Separation Sciences

1. Introduction: Fundamentals of Distribution Equilibrium

2. Gas Chromatography (Chapter 2 & 3)

3. Liquid Chromatography (Chapter 4 & 5)

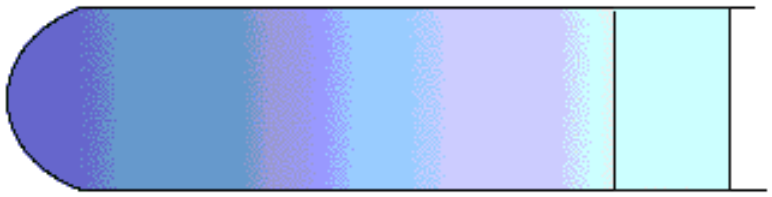
4. Other Analytical Separations (Chapter 6-8)
    a. Planar chromatography
    b. Supercritical fluid chromatography
    c. Electrophoresis
    d. Centrifugation
    e. Field Flow Fractionation

Centrifugation

1. Introduction. *Centrifugation* is a technique in which solutes are separated by their different rate of travel (or sedimentation) in a centrifugal field.

2. Centrifugation is widely used in biological separation.
The solutes are usually cells, Sub-cellular organelles, viruses, large molecules such as proteins and nucleic acids.

Theodor Svedberg



5 to 30 % Sucrose gradients Protein sample

Molecules sediment according to their size, shape and density.

2. Theory of centrifugation

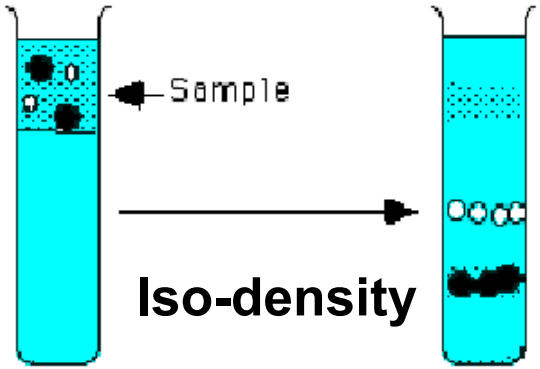
Sedimentation rate of solutes is determined by their *size, shape, and density*, and the density and viscosity of media. The ability of an solute to centrifugal (or gravitational) field can be described by sedimentation coefficient (S = v/C): v linear velocity of a solute, C: centrifugal acceleration.

For a spherical solute:

$$S = 2/9\ r^2(d-d_o)/\eta$$

Centrifugal force

S is the sedimentation coefficient and is usually expressed in Svedbergs (S) or 10^{-13} sec.
r: radius of the solute, d: density of the solute, d_0: density of media, and η :viscosity of the media.



$$\frac{S_2}{S_1} = \left(\frac{r_2}{r_1}\right)^2 * \frac{d_2 - d_0}{d_1 - d_0}$$

Sedimentation coefficients of two solutes in a centrifugal field.

3. Why do we need ultracentrifuge for separations?

Sample	Sed. coeff (S)
whole cells	10^6
cell nuclei	10^5
Mitochondria	10^4
Ribosomes	30, 50 S
soluble proteins	1 - 5 (globular) 5 - 20 (elongated)

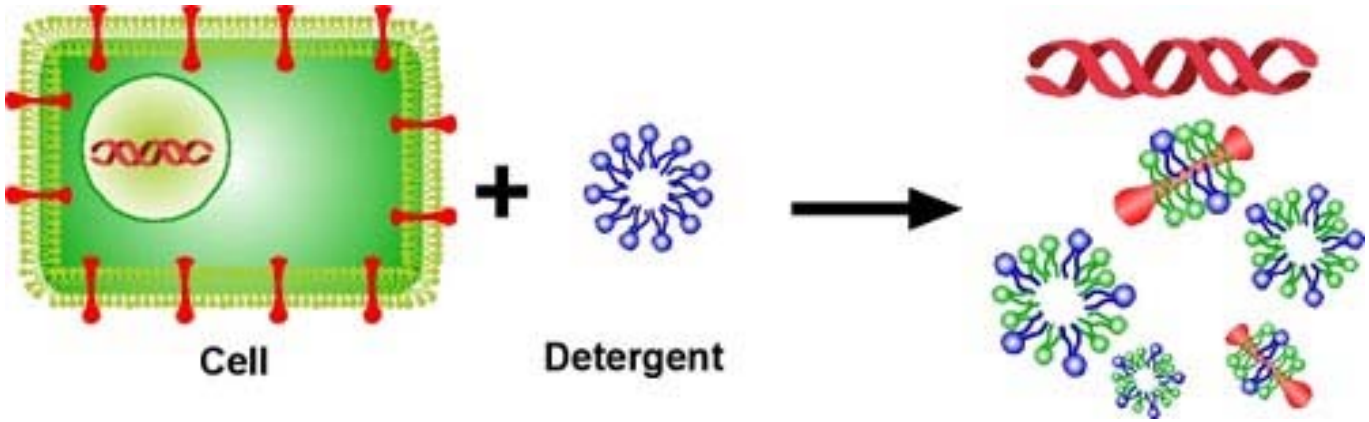
$$S = v/C$$

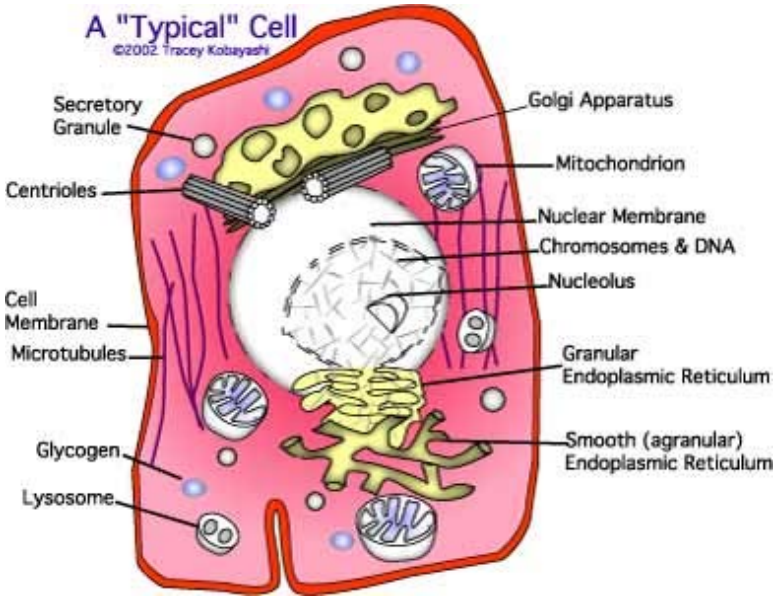
$$C = v/S$$

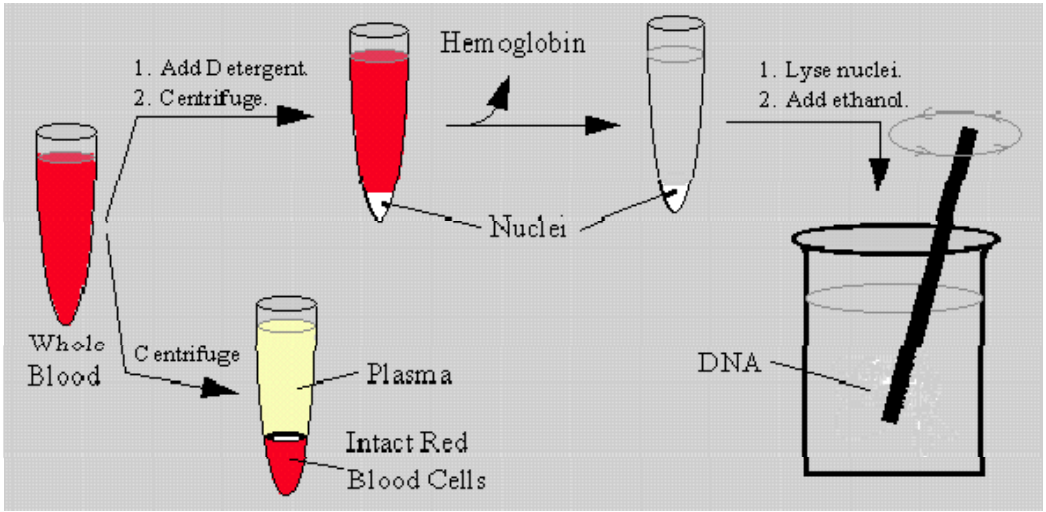
For v = 0.01 mm/s,

C?

4. Biological Applications



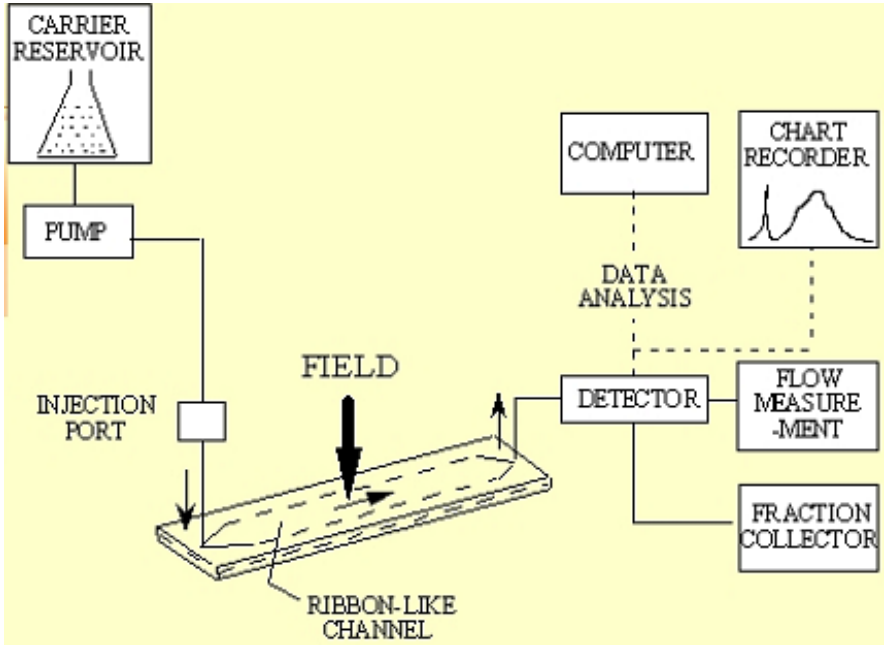




Field Flow Fractionation

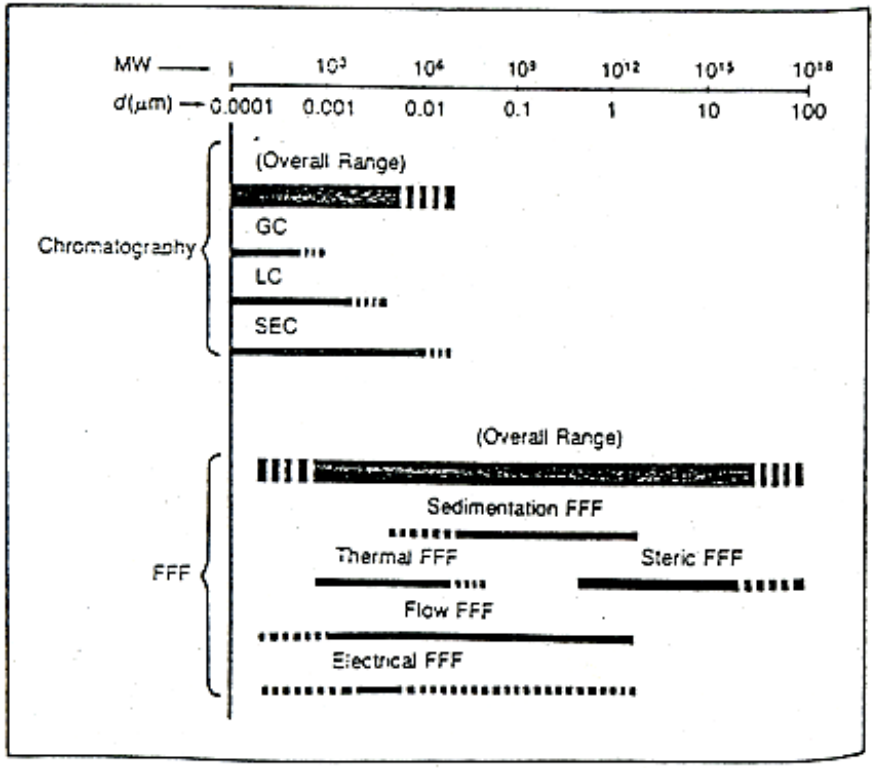
1. Introduction

a. Field flow fractionation (FFF) is a technique in which solutes are separated by their different rate of flow (or travel) through a channel to which a perpendicular field is applied.



b. This technique was first proposed by J. Calvin Giddings in 1966. It is current an area of active research and development.

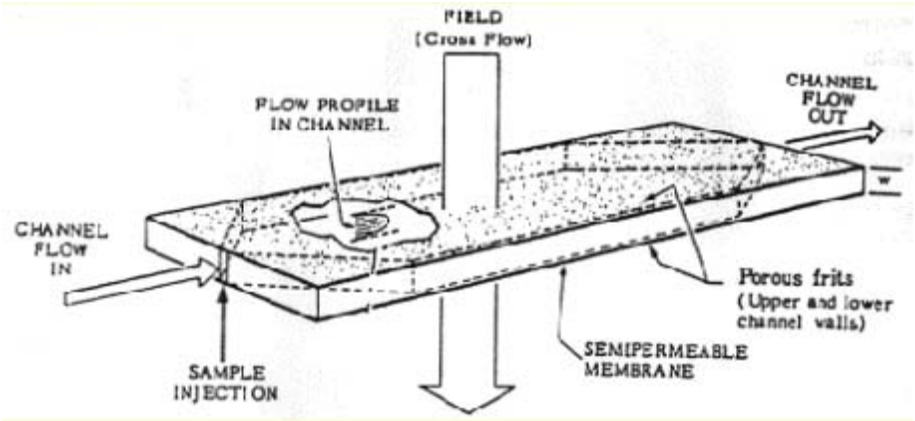
c. One of the major advantages of FFF is that its various techniques can be used to separate molecules over a 10^{15}-fold range of molecular weights. It is very useful for separation of colloids, proteins, polymers, and nanoparticles.

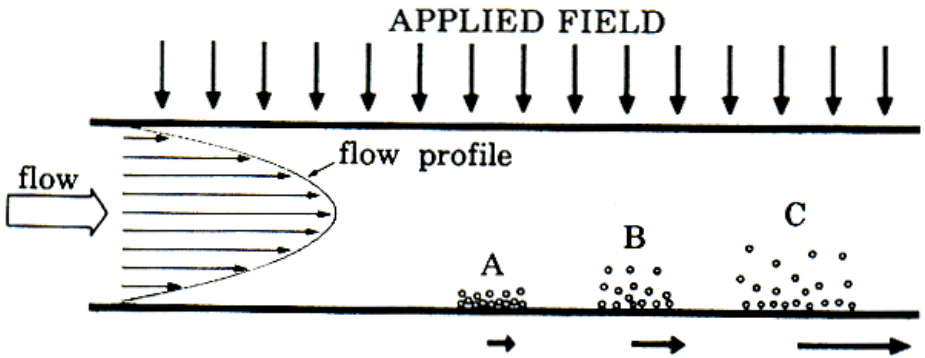


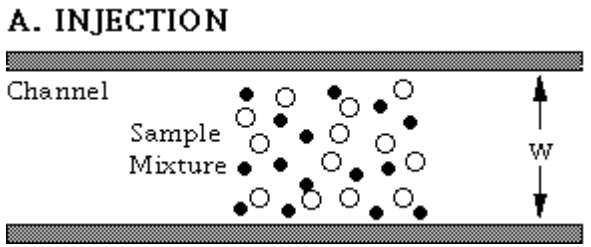
d. Most separation in FFF can be easily described in terms of fundamental physical parameters (e.g. diffusion coefficients, electrophoretic mobility, or sedimentation coefficient). This makes it easy to predict and optimize FFF separations.

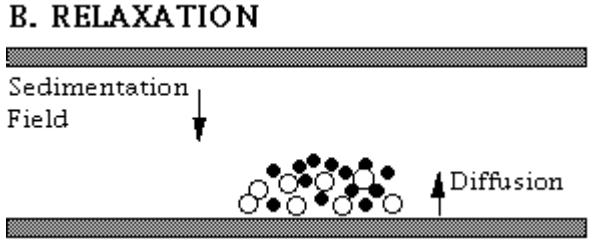
2. Theory

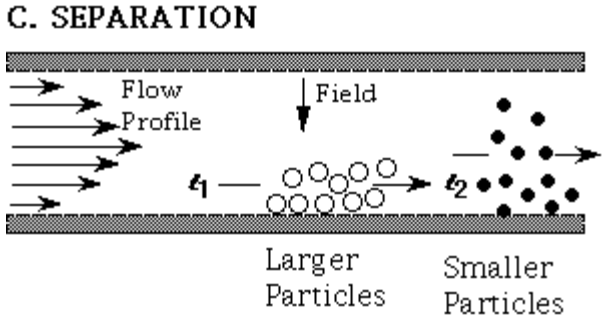
The basis of FFF is the presence of a laminar flow profile in a ribbon-shaped flow channel.





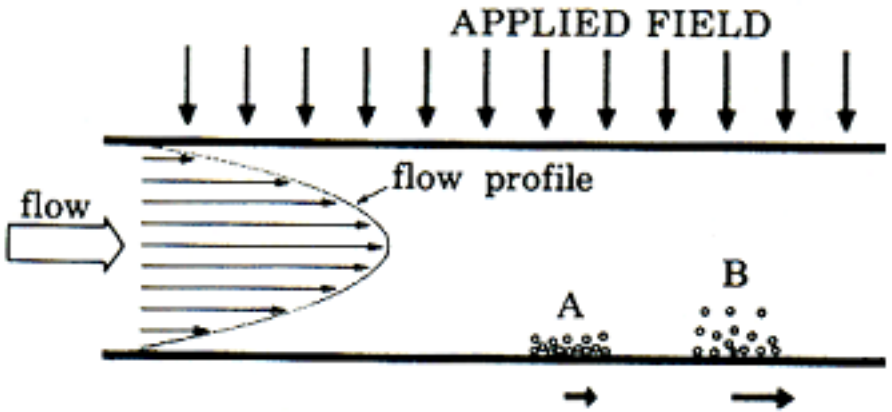






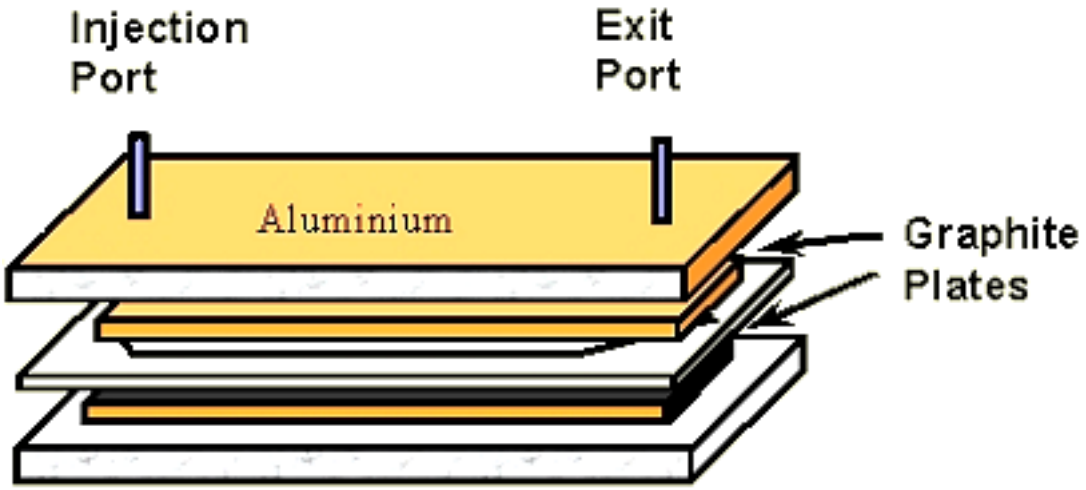
3. Types of FFF:

a. Electrical FFF
b. Sedimentation FFF
c. Thermal FFF
d. Flow FFF



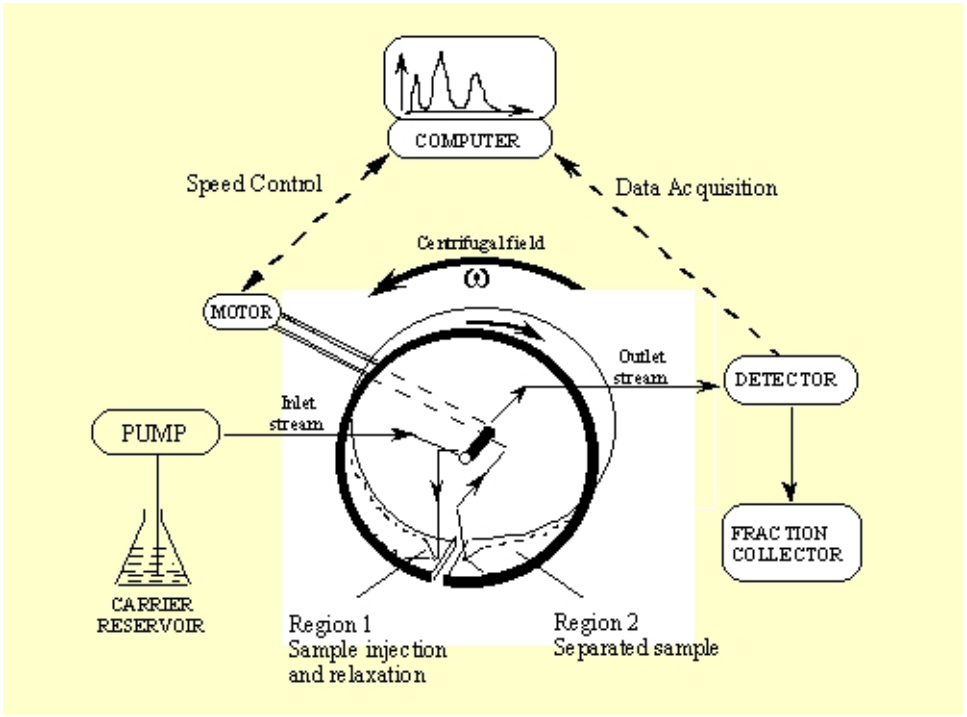
a. Electrical FFF

Electrophoretic mobility



b. Sedimentation FFF

It is used to separation of solutes with Molecular weights ranging from 10^6 to 10^{13} Dalton. (polymer particles, viruses, and entire sequences of Single vs. double stranded DNA.



Sedimentation coefficient

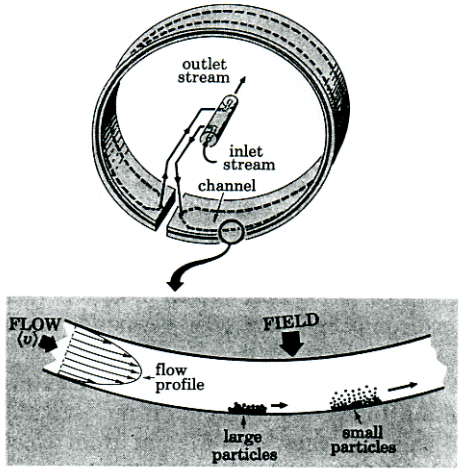


Figure 9.8. Schematic of apparatus for sedimentation FFF.

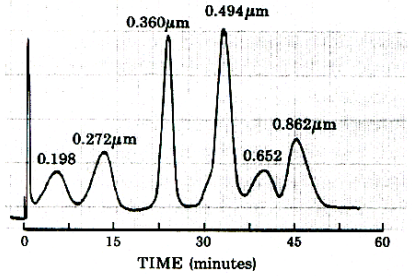


Figure 9.9. Separation of polystyrene latex beads of indicated diameters in the colloidal size range by SdFFF programmed from 1500 to 75 rpm at 2 mL/min flowrate. (Courtesy of Bhajendra N. Barman, FFFractionation. Inc.)

c. Thermal FFF

i. The external field used in this technique is a temperature gradient across the flow channel. This is produced by heating or cooling the upper and lower walls of the flow channel to different degrees.

ii. In the presence of this temperature gradient, solutes will undergo "thermal diffusion" and migrate within the gradient to different extents. This technique is effective for the separation of synthetic plymer with MW. 10^3 ~ 10^7 D in organic solution.

d. Flow FFF

Instead of an external field, a slow transverse flow of the carrier liquid is used in this technique. Flow FFF has been applied to a variety of solutes including proteins, polymers and colloidal particles.

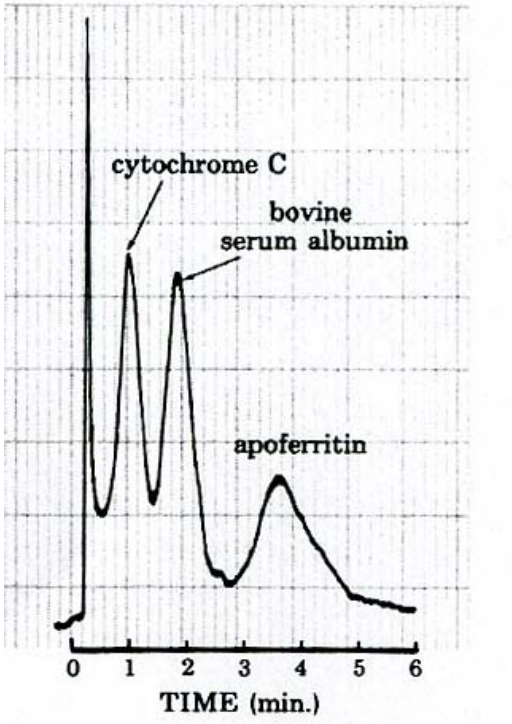


Figure 9.10. Rapid separation of three proteins by flow FFF at a channel flowrate of 8.0 mL/min and a crossflow rate of 6.8 mL/min.

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