

**Sedimentation (Under a Gravitational Potential)**

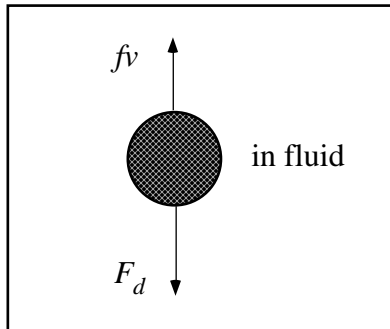


Figure 4-1

$$\text{driving force} = f v = m \frac{dv}{dt} \quad (\text{force} = ma)$$

In gravitational potential,

$$F_d = \text{driving force} = mg - m\bar{v}_2\rho g = m(1 - \bar{v}_2\rho)g$$

where  $g$  = gravitational acceleration

$m$  = mass of particle

$f$  = frictional coefficient

$\bar{v}_2$  = partial specific volume of particle

$$= \left( \frac{\partial \mathcal{V}}{\partial g_2} \right)_{T, \rho_1, g_1}$$

$\rho$  = density of viscous medium

$v$  = velocity of sedimentation

Note that

$$m\bar{v}_2 \equiv \text{effective volume of particle}$$

so that  $m\bar{v}_2\rho g$  = buoyant force

Need to include buoyant force!

Therefore,

$$m(1 - \bar{v}_2\rho)g - fv = m\frac{dv}{dt}$$

When the frictional force balances the driving force,  $\frac{dv}{dt} = 0$  and the particle acquires its terminal velocity ( $v_t$ ).

$$v_t = \frac{m(1 - \bar{v}_2\rho)g}{f}$$

### **Sedimentation (in an Ultracentrifuge)**

The same principles and formulae apply except that  $g$  is replaced by  $\omega^2 x$ .

$$v_t = \frac{m(1 - \bar{v}_2\rho)\omega^2 x}{f}$$

where  $\omega \equiv$  speed of rotation (in radians / sec)

$x \equiv$  distance from the axis of rotation

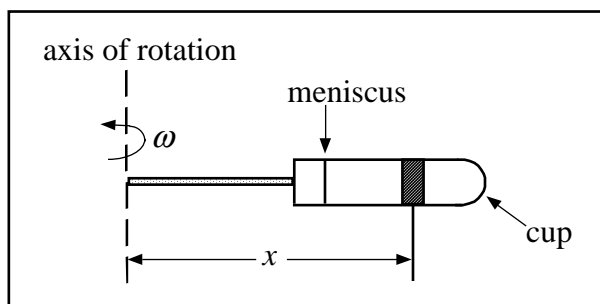


Figure 4-2

### **Sedimentation Coefficient (S)**

$$S = \frac{v_t}{\omega^2 x} = \frac{m(1 - \bar{v}_2\rho)}{f}$$

where  $S \equiv$  terminal velocity / unit acceleration

Sedimentation coefficients have units of sec.  $10^{-13}$  sec is called 1 svedberg (or 1  $S$ ).

T. Svedberg pioneered research on sedimentation in an ultracentrifuge.

$$1 S \equiv 10^{-13} \text{ sec}$$

## Determination of Sedimentation Coefficient

### (1) Boundary Sedimentation

Boundary sedimentation is sedimentation of macromolecules in a homogeneous solution.

- Begin with a homogeneous solution of macromolecules.
- As the solution is spun in the ultracentrifuge and macromolecules move down the centrifugal field, a solution-solvent boundary is generated. The boundary can be monitored by refractive index, color (absorption) etc.

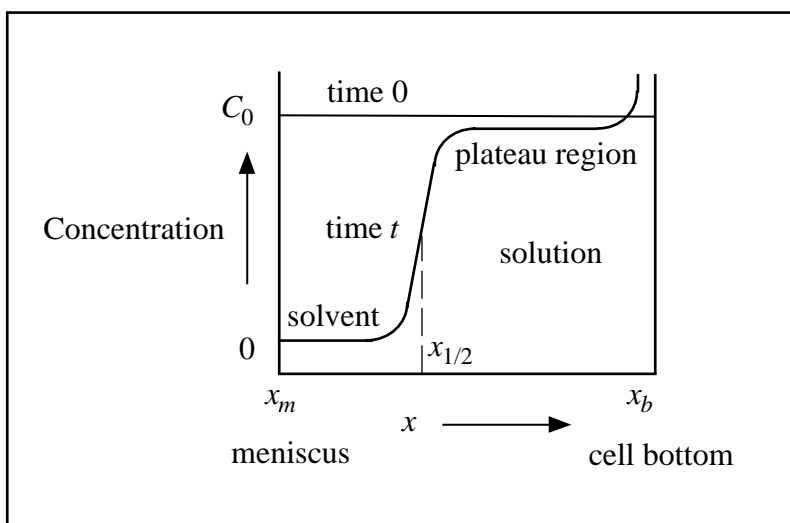


Figure 4-3

- By following the boundary with time, the sedimentation coefficient can be determined.

$$\begin{aligned}
 S &= \frac{v_t}{\omega^2 x} = \frac{dx_{1/2} / dt}{\omega^2 x_{1/2}} = \frac{1}{\omega^2} \frac{d \ln x_{1/2}}{dt} \\
 &= \frac{2.303}{\omega^2} \frac{d \log x_{1/2}}{dt} \\
 &= \frac{2.303}{\omega^2} (\text{slope})
 \end{aligned}$$

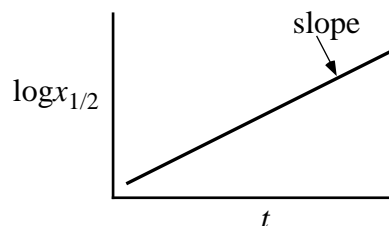


Figure 4-4

**(2) Zone Sedimentation**

- (a) A thin layer of a solution of the macromolecule(s) is placed on top of a solvent containing sucrose (sucrose solution).

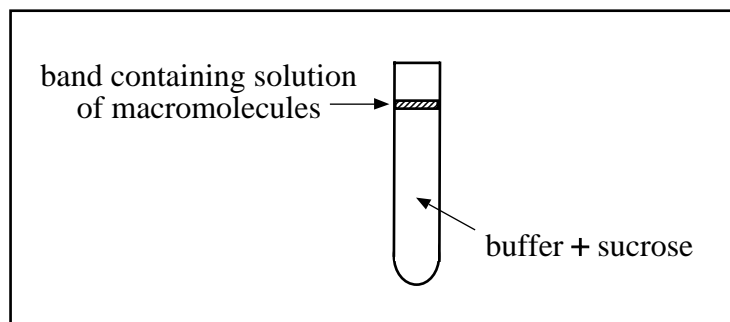


Figure 4-5

- (b) As the sample is spun in the centrifuge, a band containing macromolecules will move down the centrifugal field. Also, a sucrose gradient will have developed. The sucrose gradient ensures that the density of the “solvent” is always greater than the density of the sedimenting zone. This ensures the stability of the band.

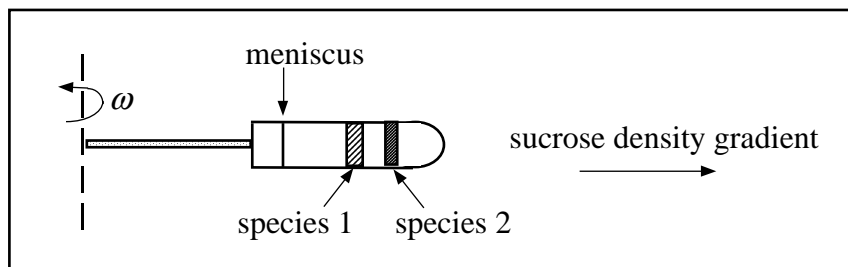


Figure 4-6

- (c)  $S$  is determined from the displacement of the band(s) with time in the centrifuge tube.

**Frictional Coefficient**

Once  $S$  is determined,  $f$  can be determined from

$$S = \frac{m(1 - \bar{v}_2\rho)}{f}$$

since  $m$ ,  $\bar{v}_2$ , and  $\rho$  can be determined experimentally.

### **Sedimentation-Equilibrium**

- (1) Consider sedimentation of a homogeneous two-component solution (a solute plus solvent) in an ultracentrifuge. Because of sedimentation, a concentration gradient is generated. Diffusion sets in. Since transport by sedimentation and diffusion go in opposite directions, eventually an equilibrium concentration is generated by ultracentrifugation. This occurs when

$$\frac{k_B T}{C} \frac{dC}{dx} = m (1 - \bar{v}_2 \rho) \omega^2 x \quad \text{or}$$

$$\frac{RT}{C} \frac{dC}{dx} = M (1 - \bar{v}_2 \rho) \omega^2 x \quad \text{or}$$

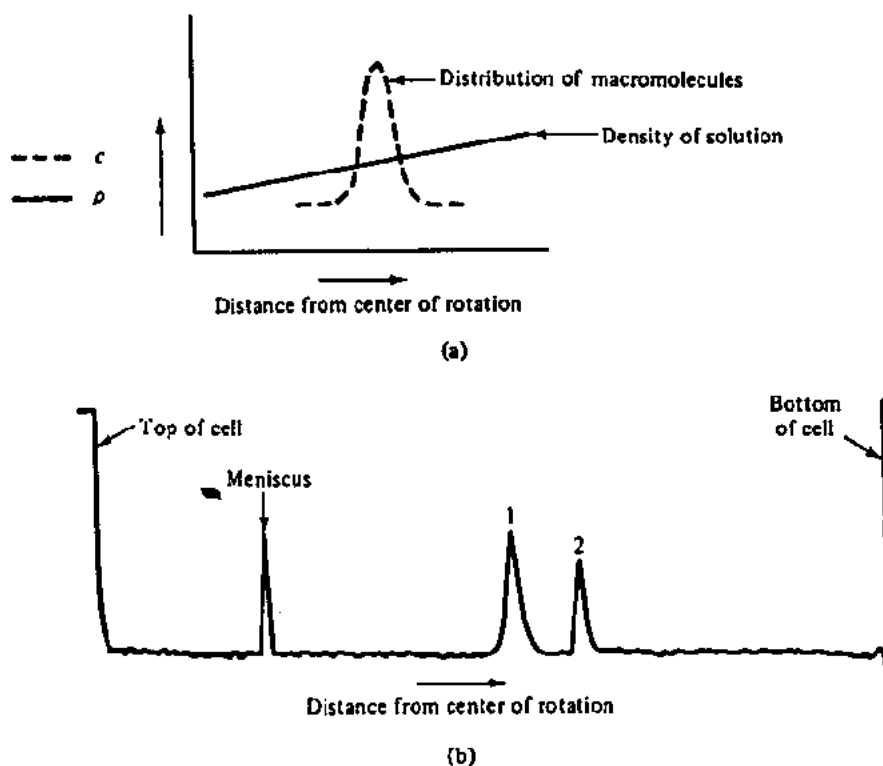
$$M = \frac{4.606 RT}{\omega^2 (1 - \bar{v}_2 \rho)} \frac{d \log C}{dx^2}$$

Measure  $\log C$  vs  $x^2$  to obtain  $M$ !

- (2) Another way to reach sedimentation equilibrium is when the macromolecule becomes “buoyant” in a density gradient. Under this condition,

$$(1 - \bar{v}_2 \rho(x)) = 0 \quad \text{and} \quad v_t \text{ or } S = 0$$

- (a) In these experiments, a density gradient is established by adding concentrated salt solution, e.g. CsCl, to the solution of macromolecule(s).
- (b) The solution is then spun at high speeds ( $\omega$ ).
- (c) The various macromolecular species will form bands at points in the salt gradient where the macromolecules become buoyant; i.e., at  $x$ 's where  $(1 - \bar{v}_2 \rho(x)) = 0$  for the species. Many biological macromolecules have “buoyant densities” sufficiently different that they can be separated or resolved by density-gradient centrifugation.



**Fig. 6.13** Density-gradient centrifugation. (a) A macromolecular species in a concentrated salt solution of an appropriate density is spun in an ultracentrifuge. The solution was initially homogeneous. After a certain time, equilibrium is reached. The concentration of the salt, and consequently, the density of the solution, increases with increasing distance from the center of rotation. The macromolecular species forms a band at a position at which the solvated molecules are buoyant. (b) An actual tracing of two DNA species in a CsCl solution. The initial homogeneous solution has a density of  $1.739 \text{ g cm}^{-3}$ . After 17 h at  $44,770 \text{ revolutions min}^{-1}$  and  $25^\circ\text{C}$ , the DNA species form two sharp bands. Species 1 is a bacterial virus DNA with a molecular weight  $20 \times 10^6$ . Species 2 is the same DNA except that it contains a heavier isotope of nitrogen ( $^{15}\text{N}$  rather than the usual  $^{14}\text{N}$ ). The substitution of  $^{14}\text{N}$  by  $^{15}\text{N}$  increases the buoyant density of this DNA by  $0.012 \text{ g cm}^{-3}$ .

Figure 4-7

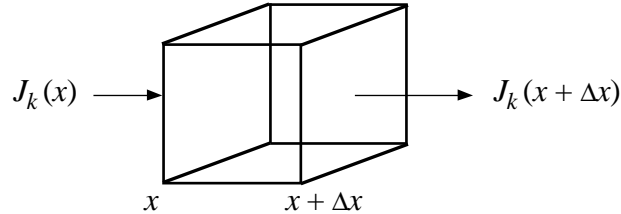
**Fick's Second Law**

Figure 4-8

If  $J_k(x) \neq J_k(x + \Delta x)$ , there will be an accumulation or depletion of the molecular species  $k$  from the zone. From mass balance,

$$- [J_k(x + \Delta x) - J_k(x)] \Delta t = \Delta C_k \cdot 1 \cdot \Delta x, \quad 1 \cdot \Delta x = \Delta V$$

$\Delta C_k$  is in moles / cc

$$- \frac{\Delta J_k^x}{\Delta x} = \frac{\Delta C_k}{\Delta t}$$

But  $J_k^x = -D \frac{dC_k(x)}{dx}$

Therefore

$$D \frac{d^2 C_k(x, t)}{dx^2} = \frac{\partial C_k(x, t)}{\partial t} \quad \text{Fick's Second Law of Diffusion}$$

or  $D \nabla^2 C_k(\vec{r}, t) = \frac{\partial C_k(\vec{r}, t)}{\partial t}$  in 3- dimensions isotropic medium

Solution to differential equation for a plane source:

- (a) 1-D only.
- (b) As  $t \rightarrow 0$ ,  $C(x, 0) = 0$  for all  $x$ 's except at  $x = 0$ , where  $C(0, 0) \rightarrow \infty$ .
- (c) Solution

$$C(x, t) = \alpha t^{-1/2} \exp\left(-\frac{x^2}{4Dt}\right)$$

where  $\alpha \equiv$  a constant

- (d) To obtain  $\alpha$ , use normalization condition, i.e., conservation of molecules of that species.

$$\begin{aligned}
 N = \text{number of molecules} &= \int_{-\infty}^{\infty} C(x,t) dx \\
 &= \alpha \int_{-\infty}^{\infty} t^{-1/2} \exp\left(-\frac{x^2}{4Dt}\right) dx = 2\alpha(\pi D)^{1/2} \quad \text{or} \\
 C(x,t) &= \frac{N}{2(\pi Dt)^{1/2}} \exp\left(-\frac{x^2}{4Dt}\right) \quad \text{Gaussian in } x!
 \end{aligned}$$

(e) Probability of finding molecules at position  $x$  at time  $t \equiv P(x,t) \equiv \frac{1}{N} C(x,t)$

This result can be used to calculate mean-square displacement of the molecules from the origin

$$\langle x^2 \rangle = \int_{-\infty}^{\infty} x^2 P(x,t) dx = 2Dt$$

$$\langle x^2 \rangle = 2Dt$$

(f)

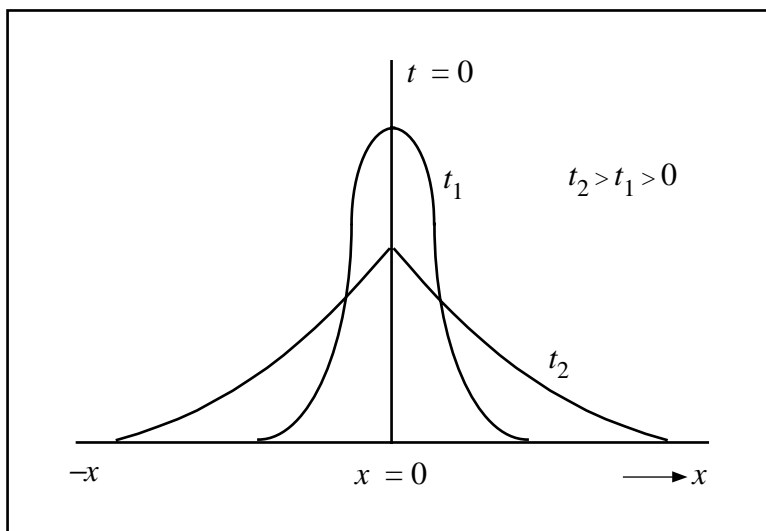


Figure 4-9



**How to Measure  $D$ ?**

(a)

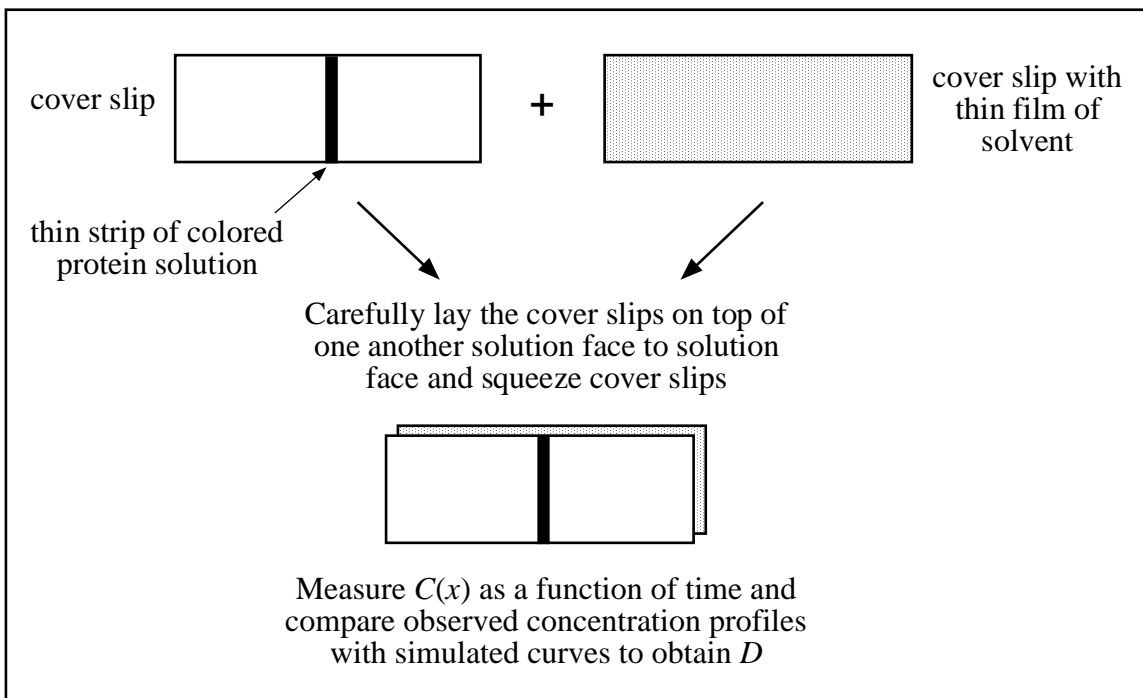


Figure 4-10

(b) In 2-D, replace the plane source by a circular patch.

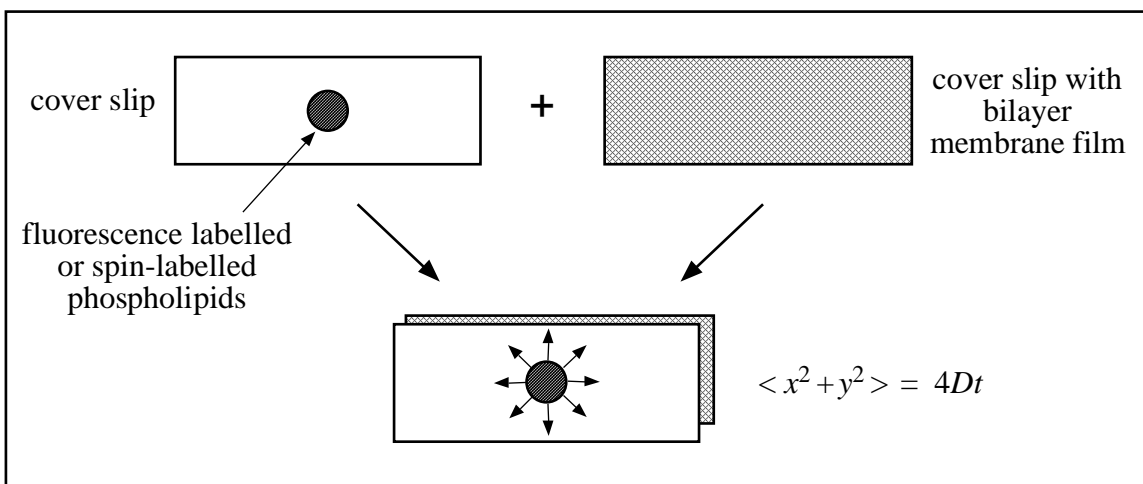


Figure 4-11

The lateral diffusion coefficient of lipids in bilayer membranes is determined in this fashion ( $D \sim 10^{-8} \text{ cm}^2/\text{sec}$ ).

$$\langle x^2 + y^2 \rangle = 4Dt = 4 \times 10^{-8} \text{ cm}^2 / \text{sec} (t)$$

$$\langle r^2 \rangle = 4 \times 10^{-8} \text{ cm}^2 \text{ in 1 sec}$$

$$\langle r^2 \rangle^{1/2} = 2 \times 10^{-4} \text{ cm in 1 sec}$$

$$= 2\mu \quad (\mu = \text{microns})$$

(c) Quasi-Electric Light Scattering

Dephasing of a coherent laser beam of light frequency  $\nu_0$  due to Doppler broadening of scattered light.

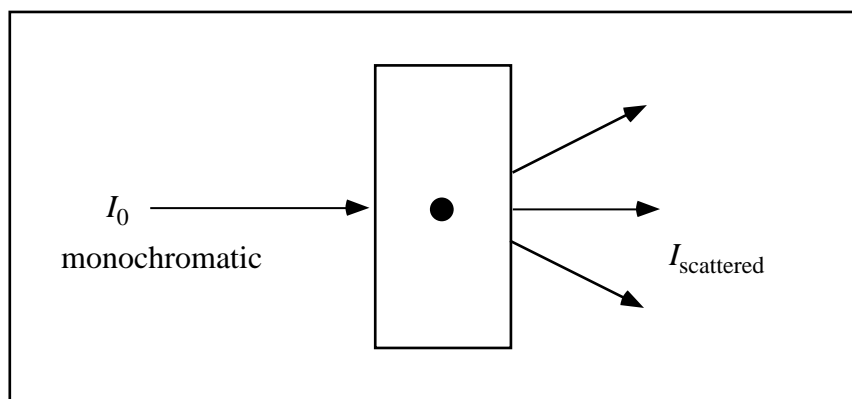


Figure 4-12

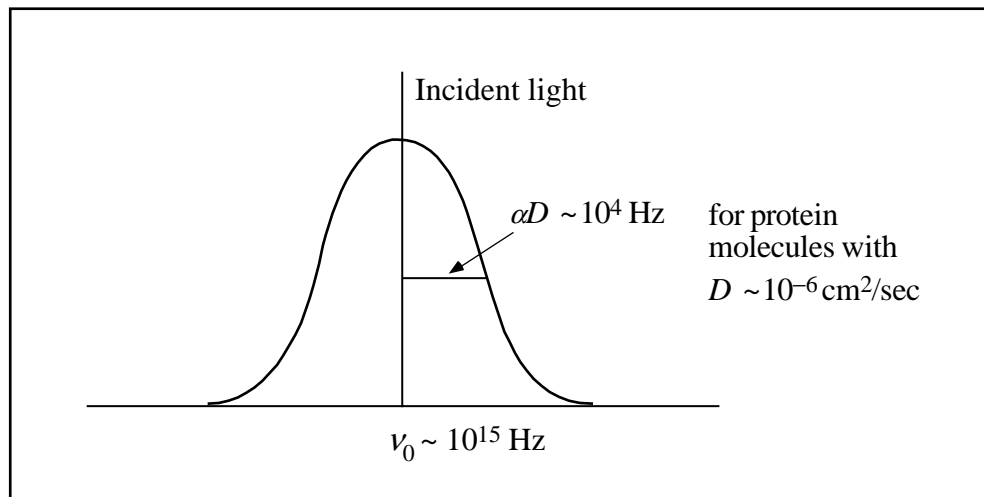
**Intensity of Scattered Light**

Figure 4-13

Will elaborate on later.