BIOC404- Lecture 5 Jan. 13th, 2006
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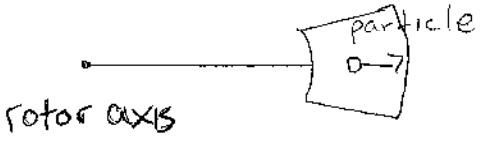
Sedimentation profile of a single particle

We began the class discussing the forces a single particle undergoes during centrifugal sedimentation. Last class, we learnt that the angular velocity, ω, equals 2π multiplied by the revolutions per minute, all divided by 60 [ω=2π(rpm)/60].



The force on a particle in a vacuum would be; $F_{centrifugal} = m_{particle} r \omega^2$.

As an aside, Dr. Silvius explained how we can convert from rpm to "g" using a pretty simple formula. Although he said he wouldn't test it directly, it seems like something that could come up.
So the centrifugal force, as acceleration, $r\omega^2$ could be written as a multiple of "g", such that

$G = r\omega^2 / (9.81 cm^2 sec^{-1})$

Movement of a particle in a solution in a centrifuge

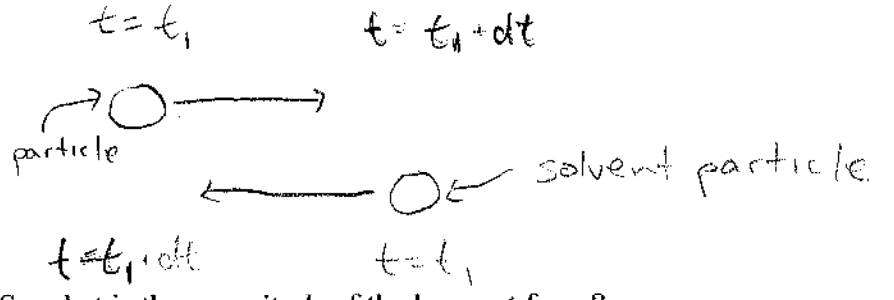
Now we will look at the case where the particle is moving in a space that is not a vacuum. For this movement, we are assuming a **small displacement of the particle**, as well as **a small change in the velocity**, however, the **velocity** remains **relatively constant**.

In a steady state form of this system, $F_{net} = F_{centr} + F_{buoyant} + F_{frictional}$
Since we are in a steady state, the $F_{net}=0$, therefore, we have $F_{centr} + F_{buoyant} + F_{frictional}=0$.

We know from earlier classes that the frictional force is equal to $-fv$.

So what exactly is the **buoyant force**?

This force can be described as the force needed to move on the $-r$ direction a volume of solvent equal to the volume of the particle that is moving in the $+r$ direction. Basically, in order for the system to compensate for the movement of the particle due to the centrifugal force, solvent must move in the opposite direction to counteract it (seen in "drawing" below. Therefore, the **buoyant force** *opposes* the centrifugal force.



So what is the *magnitude* of the buoyant force?
$F_{buoyant} = -r\omega^2$ (mass of the displaced solvent) … very similar to F_{centr}
$= -r\omega^2$ (V_h of the particle · $\rho_{solvent}$)

Remember that $F_{centr} = r\omega^2 (m_{particle})$, where $m_{particle} = (M / N_o) \cdot (1 + \delta_{water})$, which is equivalent to the mass of 1 **hydrated particle.**
So we now have all three terms of the F_{net} formula, such that:

$F_{net} = r\omega^2(M/N_o)(1 + \delta_w) - r\omega^2 (M/N_o)(V_p + \delta_w/\rho_{solvent})\rho_w - fv$

Since, $F_{net}=0$, we have:

$r\omega^2(M/N_o)(1 + \delta_w) - r\omega^2 (M/N_o)(V_p + \delta_w/\rho_{solvent})\rho_w = fv$

If we isolate terms, we get:

$r\omega^2(M/N_o)(1 + \delta_w - V_p\rho_{solvent} - \delta_w(\rho_w / \rho_{solvent}) = fv$

If the solvent density, ρ_{solv}, is equal to or close to that of the density of water, ρ_w, then the ratio reduces to 1 in the above equation further simplifying it to:

$r\omega^2(M/N_o)(1 + \delta_w - V_p\rho_{solvent} - \delta_w) = fv$

The **densities** are only similar when using a *dilute* buffer or salt solution and **does not apply** for solutions containing glucose, glycerol, etc. that do not have densities similar to water.

If we isolate v, we get:

$v = r\omega^2(M/N_o f)(1 - V_p\rho_{solvent})$

The point of this exercise is to eliminate $r\omega^2$ from the equation, which is done by defining the sedimentation coefficient to be equal to $v/r\omega^2$ ($S = v/r\omega^2$).

Therefore, from the above equation, we could also write the sedimentation coefficient as:

$S = M(1 - V_p\rho_{solvent})/ N_o f$ – this equation is entirely based on macroscopic quantities.

Through substitution of $F=kT/D$ and $R=N_o k$, the equation could be rewritten as:

$S = MD(1 - V_p\rho_{solvent})/ RT$ **Svedberg Equation**

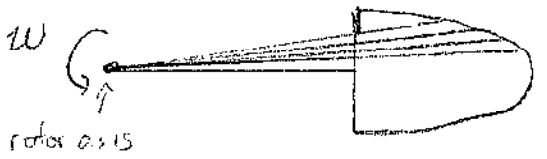
Therefore, by measuring S, D and V_p, we would be able to obtain the molecular weight of a single "dry" particle of the macromolecule.

This is a very good estimate of molecular weight as we made only **one assumption,** which was that the solvent density was similar to that of water. This technique provides a much *better estimate* of molecular weight compared to gel electrophoresis.

Measuring S for a population of particles
We are going to measure S using **boundary sedimentation.**
To begin, we start with a cell filled with a homogeneous solution of particles in the buffer. This means that no gradients exist in the solution. For this experiment, we will monitor the distribution of the particles, generally through absorbance, which can be measured at 230nm or 280nm for proteins and 260nm for nucleic acids. We can use the absorbance because **absorbance is proportional to the concentration** of the particles.

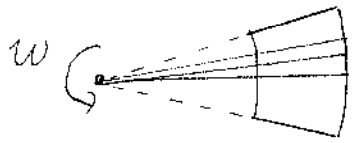
Two complexities exist with this method: 1) diffusion occurs in tandem with sedimentation, and 2) the shape of the cell.

First, we will look at the *preparative* ultracentrifuge. (top view)



As can be seen in the figure, the lines of force generated from the centrifugal motion are radial in respect to the center. Due to the nature of these force lines and the shape of the tube, the particles are pushed into the walls of the tube. This causes an accumulation of the particles at the walls of the tube, which can lead to aggregation and/or local density perturbation. If the local density of an area increases, this will lead to these particles sedimenting faster or further than they should leading to incorrect sedimentation coefficient calculations.

Next we look at the *analytical* ultracentrifuge.

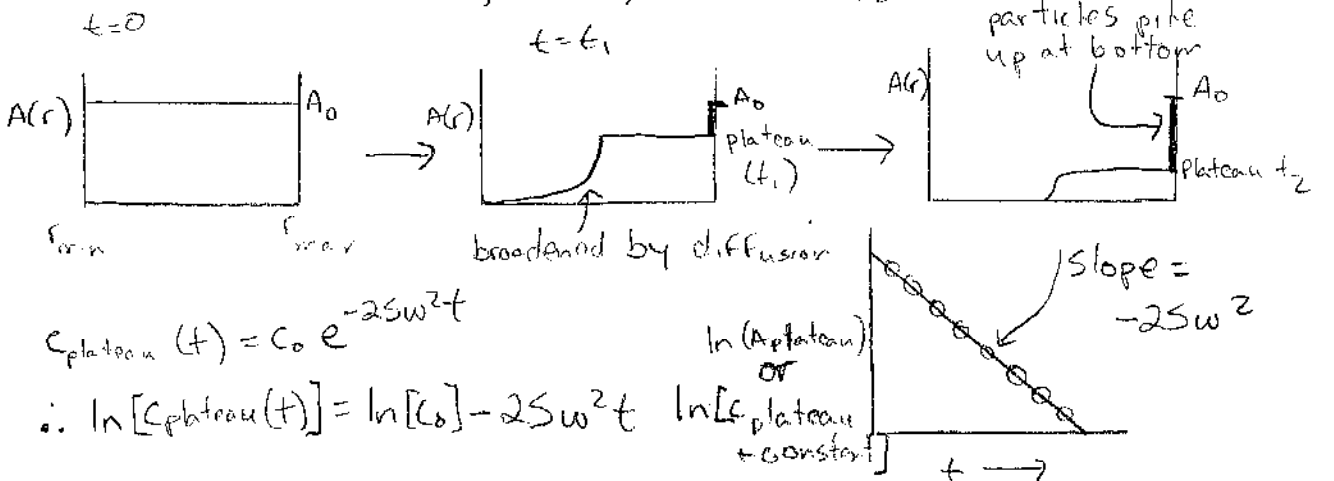


All force lines hit bottom of tube - not the walls

Unlike with the preparative tube, the walls of the analytical tube are parallel to the centrifugal force lines, so we do not have to worry about aggregation or local density perturbation.

Boundary Sedimentation – one form of sedimentation- velocity measurement

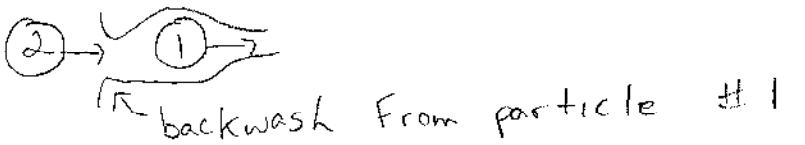
The following figure demonstrates how at time 0(t_0), there is a homogeneous solution of particles, whereas at time 1 (t_1), the particles have begun to sediment and there is no sharp boundary where the particles begin or end due to the diffusion occurring in the cell. At time 2 (t_2), most of the particles have piled at the bottom and the concentration plateau has further decreased. $A(r) \propto C(r) \therefore A_0 \propto C_0$



The boundary is tough to deal with because diffusion causes it not to be sharp.

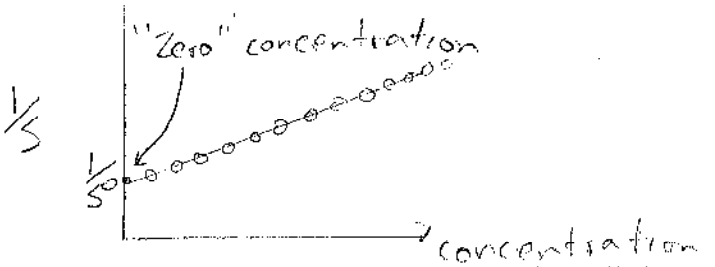
Correcting S- values for particle-particle interactions

This interaction was concentration dependent because it is based on particle collision and "backwash" that is caused from particle movement (see figure).



Concentration dependent effects on S usually obey a function that

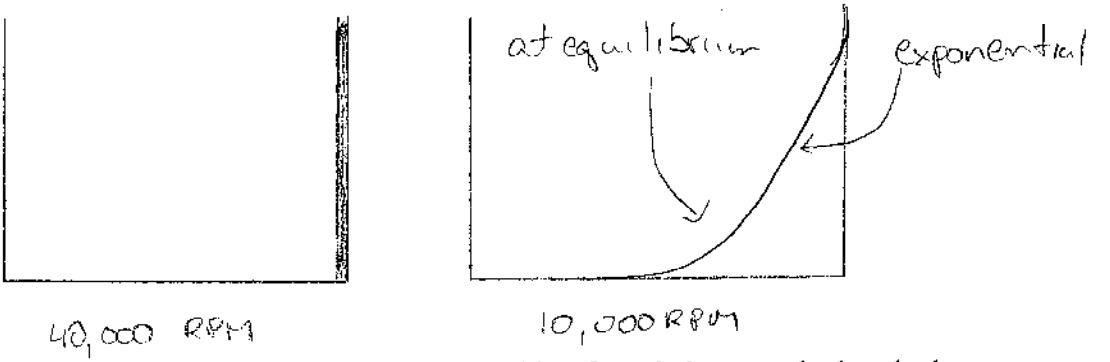
$$S=S^o/(1+kc) \rightarrow 1/S= 1/S^o + kc/S^o$$



S^o represents the sedimentation coefficient when all the concentration dependent effects have been accounted for.

Sedimentation- equilibrium

Equilibrium distribution of a protein (or a nucleic acid) in solution



Entropy acts through diffusion to prevent everything from being smashed at the bottom of the tube/cell.

So the ΔG to move one particle from an arbitrary position, r_1, to a second arbitrary position, r_2, equals **zero** for all $[r_1,r_2]$

$\rightarrow \Delta G= kT \ln\{c(r_2)/[c(r_1)]\}$ + the work to move a particle from r_1 to r_2 against force.

The work term of the equation is equal to:

$$-\int_{r_1}^{r_2} (F_{centr} + F_{buoyant})\, dr$$

This work term is negative because the particle is moving down with the gravitational field.

$$\text{So, } \Delta G = 0 = kT \ln\left[\frac{c(r_2)}{c(r_1)}\right] - \int_{r_1}^{r_2}\left[r\omega^2\left(\frac{M}{N_0}\right)\left(1-\bar{v}_p\rho_{solvent}\right)\right]dr$$

$$\text{Thus, } \ln\left[\frac{c(r_2)}{c(r_1)}\right] = \left(\frac{1}{kT}\right)\left[\frac{\omega^2 M}{N_0}\left(1-\bar{v}_p\rho_{solvent}\right)\left(\frac{r_2^2-r_1^2}{2}\right)\right]$$

$$\Rightarrow \ln\left[\frac{c(r_2)}{c(r_1)}\right] = \frac{M\omega^2\left(1-\bar{v}_p\rho_{soln}\right)}{2RT}\left(r_2^2-r_1^2\right) \qquad R = kN_0$$

Remember: We are dealing with a single uniform population of molecules and we are **assuming** that the solvent density is approximately equal to the density of water. Therefore, we must be in a dilute salt or buffer solution and not solutions containing glyercol, glucose, etc.