University of Washington
Department of Chemistry
Chemistry 452/456
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Lecture 24 8/16/06

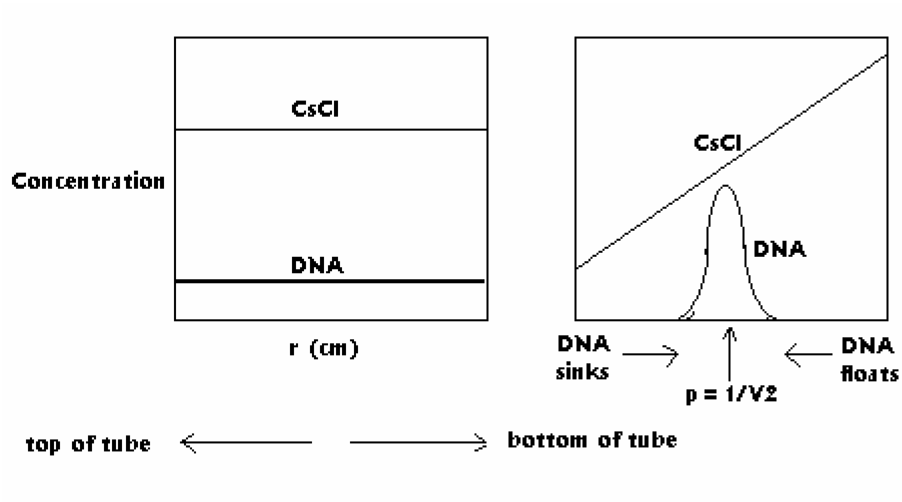
A. Thermodynamics in a centrifugal Field: Equilibrium Centrifugation

- A macromolecular solution subjected to a centrifugal field will quickly attain a steady state condition in which transport of solute mass occurs at constant velocity. However, after a long enough time, the system will attain equilibrium…at which point net transport will cease. Then the transport velocity is zero.
  - The condition of equilibrium requires that the free energy, i.e. the system's chemical potential, which is the sum of the chemical and centrifugal potentials, is minimum. Equivalently its derivative with respect to r is 0 at equilibrium.
    - For convenience designate the total potential of the system μ_{total}.
    - $\mu_{total} = \mu_{chemical} + \mu_{centrifugal} = G^0_{solute} + RT\ln C(r) + U(r)$
      - C is the solute concentration at position r in the centrifuge tube.
      - U is the centrifugal potential at position r in the centrifuge tube.
    - At equilibrium
      $$\frac{d\mu_{total}}{dr} = \frac{d}{dr}(\mu_{chemical} + \mu_{centrifugal}) = \frac{dG^0_{solute}}{dr} + RT\frac{d(\ln C(r))}{dr} + \frac{dU(r)}{dr} = 0$$
      - $U(r) = -\int (F_{buoyancy} + F_{centrifugal})dr = -\int (m - m_0)\omega^2 r dr = -\frac{(m-m_0)\omega^2 r^2}{2}$
    - $\therefore RT\frac{d(\ln C(r))}{dr} = -\frac{dU(r)}{dr} = F_{buoyancy} + F_{centrifugal} = (m - m_0)\omega^2 r$
      - Integrating: $\ln\left(\frac{C}{C_0}\right) = \frac{(m-m_0)\omega^2}{2RT}\left(r^2 - r_0^2\right) = \frac{\omega^2 M_2\left(1-\overline{V}_2\rho\right)}{2RT}\left(r^2 - r_0^2\right)$
      - This equation means that when a solution reaches equilibrium in a centrifugal filed, generated by spinning the samples at an angular frequency w, a concentration gradient will be generated. This experiment can be used to measure macromolecular masses or separate components of a mixture.

B. Equilibrium Sedimentation in a Density Gradient

- A second type of equilibrium centrifugation has proven very useful in the study of nucleic acids. Suppose a solution of a macromolecule (e.g. DNA) also contains a salt such as CsCl. Initially the salt and the DNA have uniform concentrations. Once the centrifugation has commenced the salt quickly reaches equilibrium…

- The CsCl will reach equilibrium described by the equilibrium centrifugation equation:
$$\ln\left(\frac{C_{CsCl}}{C^0_{CsCl}}\right)=\frac{M_{CsCl}\omega^2\left(1-\bar{V}_{CsCl}\rho\right)}{2RT}\left(r^2-r_0^2\right)$$
- Because of the equilibrium condition, the density of the solution will vary as a function of r, the distance from the spinning axis. Suppose at r' the solution has a



density $\rho=\frac{1}{\bar{V}_2}$, where $\bar{V}_2$ is the specific volume of the macromolecule

- At r<r' the density of the solution is less than $\frac{1}{\bar{V}_2}$ and the DNA "sinks" to the bottom of the tube, pulled "downward" by the centrifugal force
- At r>r' the density of the solution is greater than $\frac{1}{\bar{V}_2}$ and theDNA "floats" upwards toward the top of the centrifuge tube.
- At r=r' the DNA density increases…
  - Suppose the solution density gradient is roughly linear with $\rho(r)=\frac{1}{\bar{V}_2}+(r-r')\frac{d\rho}{dr}$, where dρ/dr is the density gradient of the solution near r', and is assumed to be a constant in this region.
  - The condition for equilibrium of the DNA is
  $$\frac{d\ln C_{DNA}}{dr}=\frac{M_{DNA}\left(1-\bar{V}_{DNA}\rho(r)\right)\omega^2 r}{2RT}$$
  - Note the solution density in the buoyancy correction is a function of r, defined above. Then
  $$\frac{d\ln C_{DNA}}{dr}=\frac{M_{DNA}\left(1-\bar{V}_{DNA}\rho(r)\right)\omega^2 r}{2RT}\approx\frac{M_{DNA}\left(1-\bar{V}_{DNA}\left(\frac{1}{\bar{V}_{DNA}}+(r-r')\frac{d\rho}{dr}\right)\right)\omega^2 r}{2RT}$$

- Integrate…and after some calculus… $\ln\left(\frac{C_{DNA}(r)}{C_{DNA}(r')}\right) = -\frac{\omega^2 M_{DNA}\overline{V}_{DNA}}{2RT}\frac{d\rho}{dr}r'(r-r')^2$
- Although this equation seems similar to the equilibrium centrifugation equation, it has a term $(r-r')^2$ instead of $r^2-r'^2$
- The logarithm can be removed to obtain

$$C_{DNA}(r) = C_{DNA}(r')\exp\left(-\frac{\omega^2 M_{DNA}\overline{V}_{DNA}}{2RT}\frac{d\rho}{dr}r'(r-r')^2\right)$$

$$= C_{DNA}(r')\exp\left(-\frac{(r-r')^2}{2\sigma^2}\right)$$

, whichis a bell-shaped curve with a standard deviation $\sigma^2 = \frac{RT}{\omega^2 r' M_{DNA}\overline{V}_{DNA}(d\rho/dr)}$

- Note the standard deviation increases as the salt gradient $d\rho/dr$ decrease. The utility of this technique is that it has very high resolution with respect to molecular mass. Hybrid DNA-RNA was discovered using equilibrium centrifugation in a salt gradient.