<u>Study Problems – Sedimentation</u>

1. A centrifuge rotor is spinning at 25,000. The ‘top’ of the cell is 5.5 cm from the rotor’s central axis, and the ‘bottom’ of the cell is 9.5 cm from the central axis. What are the g-forces on a particle found at the top and at the bottom of the tube?

2. A protein/RNA complex ($v = 0.71\ cm^3\ g^{-1}$) gives a sedimentation coefficient of 12.7 S in 10% sucrose, 50 mM Tris buffer, pH 7.4 at 4^oC.
(a) What will be the velocity of sedimentation of the complex under these conditions when the complex is found 6.0 cm from the central axis of a rotor spinning at 40,000 rpm?
(b) What is the $s_{20,w}$ value for this protein complex if $\rho_{solv} = 1.0419\ g\ cm^{-3}$?

3. The following data are obtained for a globular protein in 0.1 M NaCl, 10 mM phosphate, pH 7.4, $T = 5^oC$:

- Partial specific volume = $0.73\ cm^3\ g^{-1}$
- Sedimentation coefficient = 4.93 S
- Diffusion coefficient = $2.537 \times 10^{-7}\ cm^2\ sec^{-1}$

If the physical properties of this buffer are very close to those of pure water:
(a) calculate the molecular weight of the protein;
(b) assuming that the protein can be approximated as an ellipsoid of revolution, deduce what you can about the protein’s shape. Indicate any assumption(s) that you make in this analysis.

4. A pure protein ($M = 67.0$ kDa, $v_p = 0.72\ cm^3\ g^{-1}$) is centrifuged at 10,000 rpm in an analytical centrifuge, at 5^oC and in the same buffer solution as was used in the last problem. At equilibrium, if the concentration of the protein at $r = 6.5$ cm is $0.8\ mg\ ml^{-1}$, what will be the protein concentration at $r = 6.0$ cm?

Answers to Sedimentation Study Problems

1. First note that a rotor speed of 25,000 rpm is equivalent to ω = 2618 (rad) sec^{-1}.
Then using the equations from the handout (Exercise 1):

At r = 5.5 cm, the centrifugal force is equivalent to **38,400 x g**

At r = 9.5 cm, the centrifugal force is equivalent to **66,400 x g**

2. (a) (Again, note first that 40,000 rpm equates to ω = 4189 (rad) sec^{-1})

Then **v = 1.337 x 10^{-4} cm sec^{-1} or 0.481 cm hr^{-1}**

(b)

$$s_{20,w} = s_{\exp} \cdot \frac{\left(1-\bar{\mathrm{v}}_p \cdot \rho_{20,w}\right)}{\left(1-\bar{\mathrm{v}}_p \cdot \rho_{T_{\exp},\,10\%\,sucrose}\right)} \cdot \frac{\eta_{10\%sucrose}}{\eta_w} \cdot \frac{\eta_{w,\,4^o C}}{\eta_{w,\,20^o C}}$$

where the middle ratio term on the right can be evaluated at 20ºC (for which data are available from the Data Table) as (.01336 poise)/(.01002 poise). ‘Plugging in’ the other values, we obtain the answer **s = 29.6 S**.

3. (a) Remember that s = 4.50 S = **4.50 x 10^{-13} sec**

Now use equation [13b], **M = 151,920 Da** (which should really be rounded to 152 kDa or 1.52 x 10^5 Da with the proper number of significant digits)

(b) We can use two approaches, both based on calculating the frictional ratio:

(f/f_o) = 1.4973 (I leave the details to you).

First, we can calculate a **maximum possible value of F = 1.4973** (or 1.50 with the correct number of significant digits)

Second, we can obtain a ‘best-guess’ estimate of F by assuming an ‘average’ value of the hydration factor δ_w = 0.35 (g/g), then calculating **r_h = 4.022 x 10^{-7} cm and $F_{best-guess}$ = 1.3140**. This corresponds to a **prolate ellipsoid with (b/a) = 6.0** or an **oblate ellipsoid with (b/a) = 6.8** (determine the latter by interpolation from the Data Table).

4. Using equation [24] we can calculate that the ratio of concentrations at r = 6.0 vs. 6.5 cm will be **1/16.1**, so that the concentration at r = 6.0 cm will be **0.050 mg ml^{-1}**.