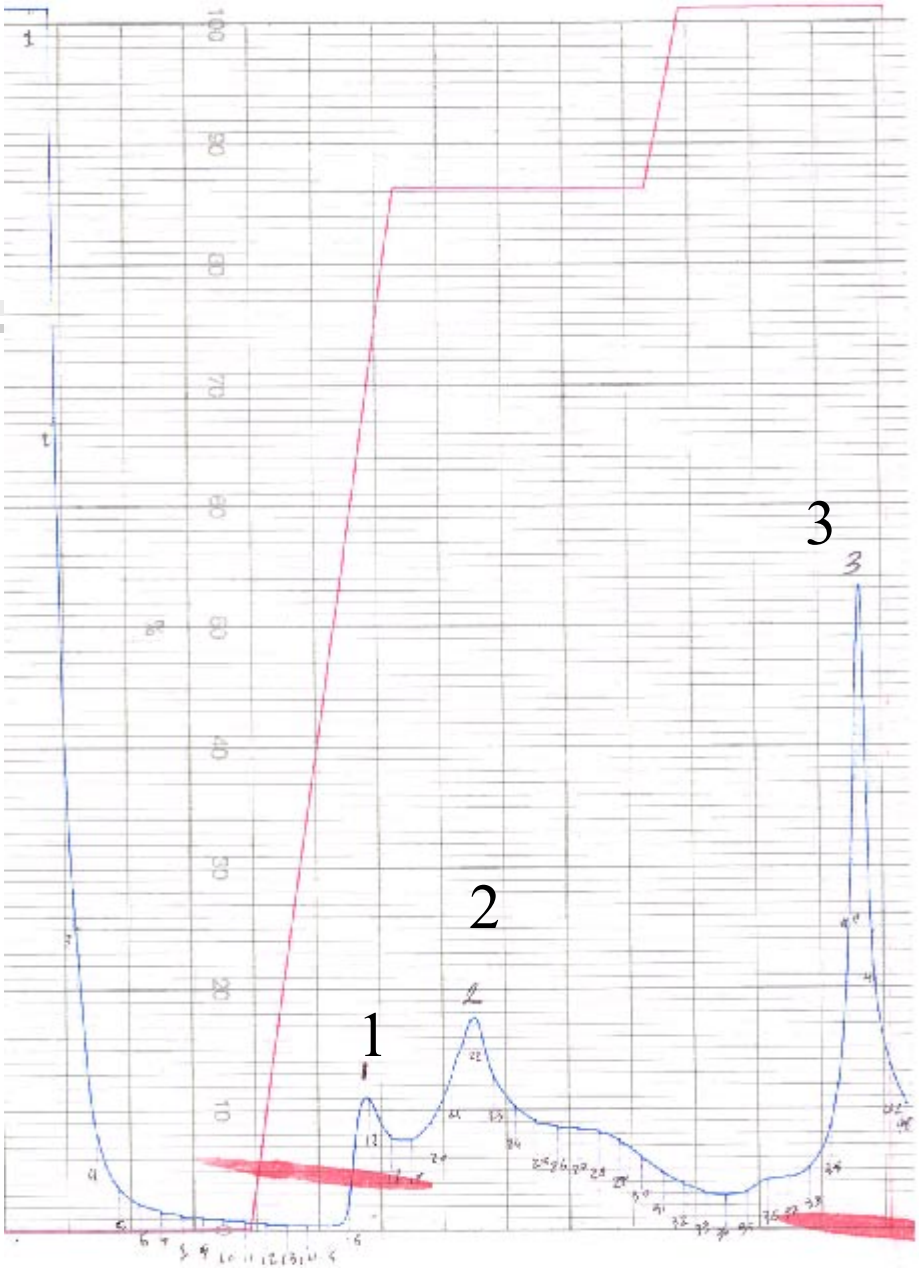


***Figure 7.** Change of 0% pH 11 to 85% pH 11 in 12 min.*

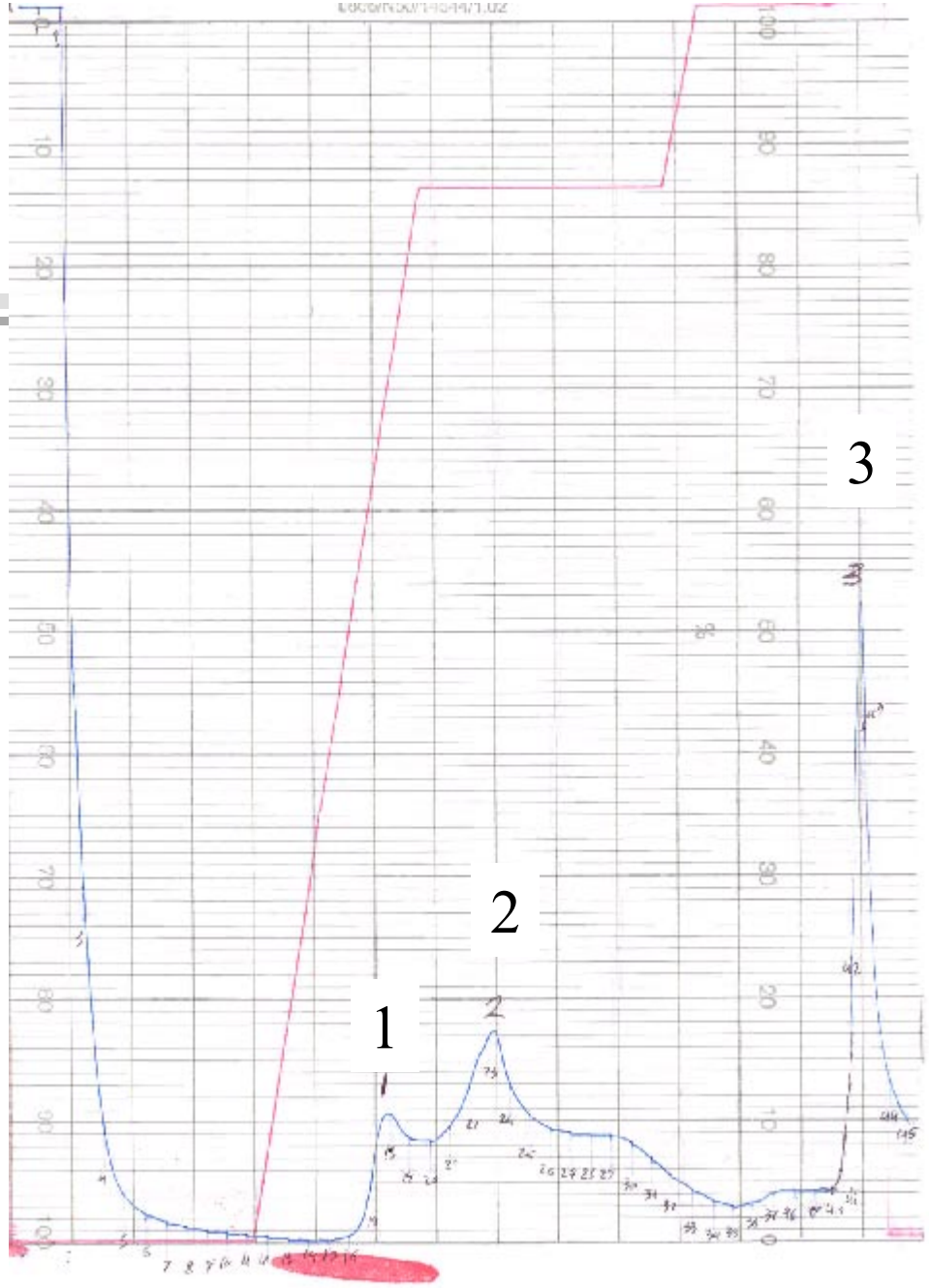


Figure 8. *Change of 0% pH 11 to 85% pH 11 in 14 min.*

4. Product Preparation / Crystallization

Crystallization is a nucleation process started from a concentrated solution:

1. Occurs when concentration exceeds saturation

2. Crystals have a well-defined morphology, large particle size

3. Homogeneous nucleation occurs when a solid interface is absent

4. Heterogeneous nucleation occurs when a foreign interface is present.

5. Secondary nucleation occurs in the presence of a crystal interface of the same solute

4. Product Preparation / Crystallization

Critical cluster or nucleus is the largest cluster of molecules just prior to spontaneous nucleation:

1. n* is the number of molecules in the critical nucleus.

2. Subcritical clusters refers to when, n < n*

3. Supercritical clusters refers to when n > n*

4. An embryo is a cluster having n = n*.

5. An embryo or critical nucleus can range from 10 nm to several μm in size.

4. Product Preparation / Crystallization

Steps in nucleation and crystal growth

$B + B \Leftrightarrow B_2 + B \Leftrightarrow B_3 + B$ .....

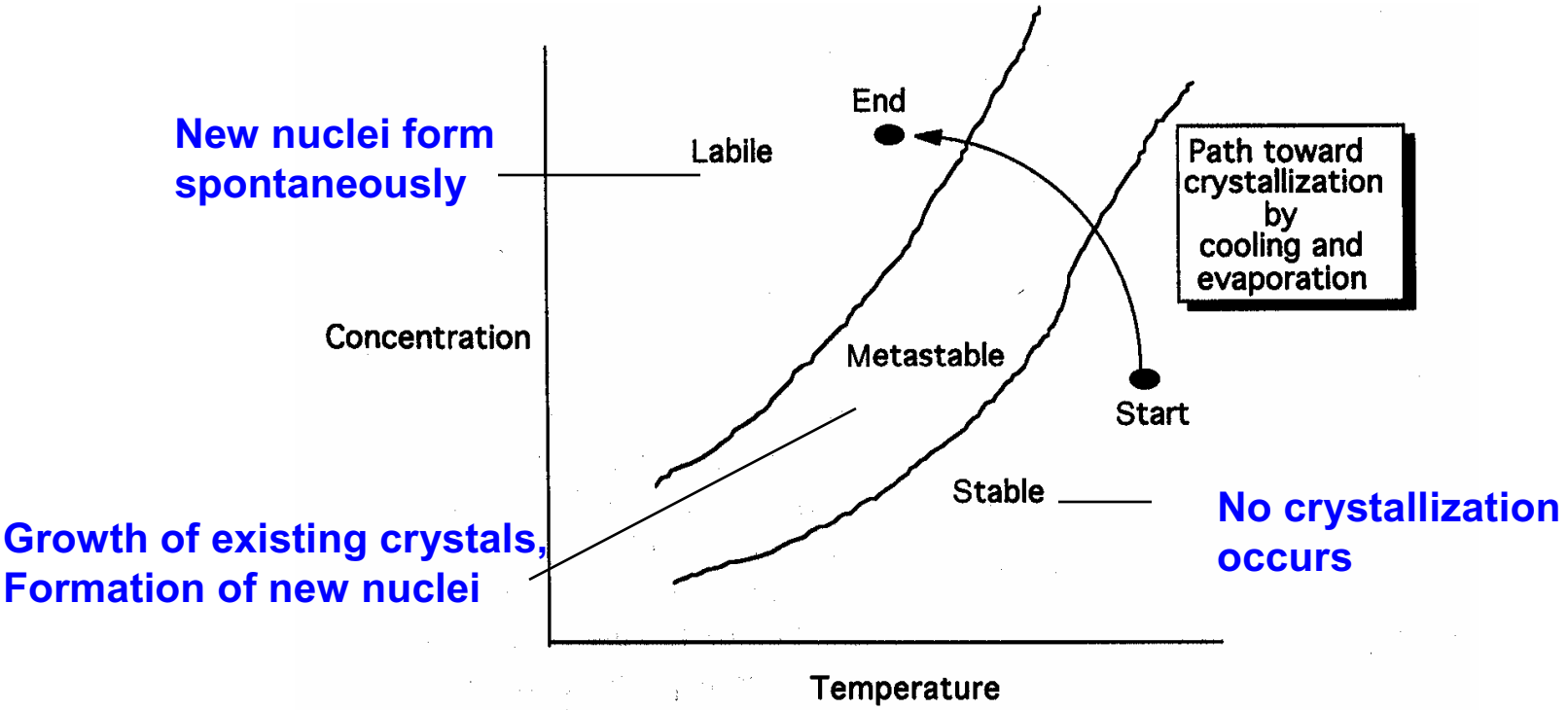
$B_{n-1} + B \Leftrightarrow B_n$ a critical cluster is formed

$B_n + B \Leftrightarrow B_{n+1} \Downarrow$ which undergoes nucleation

$B_{n+1} + B \Rightarrow$ which undergoes crystal growth

4. Product Preparation / Crystallization

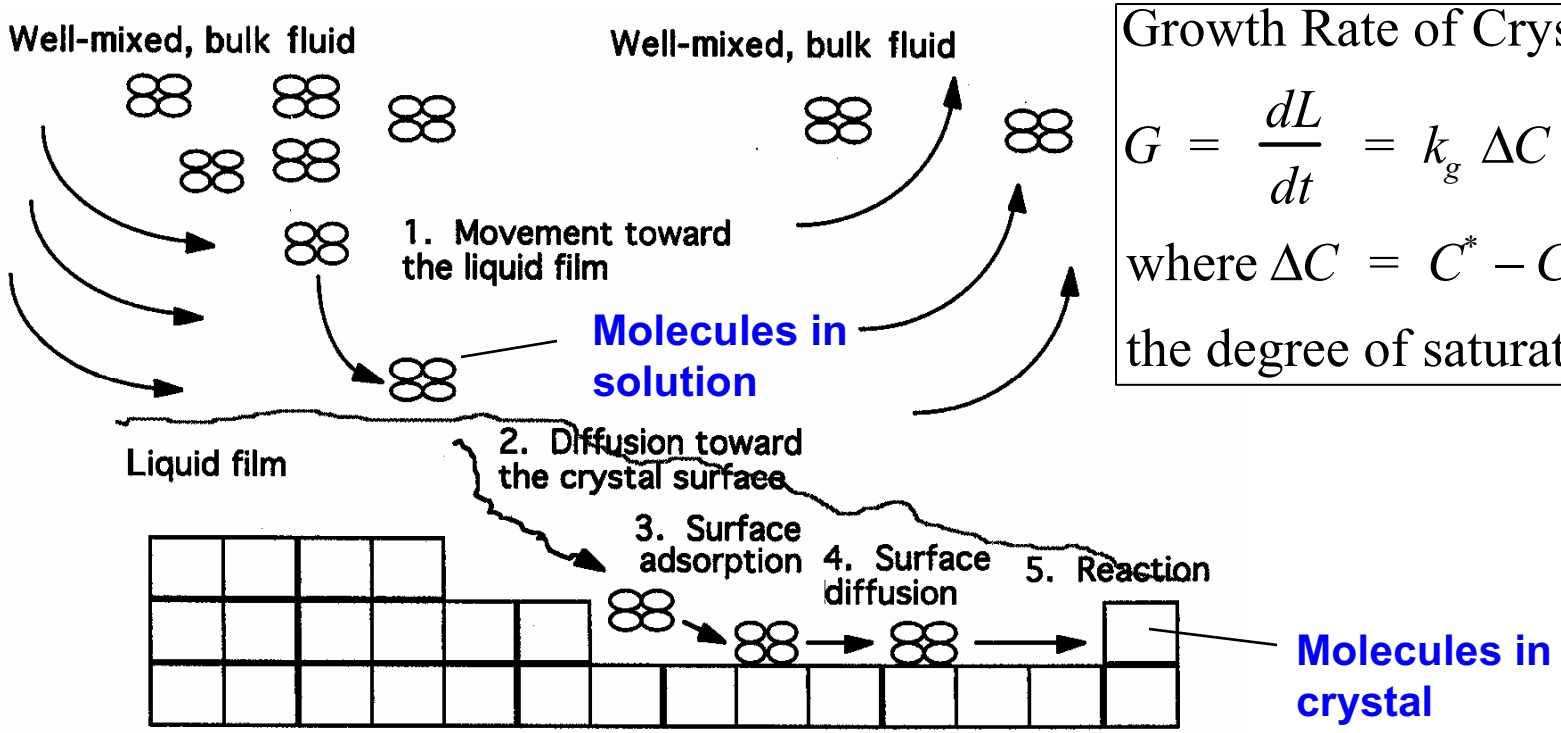
Characteristic zones of crystallization



"Bioseparation Process Science"
Garcia et al., Blackwell Science, 1999

4. Product Preparation / Crystallization

Transport Processes During Crystallization



Growth Rate of Crystals,

$$G = \frac{dL}{dt} = k_g \, \Delta C$$

where $\Delta C = C^* - C$ is the degree of saturation

"Bioseparation Process Science"
Garcia et al., Blackwell Science, 1999

4. Product Preparation / Crystallization

Thermodynamics of Homogeneous Nucleation

Free Energy Change for Homogeneous Nucleation

$$\Delta G_{Homogeneous} = \Delta G_{Surface\ formation} + \Delta G_{Clustering}$$

$$\Delta G_{Surface\ formation} = 4\pi r^2 \gamma_{sl}$$

"Bioseparation Process Science" Garcia et al., Blackwell Science, 1999, Pages127-140

where γ_{sl} is the surface tension of the solid / liquid interface

$$\Delta G_{Clustering} = -RT \ln\left(\frac{C}{C^*}\right) \Big/ \frac{4/3\pi r^3}{V_{molar,solid}}$$

The critical nucleus, r_c, is where there is a maximum in $\Delta G_{Homogeneous}$

$$\frac{d\Delta G_{Homogeneous}}{dr} = 0 = 8\pi r_c \gamma_{sl} - RT \ln\left(\frac{C}{C^*}\right) \frac{4\pi r_c^2}{V_{molar,solid}}$$

$$r_c = \frac{2\gamma_{sl} V_{molar,solid}}{RT \ln\left(\frac{C}{C^*}\right)}$$

Useful calculation when seeding a Crystallization process

4. Product Preparation / Crystallization

Rate of Formation of Nuclei,dN/dt

Nucleation is analogous to reaction kinetics,

$$B^0 = \frac{dN}{dt} = A \exp\left(- \frac{\Delta G_{\max}}{RT} \right)$$

$$= A \exp\left(- \frac{16\pi \gamma_{sl}^3 V_{\text{molar,solid}}^2}{3R^3T^3 \left(\ln\left(\frac{C}{C^*} \right) \right)^2} \right)$$

4. Product Preparation / Crystallization

Batch Crystallization, Solid Phase Balance

1. Cummulative Number of Crystals, N

versus size, L

or

2. Population Density, n

Slope of N vs L curve

A balance on n tracks the number of crystals entering and leaving a specific size range due to crystal growth.

4. Product Preparation / Crystallization

Batch Crystallization, Population Balance Equation

$$\begin{bmatrix}\text{Number of}\\ \text{crystals initially}\\ \text{within range, } L\end{bmatrix} + \begin{bmatrix}\text{Number of}\\ \text{crystals growing}\\ \text{into range, } L\end{bmatrix} = \begin{bmatrix}\text{Number of}\\ \text{crystals at end}\\ \text{within range, } L\end{bmatrix} + \begin{bmatrix}\text{Number of}\\ \text{crystals growing}\\ \text{out of range, } L\end{bmatrix}$$

$$V\, n_{\text{initial}}\, \Delta L + V\, G_1\, n_1\, \Delta t = V\, n_{\text{final}}\, \Delta L + V\, G_2\, n_2\, \Delta t$$

V is volume, ΔL is size range, G is growth rate of crystal size (dL/dt), Δt is a small time step.

subscript 1 is a smaller size range,

subscript 2 is size range for ΔL.

4. Product Preparation / Crystallization

Batch Crystallization, Population Balance Equation (cont.)

divide by V, ΔL, and Δt and allow ΔL and Δt to go to 0.

$$\frac{dn}{dt} + \frac{d(Gn)}{dL} = 0$$

Assuming G is a constant over all L

$$\frac{dn}{dt} + G\frac{dn}{dL} = 0$$

4. Product Preparation / Crystallization

Batch Crystallization, Population Balance Equation (cont.)

boundary conditions (BCs) for nucleation

at $t = 0, n = 0$

at $L = 0, n = \frac{B^0}{G}$

as $L \to \infty, n$ is finite

solve population balance equation and BCs using Laplace Transforms

$\bar{n} = \frac{B^0}{Gs} \exp\left(-\frac{Ls}{G} \right)$ in the Laplace Domain

$n = B^0 \, u\left(t - \frac{L}{G} \right)$

4. Product Preparation / Crystallization

Batch Crystallization, Cumulative Crystal Mass

M is cumulative crystal mass per unit volume

$$M = \rho_c \, k_v \int_0^L n L^3 \, dL$$

where ρ_c is density of crystal solid and k_v is a shape factor

and as $L \to \infty$,

$$M = W = \frac{1}{4} \rho_c \, k_v \, B^0 \, G^3 \, t^4$$

4. Product Preparation / Crystallization

Batch Crystallization, Cooling Curve

Determine the time - temperature relationship to achieve a constant degree of supersaturation during batch crystallization

rate of change of solute concentration = - rate of change of W

$$\frac{dC}{dt} = -\frac{dW}{dt}$$

$$\frac{dC}{dt} = -\rho_c \, k_v \, B^0 \, G^3 \, t^3$$

4. Product Preparation / Crystallization

Batch Crystallization, Cooling Curve (cont.)

to achieve a constant degree of supersaturation, the rate of temperature change must be proportional to $\frac{dC}{dt}$

$$\frac{dC}{dt} = k_T \frac{dT}{dt} = -\rho_c k_v B^0 G^3 t^3$$

integrating from the temperature that crystals start to form, T_0, at $t = 0$, we find that

$$T_0 - T = \frac{\rho_c k_v B^0 G^3 t^4}{4 k_T}$$

4. Product Preparation / Crystallization

Continuous Crystallization, Solid Phase Balances

$$\begin{bmatrix}\text{Number of}\\ \text{crystals growing}\\ \text{into range, } \Delta L,\\ \text{over a time, } \Delta t\end{bmatrix} + \begin{bmatrix}\text{Number of}\\ \text{crystals entering}\\ \text{range, } \Delta L, \text{ by flow}\end{bmatrix} = \begin{bmatrix}\text{Number of}\\ \text{crystals growing}\\ \text{out of range, } \Delta L,\\ \text{over a time, } \Delta t\end{bmatrix} + \begin{bmatrix}\text{Number of}\\ \text{crystals leaving}\\ \text{range, } \Delta L, \text{ by flow}\end{bmatrix}$$

$$V G_1 n_1 \Delta t + Q n_{\text{in}} \Delta L \Delta t = V G_2 n_2 \Delta t + V n \Delta L \Delta t$$

V is volume, ΔL is size range, G is growth rate of crystal size (dL / dt), Δt is a small time step, Q is volumetric flow rate through crystallizer.

subscript 1 is a smaller size range,

subscript 2 is size range for ΔL,

subscript in is for inlet conditions.

divide by ΔL , and Δt and allow ΔL and Δt to go to 0,

and assuming that no crystals are entering, $n_{in} = 0$,

and that G is constant.

$$V G\frac{dn}{dL} + Q n = 0$$

Restating in terms of residence time, $\tau = \frac{V}{Q}$

$$\frac{dn}{dL} + \frac{n}{G\tau} = 0$$

Boundary Condition, $L = 0$, $n = n^o = \frac{B^o}{G}$

4. Product Preparation / Crystallization

Continuous Crystallization, Solid Phase Balances

Population density solution,

$$n = n^o \exp\left(-\frac{L}{G\tau}\right)$$

$$M = \rho_c k_v \int_0^L n L^3 \, dL$$

where ρ_c is density of crystal solid and k_v is a shape factor

$$M = 6\,\rho_c k_v n^o G\tau\left(G^3\tau^3 - \left(G^3\tau^3 + G^2\tau^2 L + \frac{1}{2}G\tau L^2 + \frac{1}{6}L^3\right)\exp\left(-\frac{L}{G\tau}\right)\right)$$

and as $L \rightarrow \infty$,

$$M = W = 6\rho_c k_v n^o G^4 \tau^4$$

4. Product Preparation / Crystallization

Continuous Crystallization, Advantages

Advantages:

1. Input of solute helps to maintain a constant degree of saturation, ΔC

2. Desirable for determining growth rates and other kinetic parameters, but are not popular in industrial applications.