Analytical Ultracentrifugation

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Partial Specific Volume (v)

- Partial Specific Volume is defined as the specific volume of the solute, “which is related to volume increase of of the solution when adding dry macromolecules”

- You can calculate by using different densities of solution, and creating a graph

Partial Specific Volume (v)

ρ= density of solution

ρ_o= density of solvent

$$\rho = \rho_o + w(1-\rho_o v)$$

w= weight concentration of solute

How we find v

V is found by calculating the slope of a graph of p as a function of w

$$\rho = \rho_o + w(1-\rho_o v)$$

$$\rho - \rho_o = w(1-\rho_o v)$$

$$\rho - \rho_o / w = (1-\rho_o v)$$

$$\rho / w = v$$

Molecular Mass Sedimentation and Diffusion Equation

$s/D = M(1-v\rho_o)/ RT$

D4.19 found by combining previous equations

$M=sRT/D(1-v\rho_o)$

First Svedberg Equation

Assumption

The First Svedberg Equation works if diffusion and sedimentation friction coefficients are the same (s and D)

In reality, this is not the actual case, causes slight error in formula

Sedimentation Equilibrium

- As a solution is centrifuged for a long time, eventually the diffusion and sedimentation stop changing over time
- This is when it is at equilibrium

Lamm Equation

$(dC/dt) = -1/r[d/dr(w^2r^2sC - Dr(dc/dr])$

concentration gradient + diffusion coefficient

Sedimentation Equilibrium

Rearrange Svedberg equation, $D = sRT/m(1-v\rho_o)$

Plug into Lamm Equation, flux equal to zero at equilibrium

$[-sRT/m(1-v\rho_o)](dC/dx) + sw^2r^2C(x)=0$

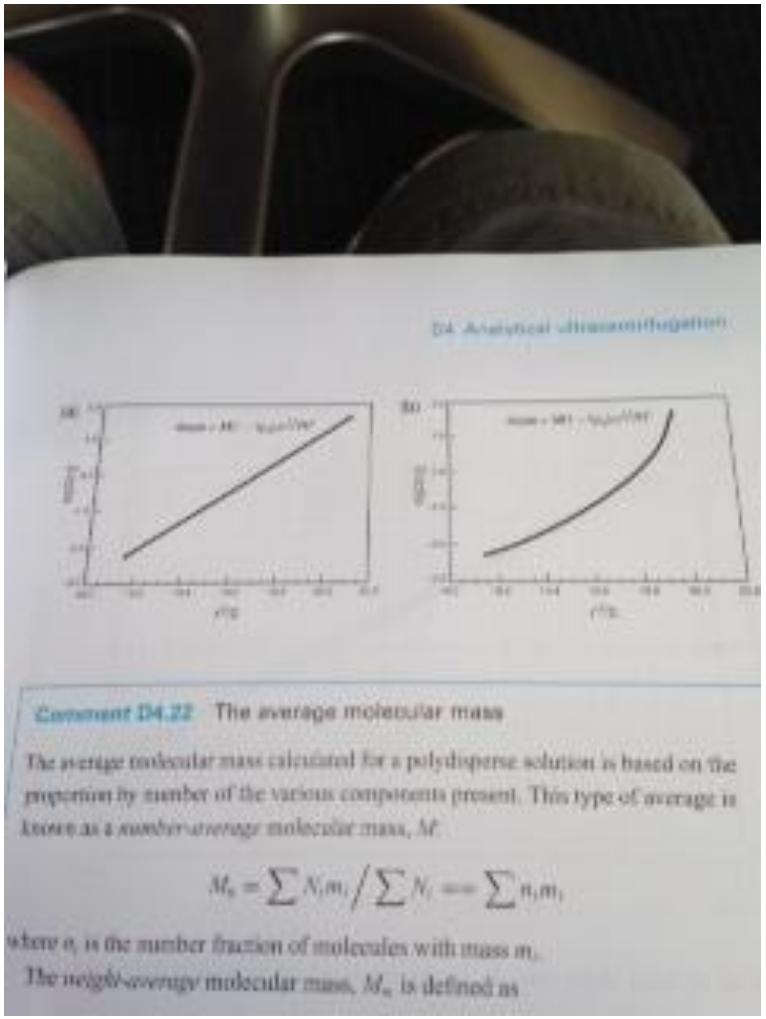
rearrange- $d\ln(C)/d(r^2/2) = M(1-v\rho_o)w^2/RT$

How to use slope

Slope of Ln[C(r)] vs $r^2/2$ graph is $M(1-v\rho_o)w^2/ RT$

As shown by graph, line is linear, meaning term is constant

Slope= Molecular weight



Number average molecular mass

Second graph is not linear because there is more than one macromolecule present

$\Sigma N_i m_i / \Sigma N_i$ is number average molecular mass

eg. 10 molecules of particle A which is 2Da and 5 molecules of particle B which is 3Da

$[(10)(2) + (5)(3)] / 15 = 2.3\text{Da} = M_n$

Weight Average Molecular Mass

$\Sigma(N_i m_i)m_i/\Sigma N_i m_i$= Weight average molecular mass

eg. 5 molecules of A which is 2Da and 10 molecules of B which is 3Da

[(5)(2)(2)+(10)(3)(3)](15)(5)= 1.47Da

Concentration dependence of average molecular mass

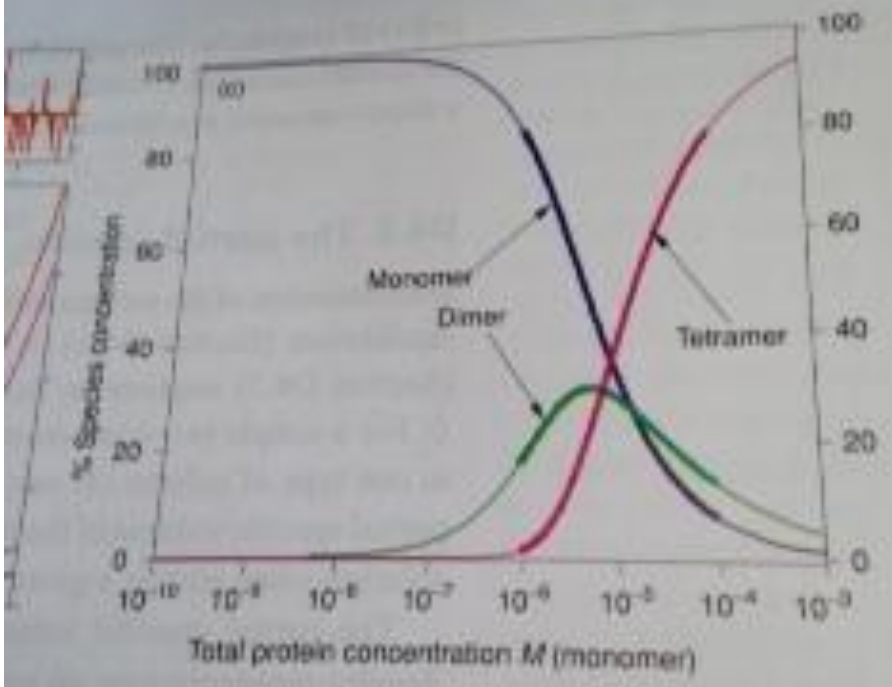
$C(r) = C(a)\exp[w^2M(1-v\rho)(r^2-a^2)/2RT]$= second svedberg equation

$\exp[w^2M(1-v\rho)(r^2-a^2)/2RT]$ known as * for now

$C(r) = C_a^* + C_b^* + C_{ab}^*$

$C_{ab} = C_aC_b/K_{ab}$

Can find equilibrium constant from sedimentation data



t V_L (immunoglobulin
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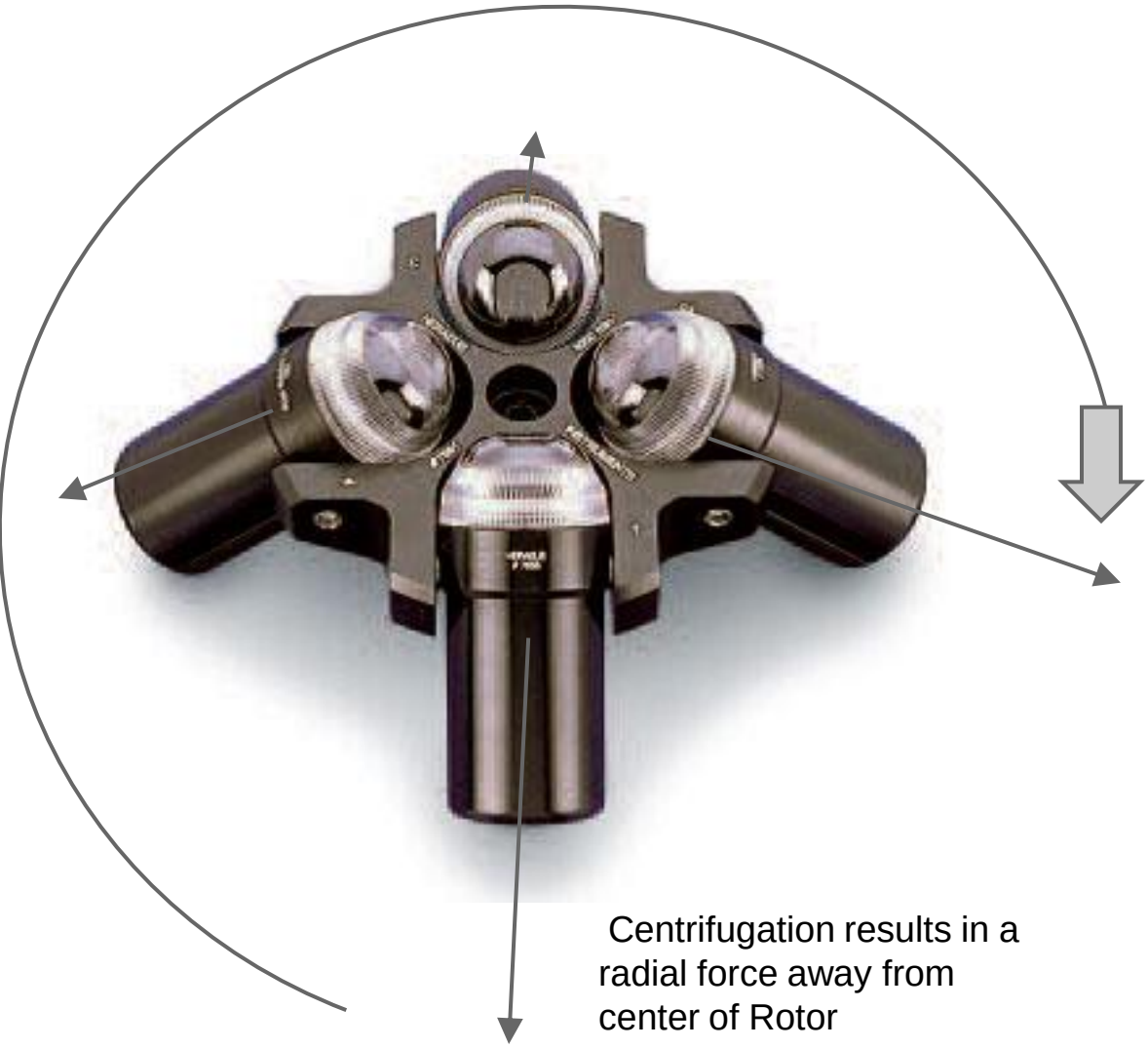
lation (After Hensley, 1996.)

A Closer Look at the Forces in Centrifugation



Centrifugation results in a radial force away from center of Rotor

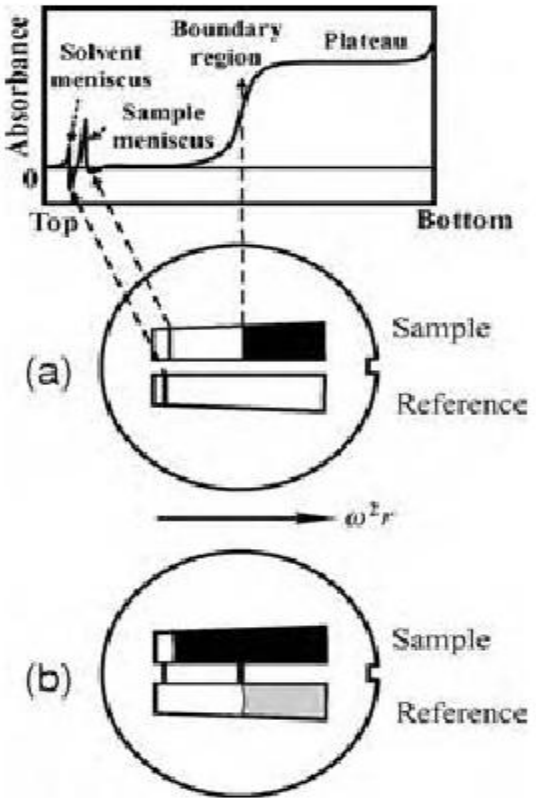


Fig. D4.2 Two types of cells used in AUC: (a) double sector-shaped cell; (b) boundary forming cell. Note the two small connecting channels between compartments. Liquid flow across the lower channel occurs only in the centrifugal field. The upper channel

Density Gradient Sedimentation: The Two Techniques

1. **Analytical Zonal Sedimentation Velocity**
    a. **High velocity, low spin time**

1. **Density Gradient Sedimentation Equilibrium**
    a. **Low velocity, High spin time**

Analytical Zonal Sedimentation Velocity

- Upon Centrifugation, the analyte particles sediment through the gradient to separate zones based on their sedimentation velocity

- Linear 5-20% sucrose gradients are a tradition choice for use as non-ionic gradient material

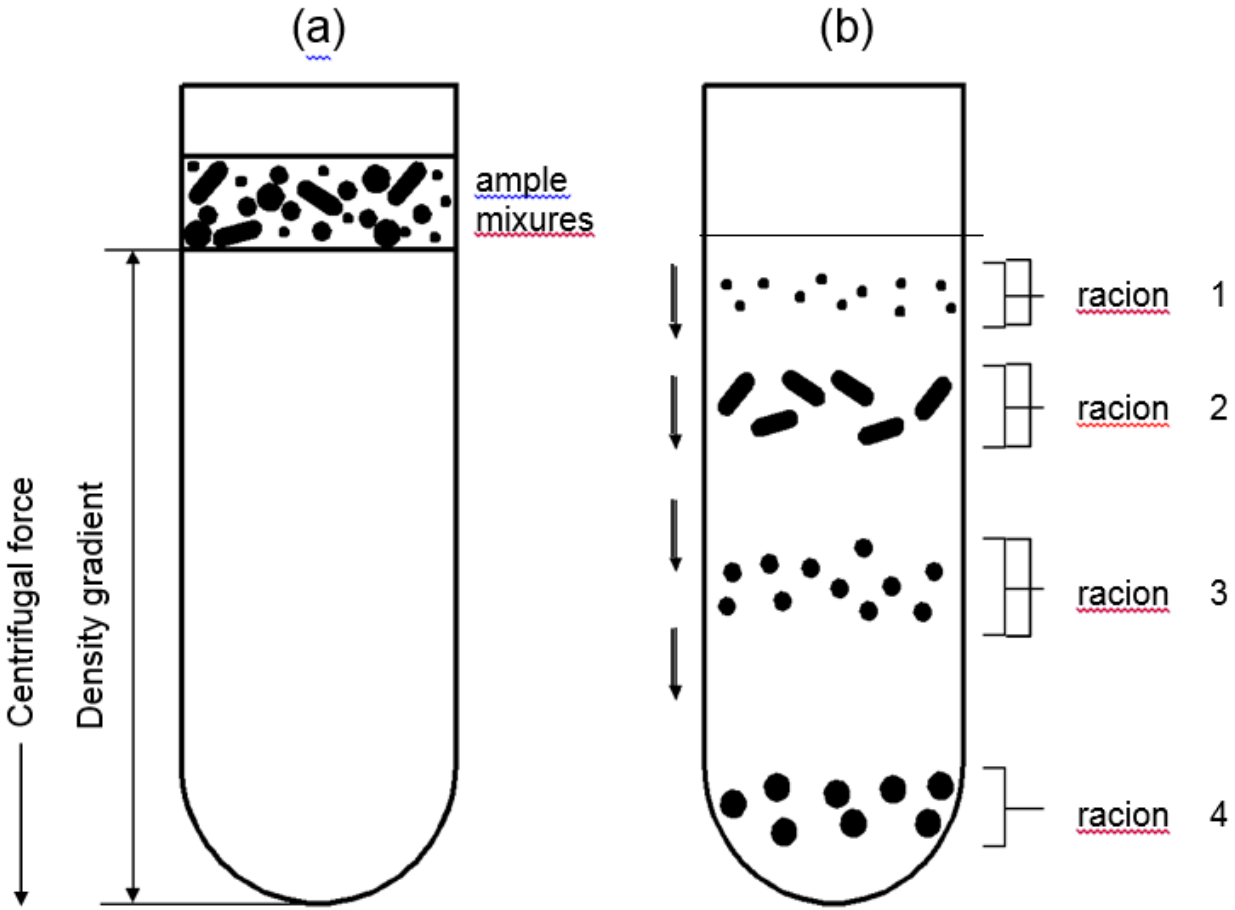
- Separates the molecules in mixtures according to their sedimentation coefficients (S’s)

The Process of Sedimentation in a Centrifugal Field

- Velocity zonal sedimentation separates molecules in the mixture according to their sedimentation coefficients.

- Analyte particles when exposed to the centrifugal field settle down through the sucrose solution until their density is equal to the density of the sucrose solution .

- A density gradient of the analyte particles results with the the densest particles migrating the farthest through the sucrose solution.



A sample containing mixtures of particles of varying size, shape and density is added on the top of a preformed density gradient. The gradient is higher in density toward the bottom of the tube. Centrifugation results in **separation** of the particles **depending on** thier **size**,**shape** and **buoyant density**. Fractions of defined volume are collected from the gradient

Example of an Automated Volume Fraction Collector

- Since Density gradient is stable upon cessation of Centrifugation, the sample tube may transferred to a fraction collector.
- A hole is poked in the bottom of the sample tube and then the density fraction are dripped into collection tubes one level at a time

Density Gradient Sedimentation Equilibrium

- Pre sample injection, a solution containing a heavy such as CsCl or RbCl is spun until a small solute density gradient forms within the cell from the force of the Centrifugal field.

- Three components are in tube/cell: solvent molecules, solutes molecules (salts) and analyte molecules.

- The small solute becomes distributed in the cell in just the same way as a large molecule

A Mathematical Description of the equilibrium sedimentation distribution

- An equation describing the equilibrium sedimentation distribution is obtained by setting the total flux equal in the cell to zero in since at equilibrium, there are no changes in concentration with time

The macromolecular concentration distribution between the meniscus at a and point r obeys an exponential law, called the second Svedberg equation:

$$C(r) = C(a)\exp[\omega^2 M(1 - \bar{v}\rho)(r^2 - a^2)/2RT] \qquad \text{(D4.23)}$$

Because of the small molecular mass of the solute molecules we can expand the Svedberg eqation and

Thus the Svedberg form of the macromolecular concentration distribution between meniscus a and point r in the cell reduces to:

$$C_3(r)/C_3(a) = 1 + M_3(1 - \bar{v}\rho)\omega^2(r^2 - a^2)/2RT \qquad \text{(D4.29)}$$

Where:

C is the concentration distribution of the analyte particles.

M is the mass of the analyte particles

v is the partial specific volume of the analyte particles

p is the density

w is the angular velocity

R is the Universal Gas Constant and T is the Temperature in the cell

Applying Equilibrium Sedimentation to prove the Semi Conservative Nature of DNA

Messelson and Stahl grew *E.coli* cells in a medium in which the sole nitrogen source was 15 N-labelled ammonium chloride.
The 15 N-containing *E.coli* cell culture was then transferred to a light 14 N medium and allowed to continue growing. Samples were harvested at regular intervals.
The DNA was extracted and its buoyant density determined by centrifugation in CsCl density gradients. The isolated DNA showed a single band in the density gradient, midway between the light 14 N-DNA and the heavy 15 N-DNA bands (Fig. D4.24(c)).
After two generations in the 14 N medium the isolated DNA exhibited two bands, one with a density equal to light DNA and the other with a density equal to that of the hybrid DNA observed after one generation (Fig. D4.24(d)).
After three generations in the 14 N medium the DNA still has two bands, similar to those observed after two generations (Fig. D4.24(e)). The results were exactly those expected from the semiconservative replication hypothesis.

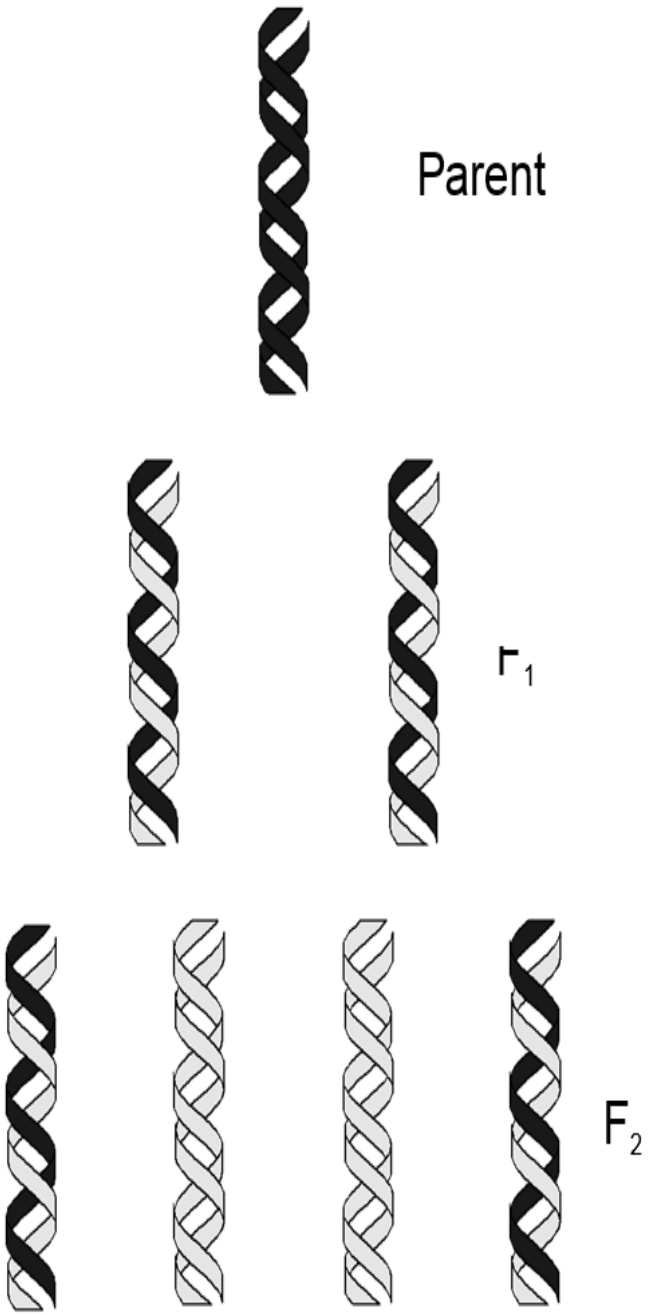


Fig. D4.23 The semiconservative mechanism of DNA replication. Each F_1 duplex contains one parent strand. The F_2 generation consists of two hybrid DNAs and two totally new DNAs. Newly replicated strands are shown in grey; parental strands in black.

(a)
(b)
(c)
(d)
(e)
F1
F2
F3

Macromolecular Shape from Sedimentation Data

- Molecules of the same shape but different molecular mass are called homologous series.
- The relationship between mass M and sedimentation coefficient s is as follows

$$s = K_s M^a$$

where K_s is a coefficient that depends on the partial specific volume. The value of the exponent, *a*, depends on the *shape* of the molecules in the series

Homologous series of quasi-spherical particles: globular proteins in water

- The frictional coefficient of a sphere of radius *R*0 under slip boundary conditions in a solvent of viscosity $\eta 0$ is given by Stokes Law:

$$f_0 = 6\pi\eta_0 R_0$$

The radius of the sphere is related to its mass via its volume, V_0

$$\frac{4}{3}\pi R_0^3 = V_0 = M\bar{\upsilon}/N_A$$

and we can write

$$f_0 = 6\pi\eta_0(3M\bar{\upsilon}/4\pi N_A)^{1/3}$$

Inserting this result into Eq. (D4.19), we obtain, after rearrangement,

$$s^* = s^0_{20,w}\bar{\upsilon}^{1/3}/(1-\bar{\upsilon}\rho) = M^{2/3}/6\pi\eta_0 N_A^{2/3}(3/4\pi)^{1/3} \qquad \text{(D4.34)}$$

The log–log dependence of s^* on M is a straight line of slope 2/3. When $s^0_{20,w}$ is expressed in Svedberg units and all other quantities in cgs units, Eq. (D4.34) becomes

$$s^* = 12.0 \times 10^{-3} M^{2/3} \qquad \text{(D4.35)}$$

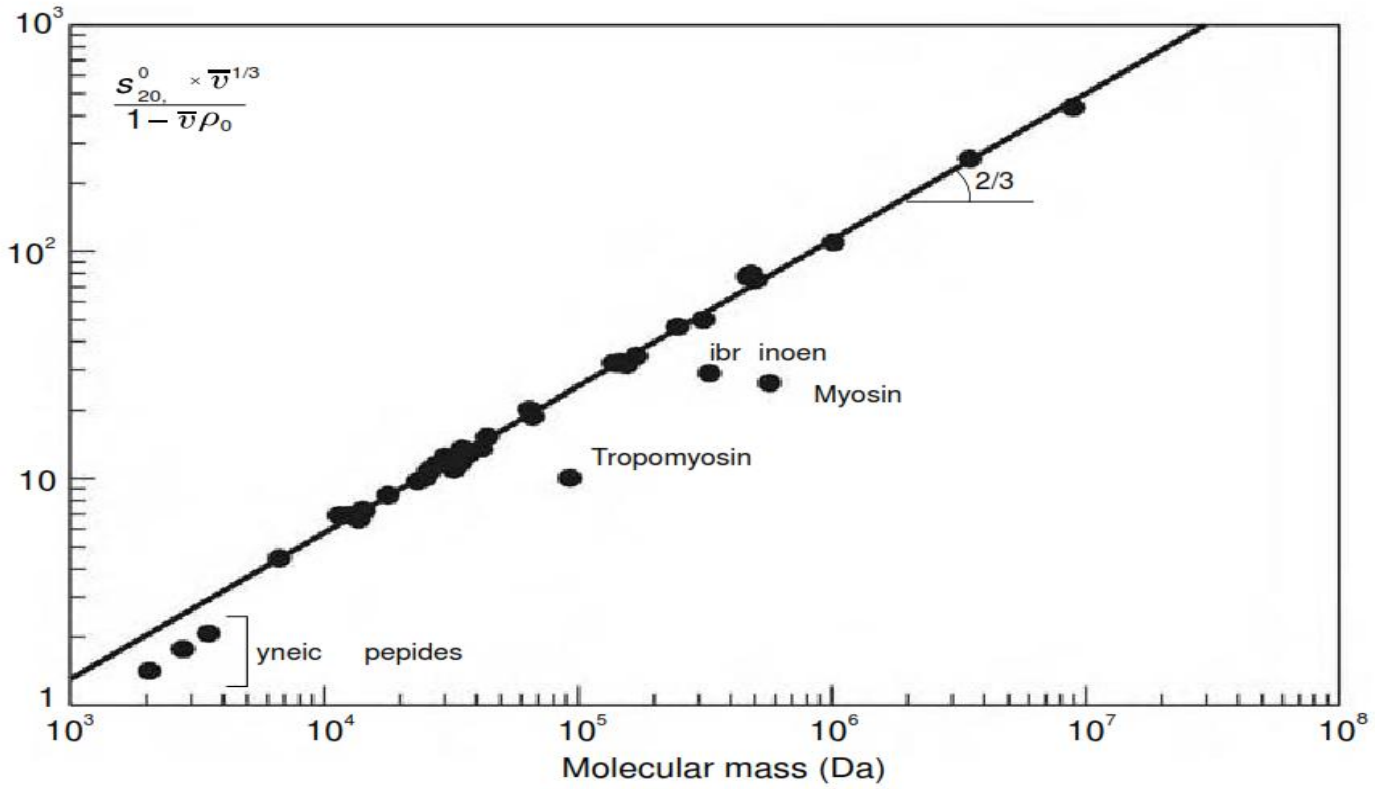


Fig. D4.25 The log–log plot of the dependence $s^0_{20,w}\bar{\upsilon}^{1/3}/(1-\bar{\upsilon}\rho_0)$ on M for proteins in a wide mass interval using the data presented in Table D4.3. For globular proteins plot the slope of this is 2/3, which is typical for quasi-spherical particles. Exceptions to the rule are: synthetic peptides (unfolded particles), tropomyosin, fibrinogen, myosin (rod-like particles).

- A good straight line fit of log s* versus log M is obtained according to the single equation:

$$s^* = 9.1 \times 10^{-3} M^{0.65}$$

- This establishes that globular proteins actually form a homologous series.

- Small deviations from perfect spherical shapes and the existence of hydration shelly modify the relation between the Stokes radius and the partial specific volume without changing the power law.

- Thus we conclude that the fact that their sedimentation behavior can be described by the single previous equation means that globular proteins are very close to spherical in shape and hydrated to abou the same extent.
- Three proteins that do not obey the equation:

$$s^* = 9.1 \times 10^{-3} M^{0.65}$$

Can you identify them?

Fig. D4.25 The log–log plot of the dependence $s^0_{20,w}\bar{v}^{1/3}/(1-\bar{v}\rho_0)$ on M for proteins in a wide mass interval using the data presented in Table D4.3. For globular proteins plot the slope of this is 2/3, which is typical for quasi-spherical particles. Exceptions to the rule are: synthetic peptides (unfolded particles), tropomyosin, fibrinogen, myosin (rod-like particles).