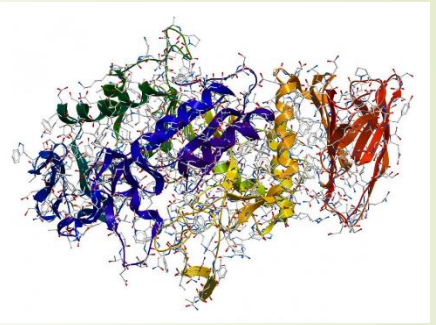
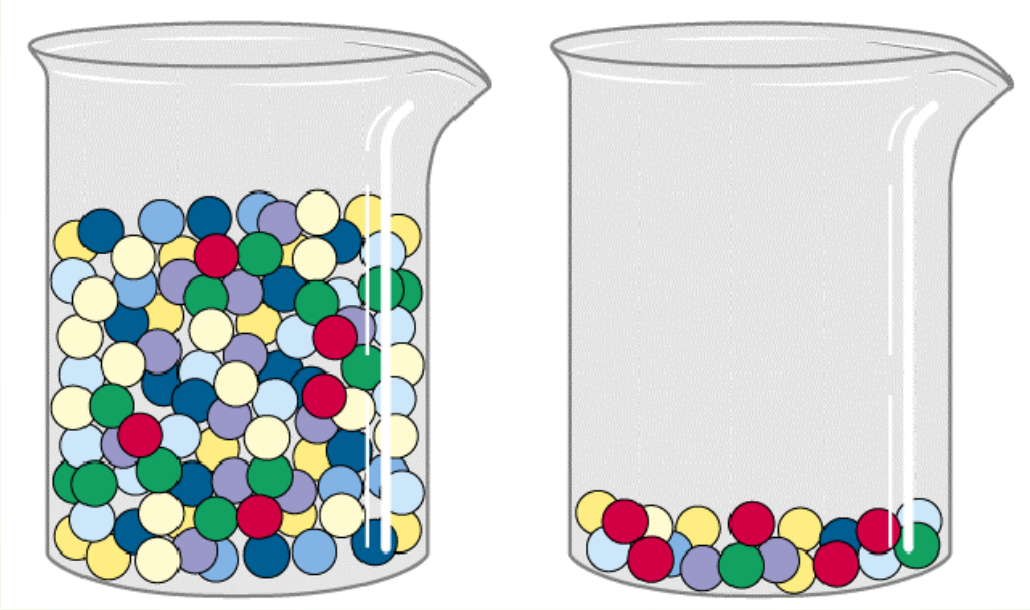


CEN-CHE 422
ENZYME ENGINEERING

Enzyme Separation and Purification Methods-3

Purification of Proteins

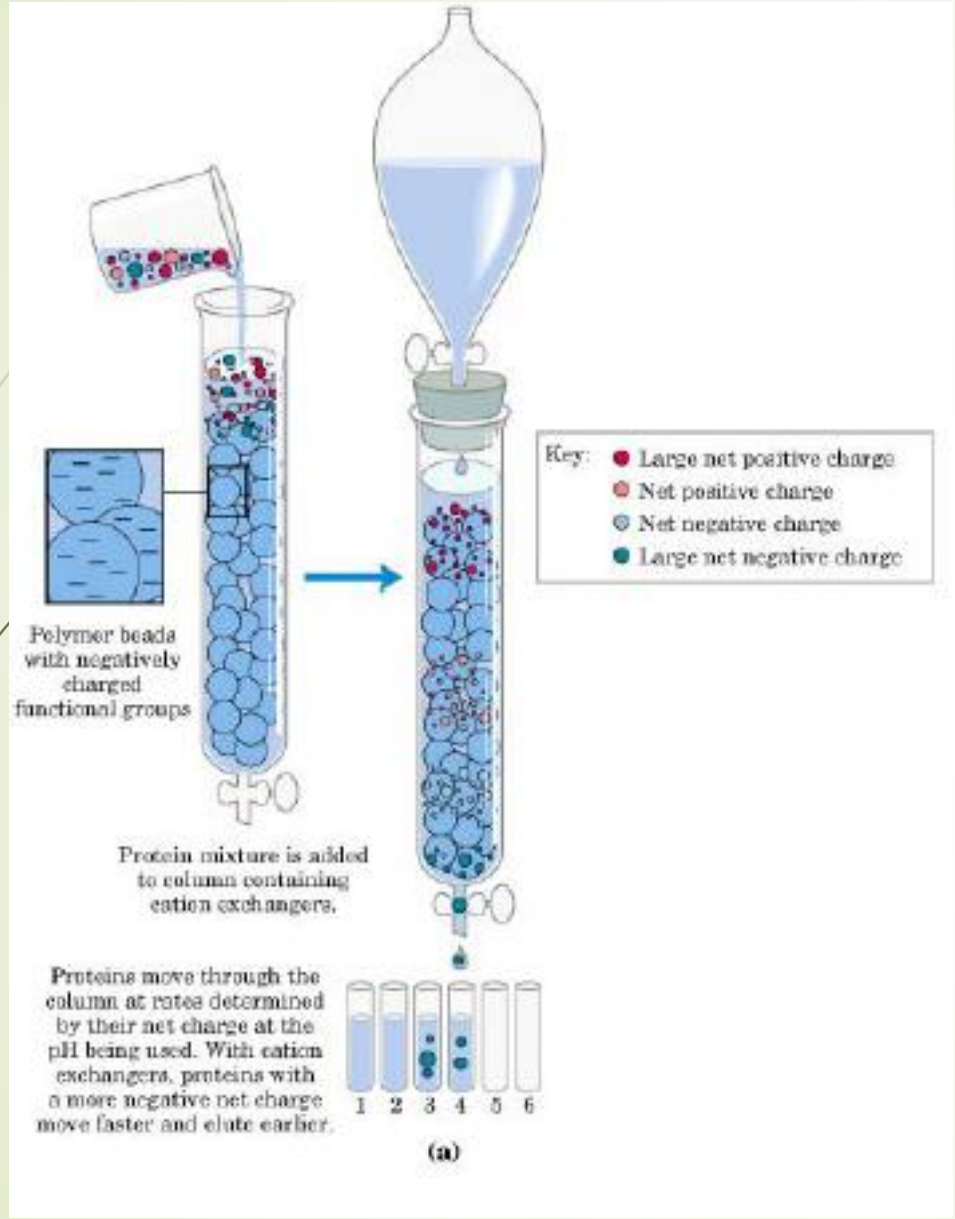
"Chromatographic Methods"



Proteins differs from each other with their size and charge

- Stationary phase
- Mobile phase

Ion Exchange Chromatography



Charge of the protein:

Zero at pH=pI ;
Negative at pH>PI
Positive at pH<pI
Each protein has a characteristic pI (isoelectric point)

Anion exchange:

Resin: Positively charged
pH>pI

The protein of interest remains in the resin and is removed by the salt gradient.
The protein with the highest pI is removed first.

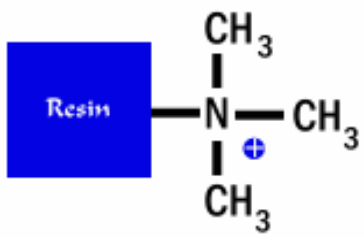
DEAE Cellulose veya (Sephadex)

Cation exchane:

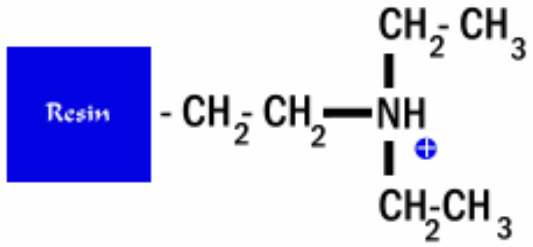
Negatively charged resin
The protein with the highest pI value is eluted last.

CM Cellulose or Sephadex

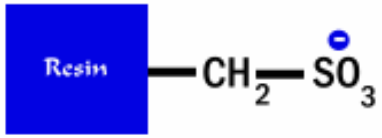
Ion Exchange Chromatography



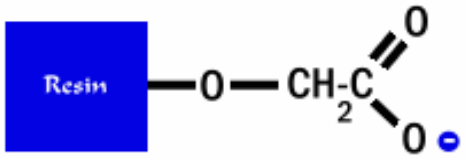
Q-anion exchanger



DEAE-anion exchanger

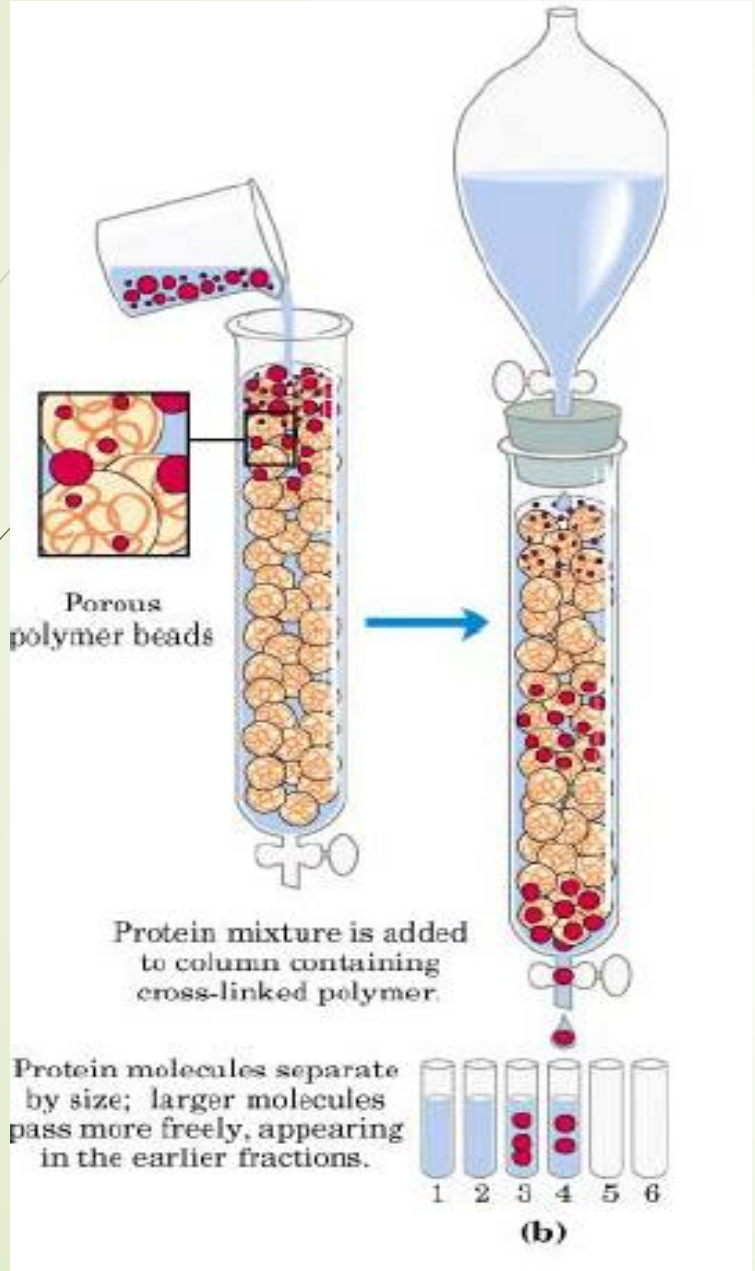


S-cation exchanger



CM-cation exchanger

Gel Filtration



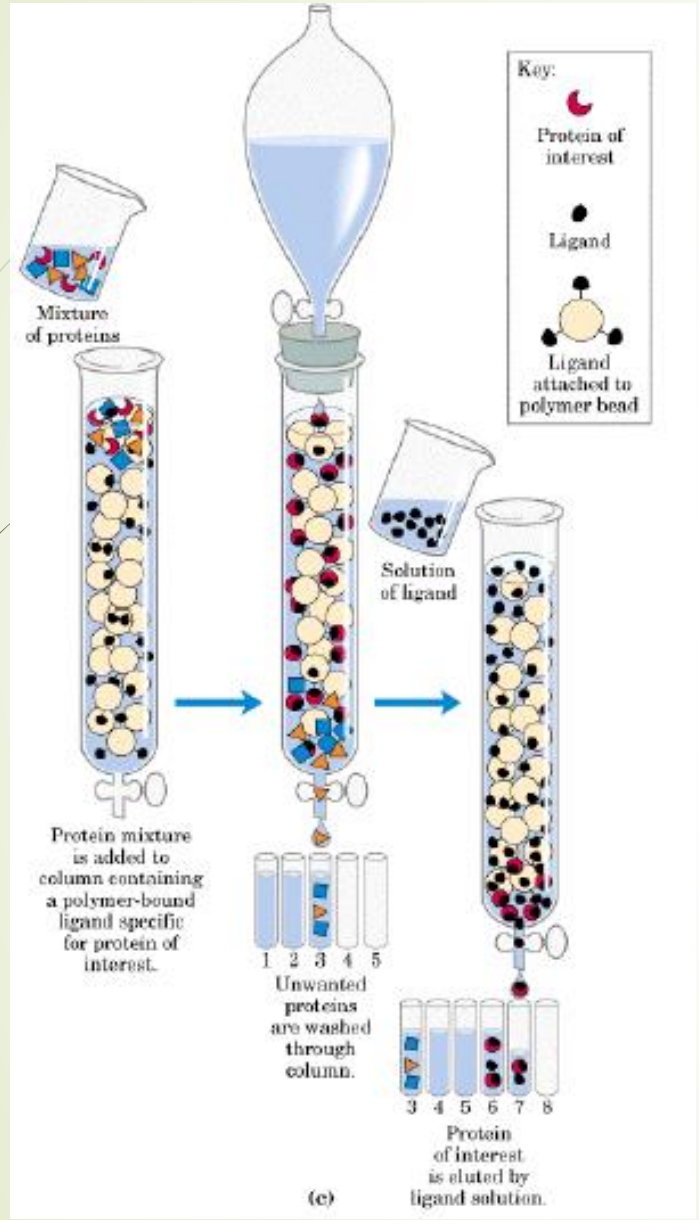
Porous beads made of different material.

Proteins that are small enough to get into the pores get inside the beads, while the larger ones pass around

It can be used for molecular weight determination.

The gel can be dextran, agar or polyacrylamide

Affinity Chromatography



A ligand that will bind the protein tightly is attached to the matrix.

Protein of interest binds, others pass

Centrifugation

Ultracentrifuge: Although the sedimentation rate depends on the size and shape of the molecule and the viscosity of the solution, the rate increases as the molecular weight increases.

Large molecules such as enzymes can be separated by ultracentrifugation in the high centrifuge area (up to 300,000 times gravity = 300,000 x g). However, this method is not very useful for separating enzymes from each other, because only small volumes (a few cm3) can be processed in the ultracentrifuge.

Centrifuge: It is widely used to remove the precipitated or insoluble material or to remove the enzyme precipitated with $(NH_4)_2SO_4$. In this case, low centrifuge areas (5000-50,000) x g are required and volumes of up to several liters are suitable.

Centrifugal force is used to separate materials that require a force greater than the force of gravity. Centrifugal force separates particles between 100-0.1 μm in diameter.

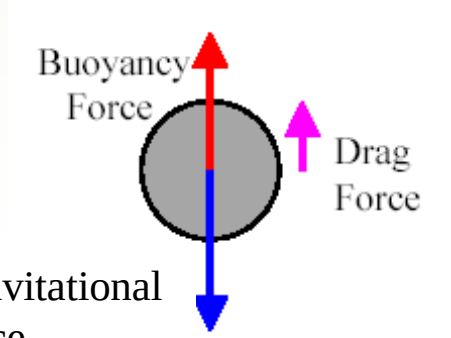
The theory of separation by gravitational force before centrifugal force should be well understood.

$$F_G = F_D + F_B$$

$$F_G = \frac{\Pi}{6} D_p^3 \rho_p \frac{g}{g_c}$$

$$F_B = \frac{\Pi}{6} D_p^3 \rho_f \frac{g}{g_c}$$

$$F_D = \frac{C_D}{2g_c} \rho_f U_o^2 A$$



ρ_f = liquid density
ρ_p= particle density
Uo=the final velocity of the particle or the relative velocity between the fluid and the particle (settling rate)
A=cross-sectional area of the particle perpendicular to the flow

$$A = (\frac{\Pi}{4}) D_p^2$$

$Re_p<0.3$ ise drag force F_D is expressed by Stokes Law:

$$F_D = 3\Pi\mu D_p U_o \frac{1}{g_c}$$

By combining two equations for F_D

$$C_D = \frac{24}{\mathrm{Re}_p}$$

$1<Re_p<10.000$ for a sphere particle C_D is approx:

$$C_D = \frac{24}{\mathrm{Re}} + \frac{3}{(\mathrm{Re})^{1/2}} + 0.34$$

Here: $$\mathrm{Re}_p = \frac{D_p U_o \rho_f}{\mu}$$

If we re-write the equation of forces:

$$3\Pi\mu D_p U_o = \frac{\Pi}{6} D_p^3 (\rho_p - \rho_f) g$$

$$U_o = \frac{g D_p^2 (\rho_p - \rho_f)}{18\mu}$$

In the centrifuge area; the final separation rate (settlement rate), of the particles (Uoc,) is given by the following equation (Centrifugal force has replaced gravity)

$$U_{oc} = \frac{r\omega^2 D_p^2 (\rho_p - \rho_f)}{18\mu}$$

$$g = r\varpi^2$$ ω = angular velocity

$$\omega = 2\Pi N r$$ r = distance from center of rotation

$$Z = \frac{r\omega^2}{g}$$ centrifuge factor

$$U_{oc} = \frac{gZD_p^2(\rho_p - g_f)}{18\mu} = ZU_o$$

To increase the particle settling rate in the centrifuge:

- Centrifuge speed should be increased
- Particle diameter should increase
- The difference between particle and liquid densities should increase
- The viscosity of the solution should be lowered