CHEM*3570 Analytical Biochemistry Centrifugation

1. For a cylinder, the moment of inertia I = ½ mr^2 (see any Physics textbook), and kinetic energy of rotation E = ½ $I\omega^2$. Consider an ultracentrifuge rotor to be a cylinder of titanium (density = 4.5), diameter = 25 cm., height = 10 cm. Calculate the kinetic energy of such a rotor, spinning at 50,000 rpm. How fast would you have to drive a 1,000 kg truck, to acquire the equivalent kinetic energy?

$I = \frac{1}{2} mr^2$ r = 12.5 cm h = 10 cm. $\therefore V = \pi r^2 h = 4{,}909\ cm^3$

$\rho = 4.5\ gm\ cm^{-3}$ $\therefore$ m = 22 kg $I = \frac{1}{2}(22\ kg)(1.25 \times 10^{-1}\ m)^2 = 0.172\ kg\ m^2$

$E = \frac{1}{2} I \omega^2$ 50,000 rpm = 833 rps $\therefore \omega = 2\pi\ 833\ rps = 5{,}236\ s^{-1}$

(Note that ω is given in radians per second)

$E = \frac{1}{2}(0.172\ kg\ m^2)(27.4 \times 10^6\ s^{-2}) = 2.36 \times 10^6\ kg\ m^2\ s^{-2} = 2.36$ MJ

1 ton = 10^3 kg $E = \frac{1}{2} mv^2$. $\therefore 2.36 \times 10^6\ kg\ m^2\ s^{-2} = 500\ kg\ v^2$

$\therefore v^2 = (2.36 \times 10^6) / (500) = 4.6 \times 10^3\ m^2\ s^{-2}$ $\therefore v = 68\ m\ s^{-1}$

That's about 250 kph, so you can see why ultracentrifuges are armour-plated!

2. A protein is studied in the analytical ultracentrifuge. At high speed (60,000 rpm), the sedimentation velocity **v** is measured as 1.58×10^{-4} cm sec^{-1} at a radius of 10.0 cm.

Estimate the sedimentation coefficient of the protein, expressing the results in Svedberg Units (**S**) where 1 S = 10^{-13} sec. If the sample cell in the ultracentrifuge is 1.5 cm deep, estimate approximately how long it takes to sediment from top to bottom.

$$\mathbf{s} = \frac{\mathbf{v}}{\omega^2\ \mathbf{r}} = \frac{1.58 \times 10^{-4}\ \mathbf{cm\ s}^{-1}}{\left(\frac{2\pi \times 60{,}000}{60}\right)^2 \mathbf{s}^{-2} \times 10.0\ \mathbf{cm}} = 4.0 \times 10^{-13}\ \mathbf{s}$$

$\therefore$ **s = 4.00 S**

Time taken to sediment 1.5 cm: First, an *approximate* calculation, assuming constant velocity:

$$\mathbf{t} = \frac{1.5\ \mathbf{cm}}{1.58 \times 10^{-4}\ \mathbf{cm\ s}^{-1}} = 9{,}500\ \mathbf{s} = 2\mathbf{h}\ 38\mathbf{m}$$

Actually, as the molecule sediments, it becomes further from the center of rotation; the g force increases with radius. Therefore, the time to sediment is more accurately given by solving the differential equation:

$$v = {dr}/{dt} = s\omega^2 r \qquad \int_{r_1}^{r_2} \frac{dr}{r} = s\omega^2 \int_0^t dt$$

r_1 and r_2 are the initial radius and radius at time t, respectively.

$$\ln\frac{r_2}{r_1} = s\omega^2 t \qquad t = \frac{\ln\left({r_2}/{r_1}\right)}{s\omega^2}$$

The factor $k = [\ln(r_2/r_1)]/\omega^2$ is an indicator of the required sedimentation time for a given rotor:

t = k/s

For: r_1 = 10 cm and r_2 = 11.5 cm: $\ln(r_2/r_1) = 0.14$.

$t = 0.14/ s\omega^2 = 0.14 / \{(4 \times 10^{-13}\ s)(2\pi \times 10^3\ s^{-1})^2\} = 8{,}860$ sec.

3. The frictional coefficient *f* for a molecule is not easy to predict, because it depends on the detailed shape and surface properties of the molecule. However, for a macroscopic hard sphere, we can apply Stokes' Law (1851): $f = 6\pi\eta r$
where η = viscosity of the solution and r is the radius of the particle.

A plastic ball of radius 1 cm. is dropped through a graduated cylinder full of cooking oil, $\eta = 20$ Pa-s ($N\ s\ m^{-2}$). The density of the ball is 0.95 and the density of the oil is 0.8. At what velocity will the ball descend?

Note: Units of η: $N\ s\ m^{-2} = kg\ m\ s^{-2}\ s\ m^{-2} = kg\ s^{-1}\ m^{-1}$

$f = 6\pi\eta r$ drag force = fv mass = $\rho V = \rho(4/3\,\pi r^3)g$ weight = mg

Let σ = density of liquid. buoyancy = σV; effective mass = $(\rho-\sigma)V$

At limiting velocity: $fv = m_{eff.}g \therefore v = m_{eff.}g/f$

$$v = \frac{m_{eff.}\, g}{f} = \frac{\frac{4}{3}\pi r^3(\rho - \sigma)g}{6\pi\eta r} = \frac{2r^2(\rho - \sigma)\, g}{9\eta}$$

$$v = \frac{2r^2(\rho - \sigma)\, g}{9\,\eta} = \frac{2\,(10^{-2}\,m)^2(.95-.8)\times 10^3\ kg\, m^{-3}(9.8\, ms^{-2})}{9\,(20\ kg\ s^{-1}\ m^{-1})}$$

$$= 1.6\times 10^{-3} m\, s^{-1} = 1.6\ mm\, s^{-1}$$

4. As a simple model, we may assume that proteins are simply hard spheres of uniform density. In this case, how will the sedimentation coefficient s vary as a function of m_0?

$$\mathbf{s = \frac{m_0\ (1 - \bar{v}\,\rho\,)}{\mathit{f}}}$$

$$m_0 \propto r^3 \therefore f \propto r \propto (m_0)^{1/3}$$

$$s \propto \frac{m_0}{f} \propto \frac{m_0}{(m_0)^{1/3}} \propto (m_0)^{2/3}$$

5. The Meselson-Stahl experiment which proved the semi-conservative model of DNA replication relied on the analysis of *E. coli* DNA labelled with the stable isotope ^{15}N (by growing the bacteria on medium made with $^{15}NH_4Cl$). What is the expected difference in density between ^{15}N and ^{14}N DNA?

Number of nitrogen atoms in bases: A: 5; C: 3; G: 5; T: 2; average = 7.5 N atoms per base pair.
Average MW of base pair ≈ 660 Da.
So Δ_{mass} = 7.5 units/ 660 ≈ 1% difference between fully ^{15}N-labelled and natural DNA;
half this difference for the semi-conservative product (one strand ^{15}N and one strand ^{14}N).