Topic:

Solution Transport of Macromolecules

Macromolecular Transport

Transport processes are irreversible processes:

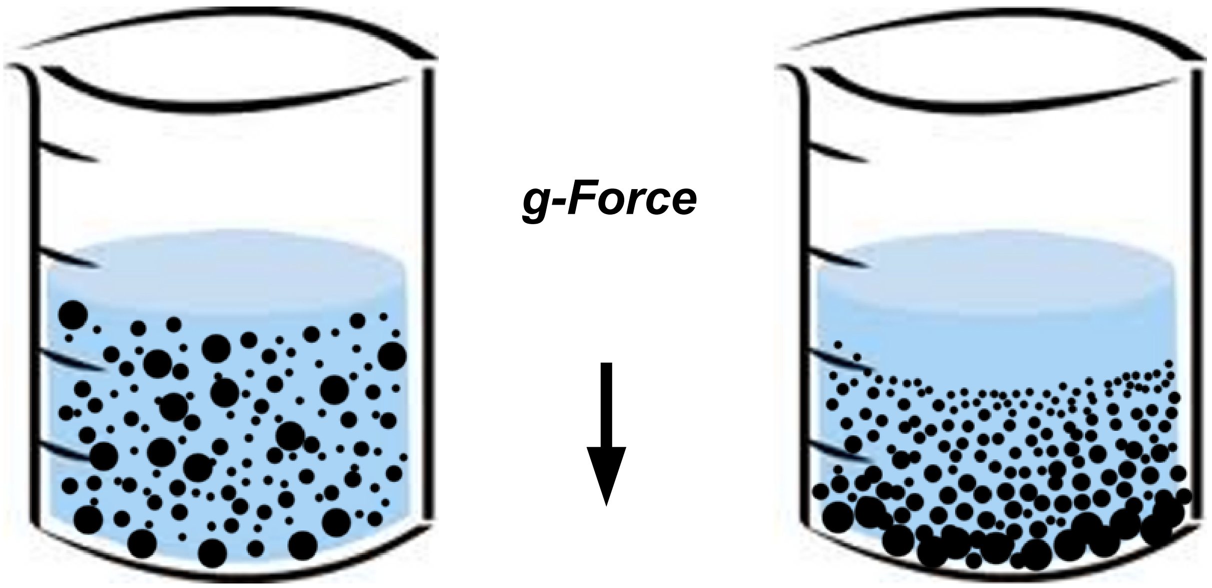
- System is in a non-equilibrium state and relaxes towards an equilibrium
- Transport occurs due to a potential applied to the system:

Process	Potential	Flow of	Equilibrium State	Experiment:
Electrical conduction	Electrostatic	Electrons	Uniform electrostatic potential	Electrophoresis
Heat Conduction	Temperature	Heat	Uniform temperature	Thermophoresis
Diffusion	Chemical Potential	Molecules	Uniform chemical potential	Light Scattering, Fluorescence Correlation, Analytical Ultracentrifugation
Sedimentation	Total potential (chemical potential + centrifugal potential energy)	Molecules	Uniform total potential	analytical ultracentrifugation

The flow is proportional to the gradient in the potential: $J_i = -L_i \frac{\partial U_i}{\partial x}$

Macromolecular Transport - Sedimentation

Sedimentation of fine sand, coarse sand and pebbles by gravity, which particle sediments fastest?



Effect of size:

Bigger particles of the same material (i.e., same density) will sediment faster than smaller particles

Sedimentation is proportional to the MOLECULAR WEIGHT

Macromolecular Transport - Sedimentation

Effect of shape:

Measure speed of sedimentation of a piece of metal:

1. solid ball →*will sediment quickly*

2. flattened into a piece of foil →*will sediment slowly*

Shape matters!

Sedimentation is inversely proportional to ANISOTROPY

Macromolecular Transport - Sedimentation

Effect of partial specific volume:

Measure speed of sedimentation of a piece of metal:

1. flattened into a piece of foil →*will sediment slowly*

2. foil loosely folded into a ball →*will float*

All particles have the same weight – so what's different?

Sedimentation is inversely proportional to BUOYANCY and PARTIAL SPECIFIC VOLUME of sedimenting particle

Macromolecular Transport - Sedimentation

Effect of Solvent Density:

Measure speed of sedimentation of a protein:

1. in distilled water → *will sediment fast*

2. in salt water → *will sediment slowly*

It's the same particle – so what's different?

Sedimentation is affected by the DENSITY of the solvent

It’s impossible for anyone to get drown in Dead Sea!

Dead Sea is ten times more salty than the Mediterranean and three times more salty the Great Salt Lake in Utah

By TelanganaToday | Published: 22nd Jul 2020 7:01 pm

Macromolecular Transport - Sedimentation

Effect of Solvent Viscosity:

Measure speed of sedimentation of a protein:

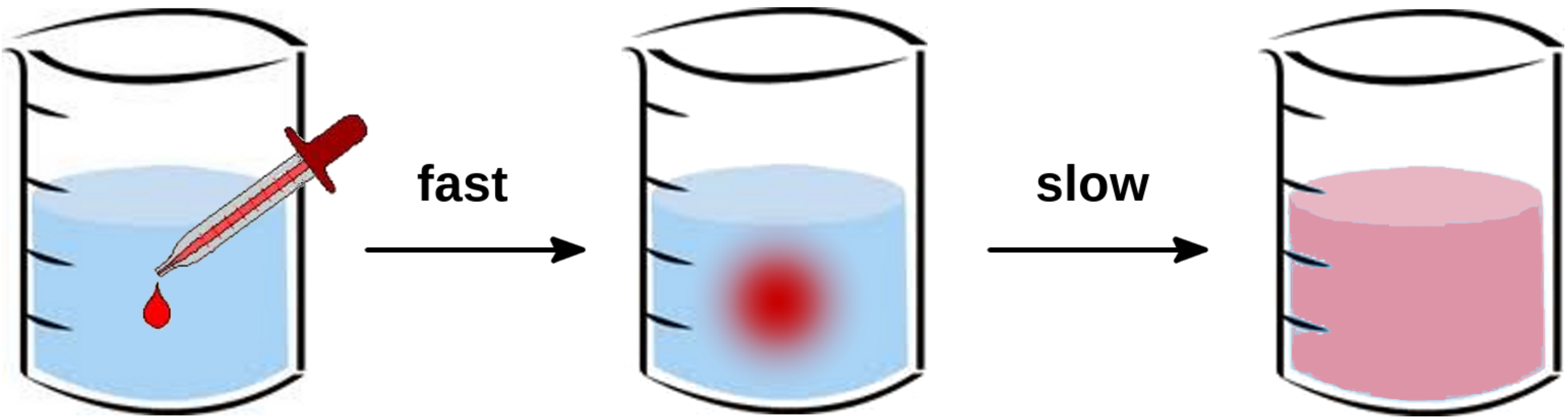
1. in distilled water → *will sediment fast*

2. in a sucrose solution → *will sediment slowly*

It's the same particle – so what's different?

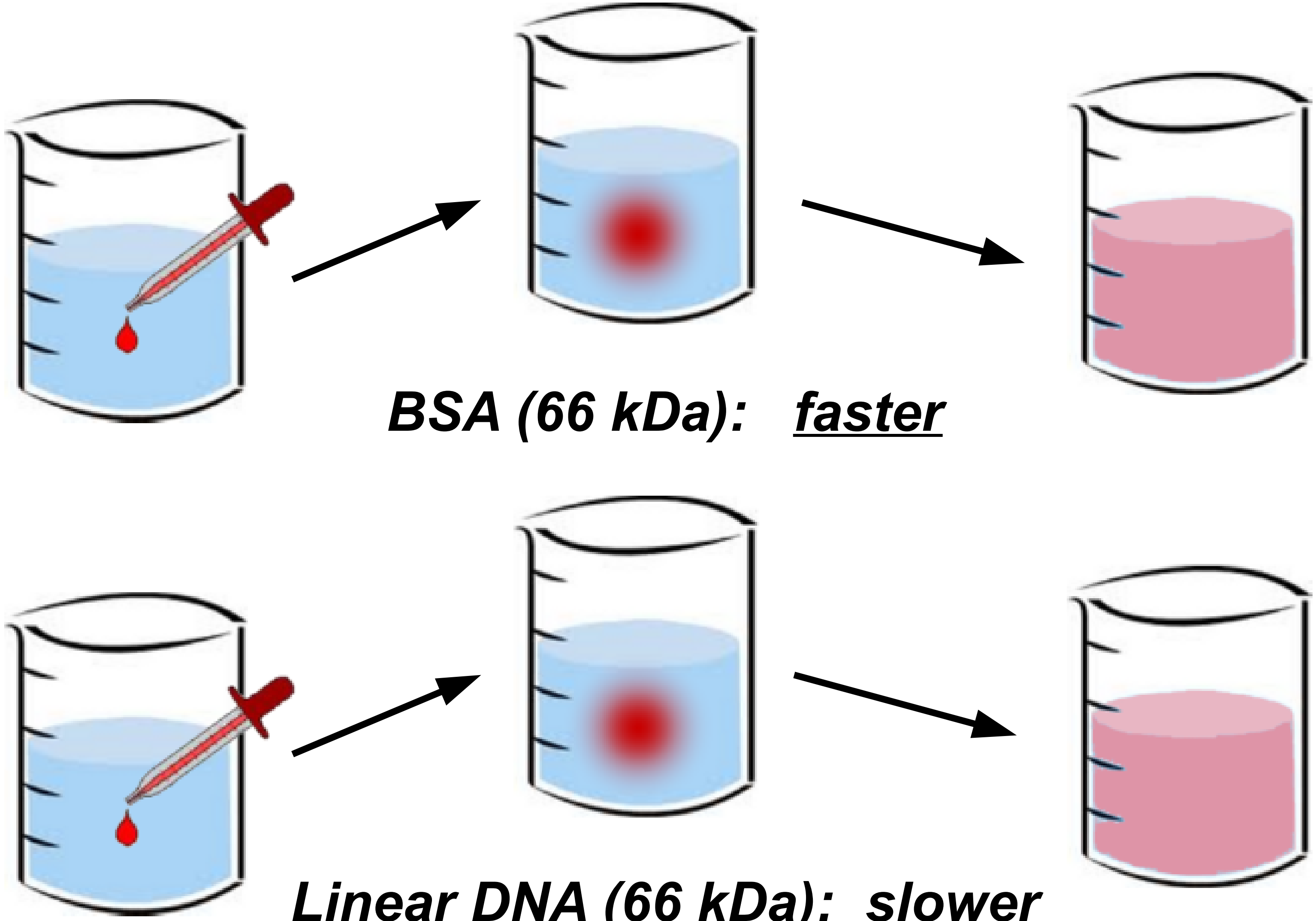
Sedimentation is affected by the VISCOSITY of the solvent

Macromolecular Transport - Diffusion



Diffusion is proportional to the CONCENTRATION GRADIENT

Macromolecular Transport - Diffusion



Diffusion is inversely proportional to the friction of particle

Effect of Solvent Viscosity:

Measure speed of diffusion of a protein:

1. in a viscous solution containg sucrose → *slow*

2. in distilled water → *fast*

Diffusion is inversely proportional to

the VISCOSITY of the solvent

Macromolecular Transport - Diffusion

Effect of Solvent Density:

Measure speed of diffusion of a protein:

1. in solution with high density →

2. in solution with low density → *no change*

Diffusion is NOT affected by the

DENSITY of the solvent

Macromolecular Transport - Diffusion

Summary:

Sedimentation:

- Contribution from the solute:
  - Friction (includes effects from hydration)
  - Mass
  - Density (hydration)
- Contribution from the buffer:
  - Density
  - Viscosity

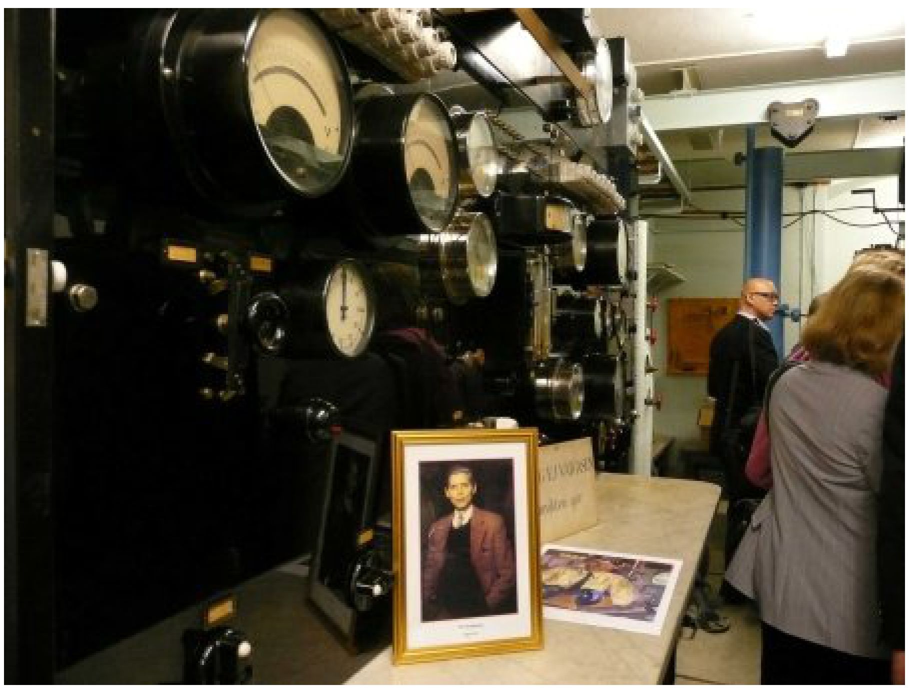
Diffusion:

- Contribution from the solute:
  - Friction
- Contribution from the buffer:
  - Viscosity

Analytical Ultracentrifugation (AUC)

Theodor “The” Svedberg, University of Uppsala, Sweden. 1884-1971
Inventor of the Analytical Ultracentrifuge
Nobel Prize in Chemistry for “work on disperse systems” in 1926

Swedish physical chemist Theodor "The" Svedberg studied the chemistry, distribution, light absorption and sedimentation of colloids and molecular compounds. In 1923 he invented the analytical ultracentrifuge, a high-speed centrifuge used to measure molecular weight of biopolymers.

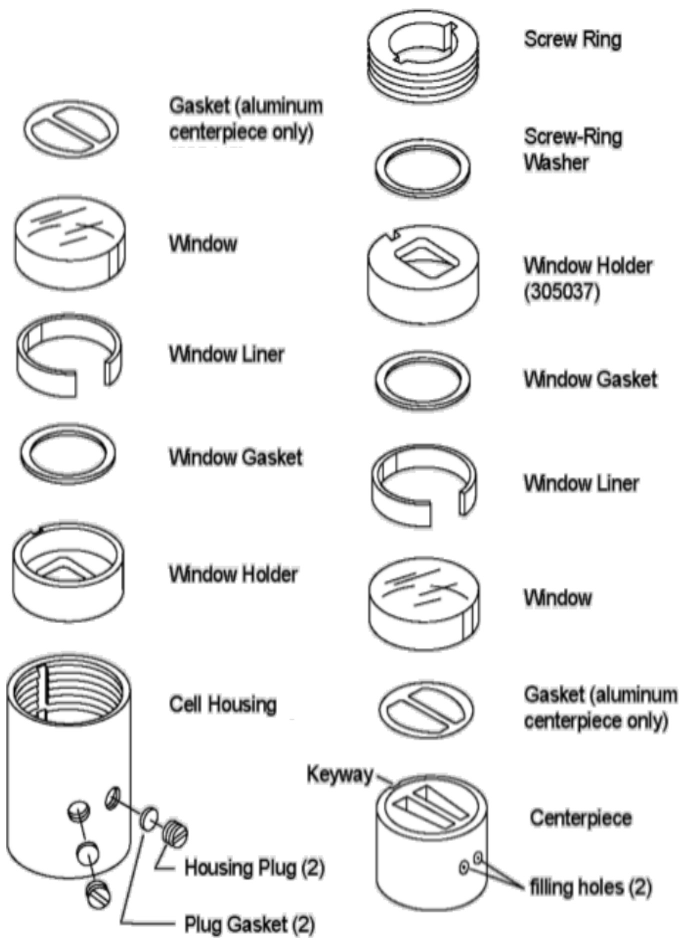


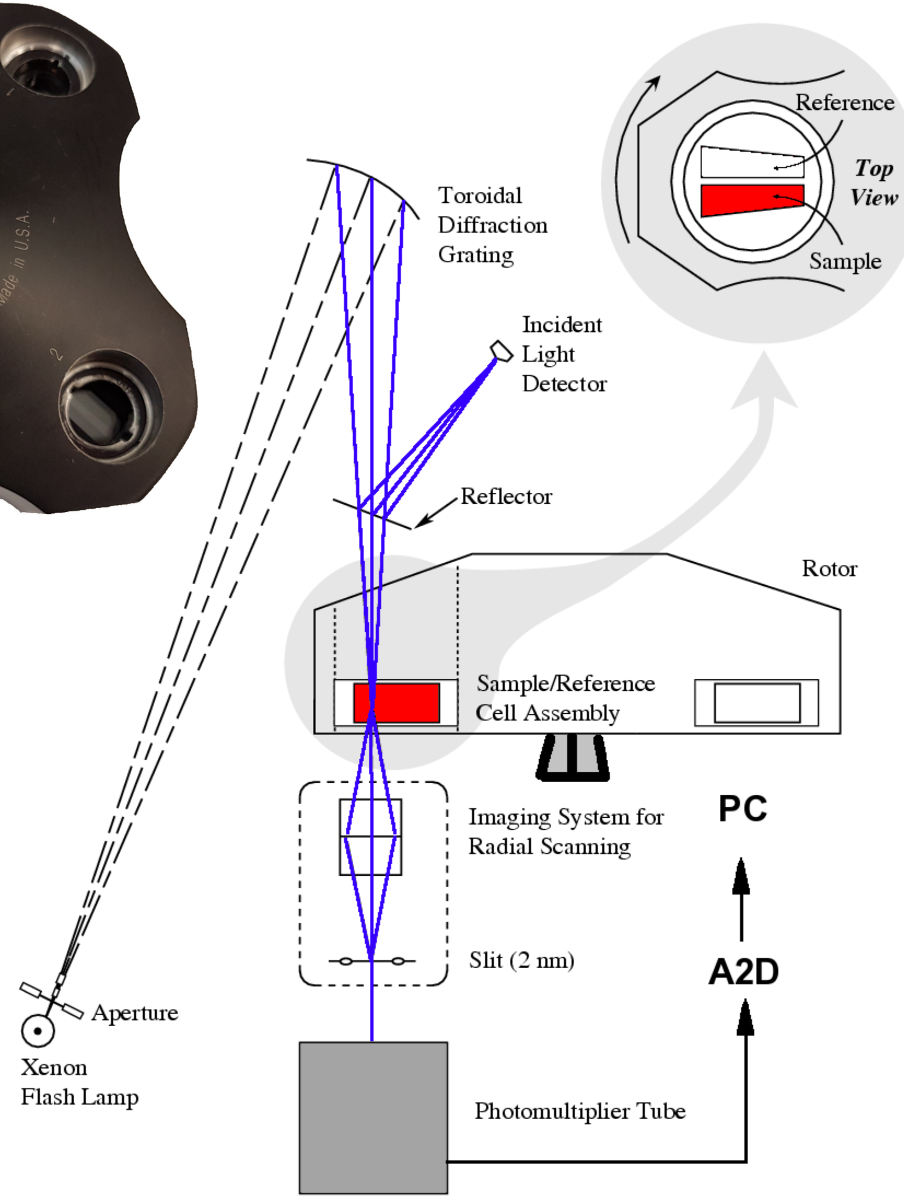
AUC History...

Beckman Optima AUC

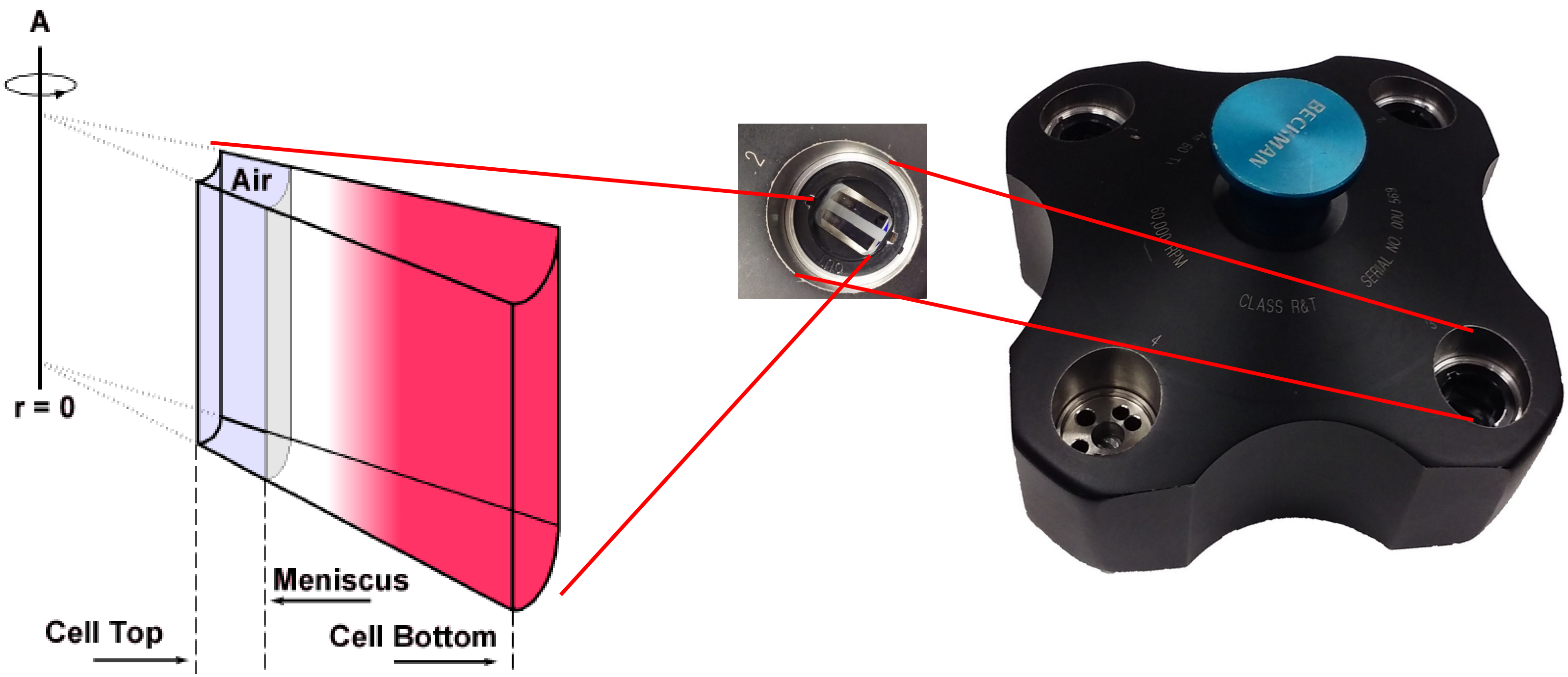
- 10 micron radial resolution
- 0.5 nm wavelength resolution
- 8 seconds per scan
- Network interface
- Multi-wavelength capable
- Built-in database for data acquisition

Macromolecular Transport - Diffusion

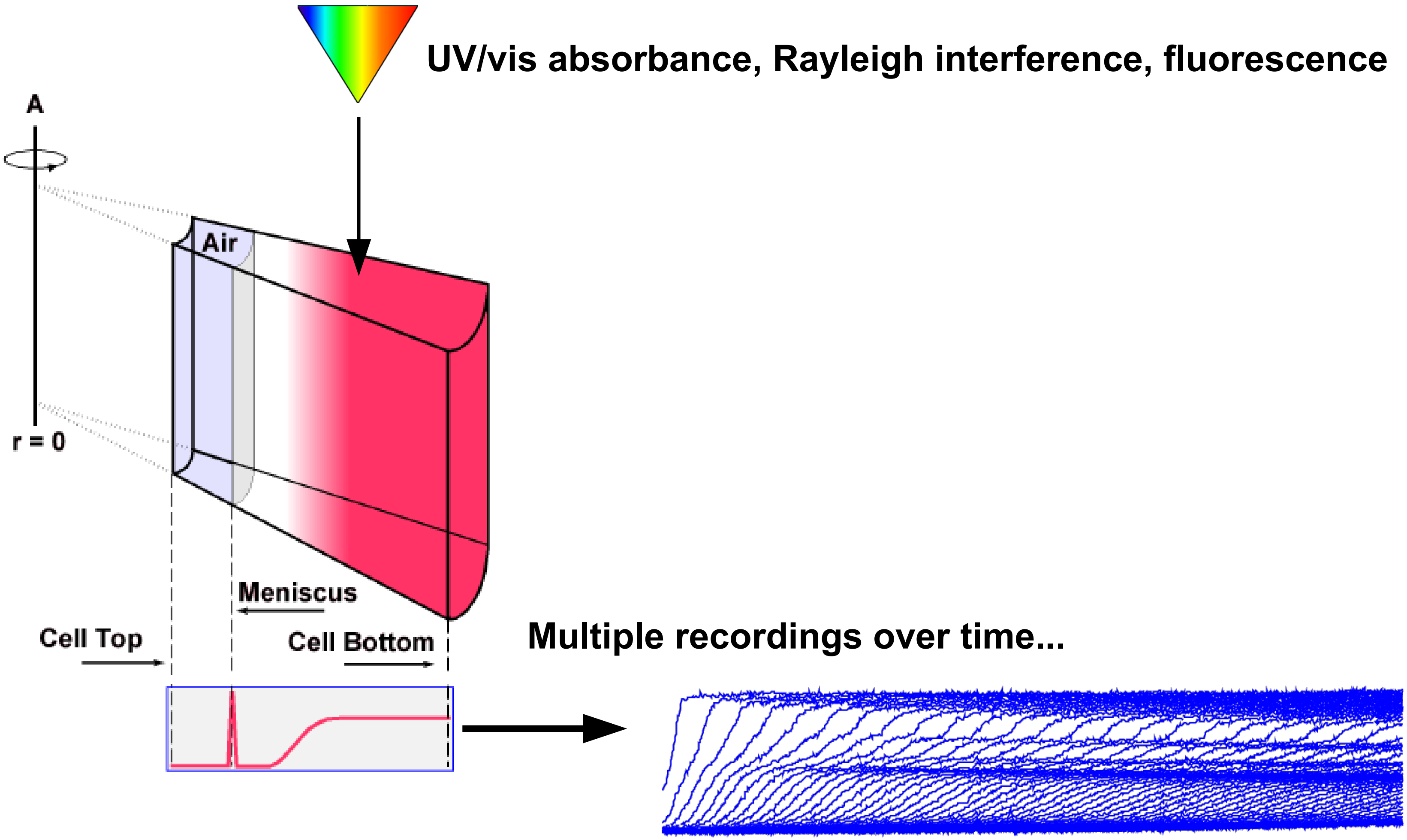




Sedimentation Instrumentation



Sedimentation Instrumentation

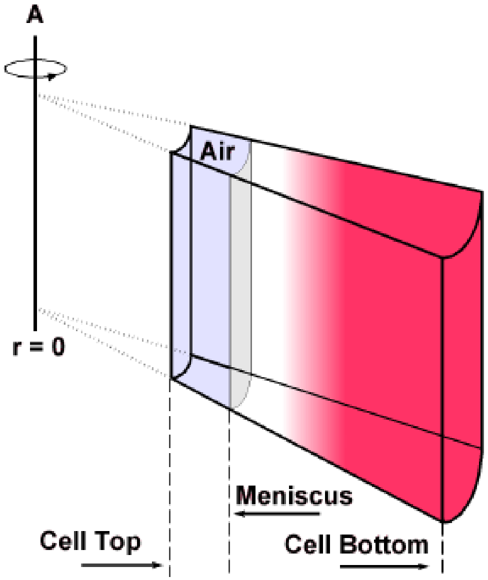


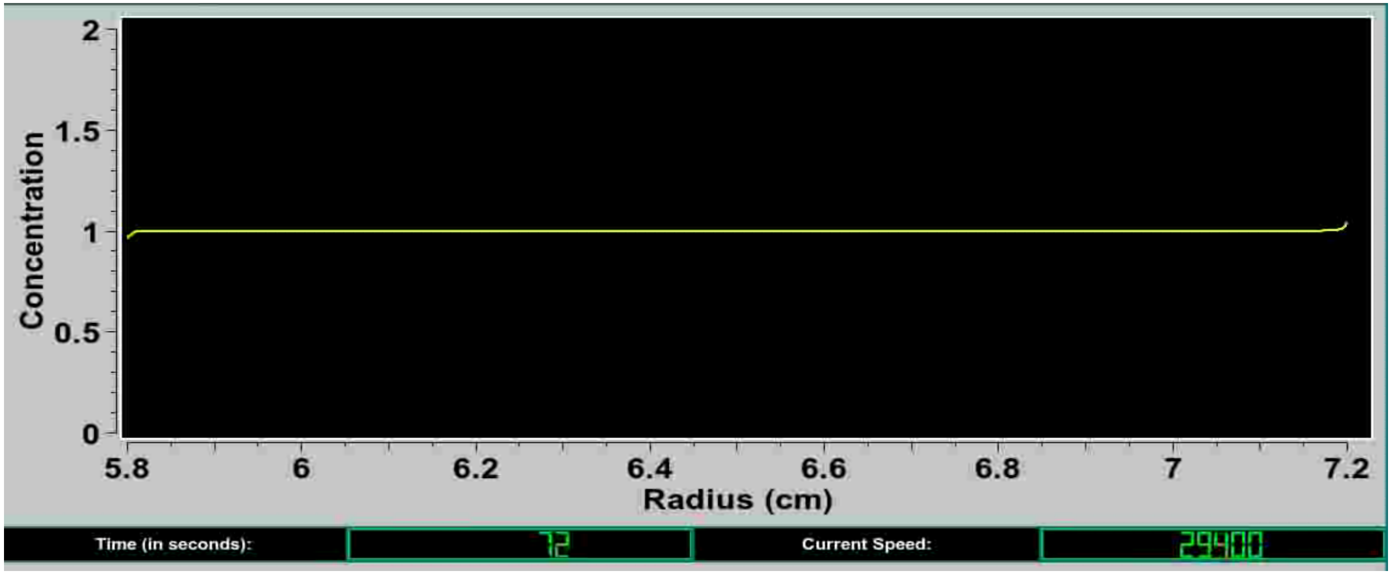
Transport Processes – Sedimentation and Diffusion

$$\left(\frac{\partial C}{\partial t}\right)_r = \frac{-1}{r}\frac{\partial}{\partial r}\left[s\,\omega^2 r^2 C - D\,r\,\frac{\partial C}{\partial r}\right]_t$$

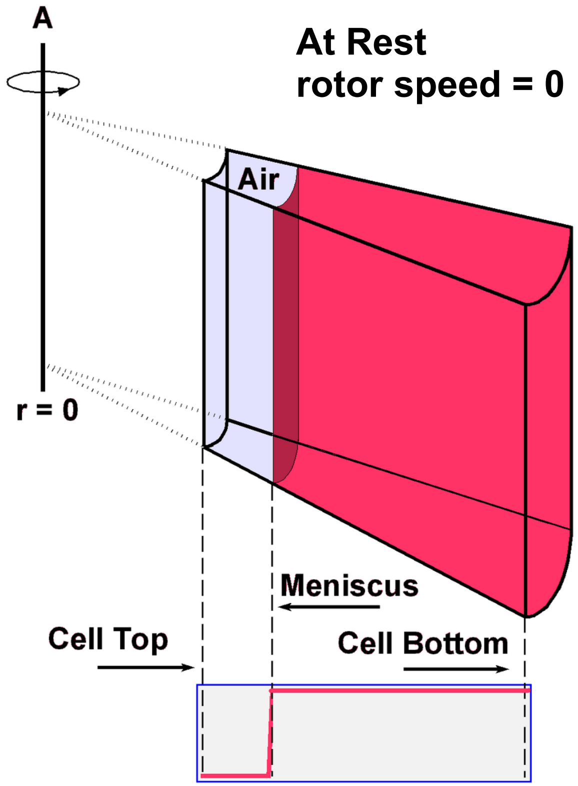
Lamm Equation, solved with finite element method

Cao, W and B. Demeler. Modeling AUC Experiments with an Adaptive Space-Time Finite Element Solution for Multi-Component Reacting Systems. Biophys. J. (2008) 95(1):54-65

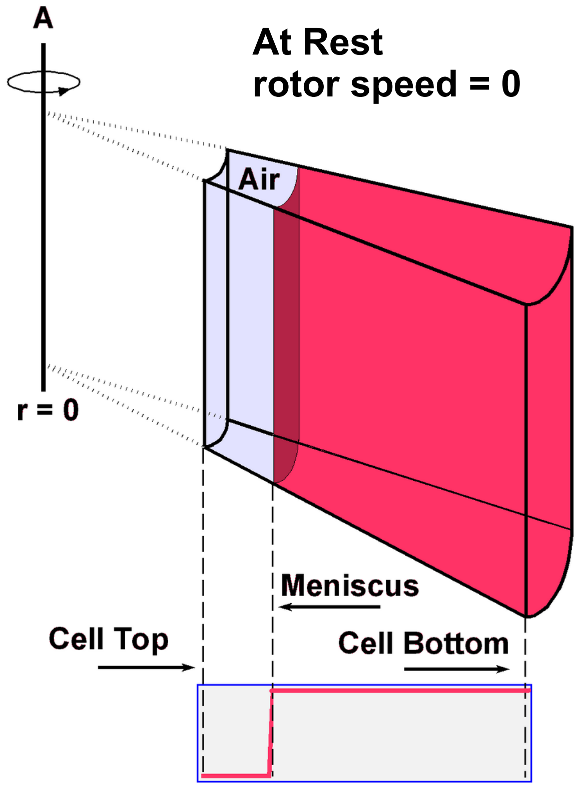


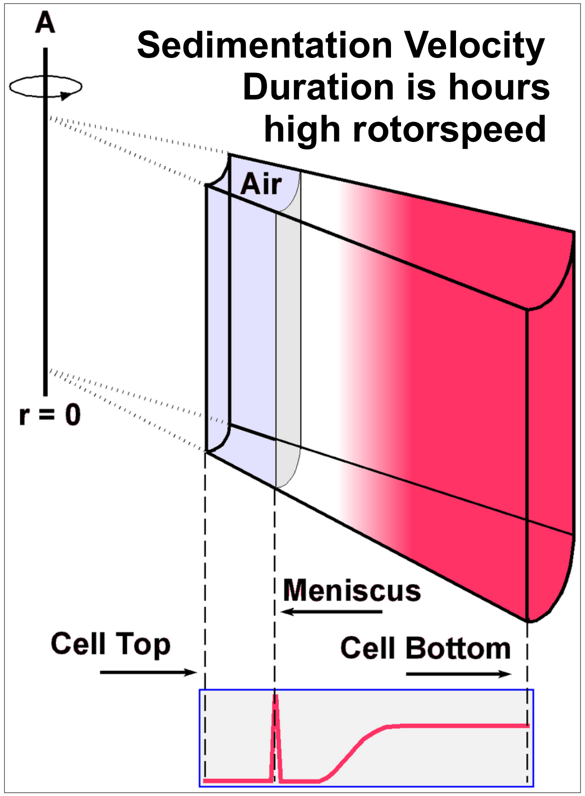


Transport Processes – Sedimentation and Diffusion



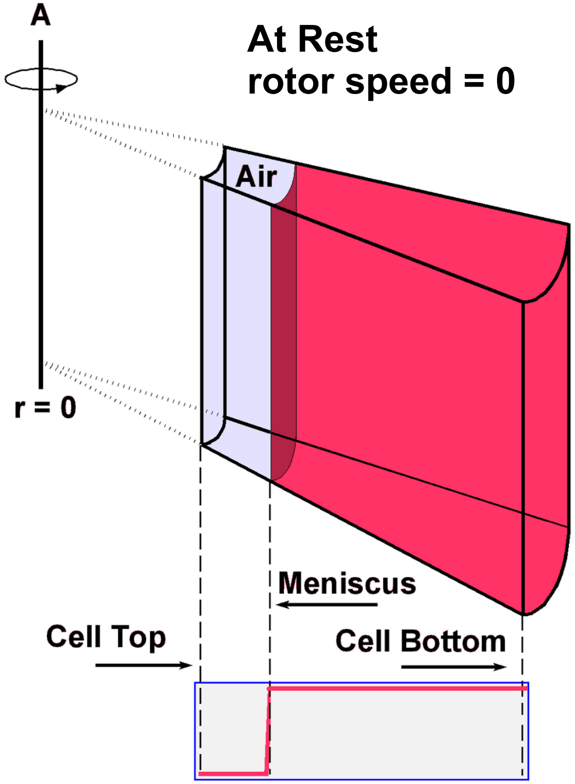
Transport Processes – Sedimentation and Diffusion

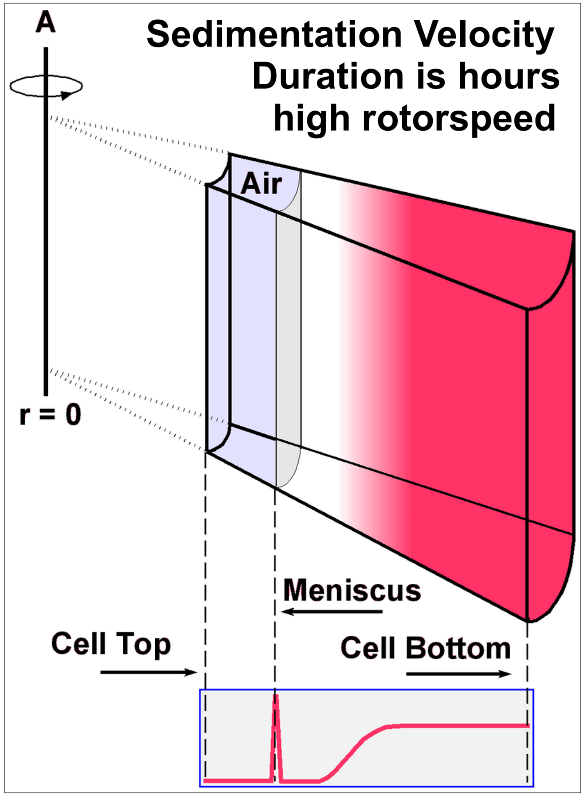




Transport Processes – Sedimentation and Diffusion

$$\left(\frac{\partial C}{\partial t}\right)_r = \frac{-1}{r}\frac{\partial}{\partial r}\left[s\omega^2 r^2 C - D r \frac{\partial C}{\partial r}\right]_t$$



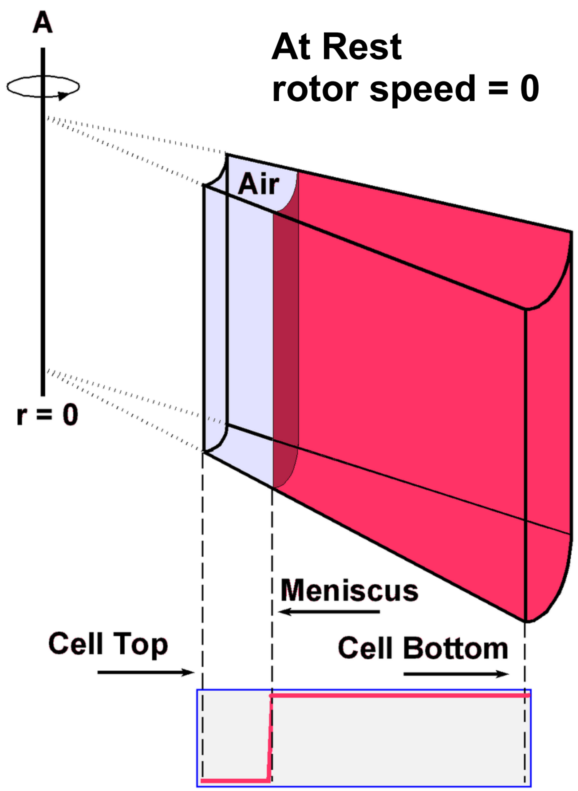


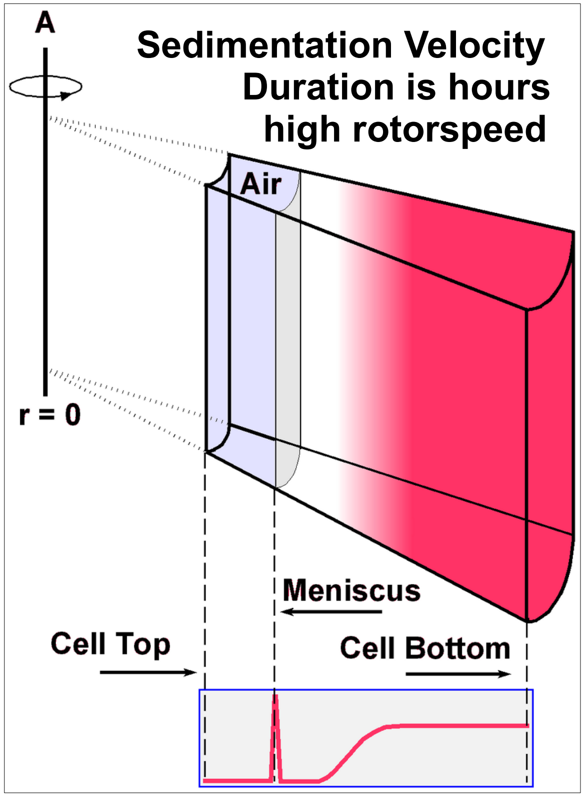
Question: ***What happens at the end of the sedimentation experiment?***

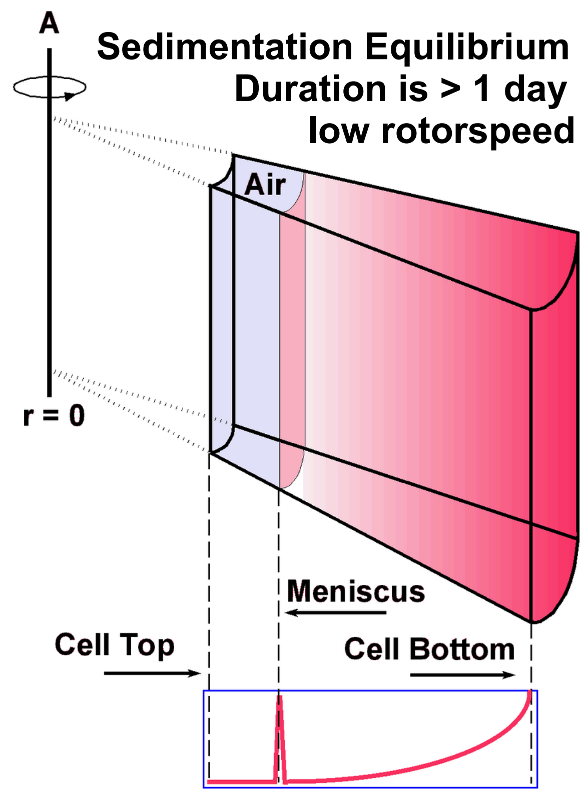
Transport Processes – Sedimentation and Diffusion

$$\left(\frac{\partial C}{\partial t}\right)_r = \frac{-1}{r}\frac{\partial}{\partial r}\left[s\omega^2 r^2 C - D r \frac{\partial C}{\partial r}\right]_t$$

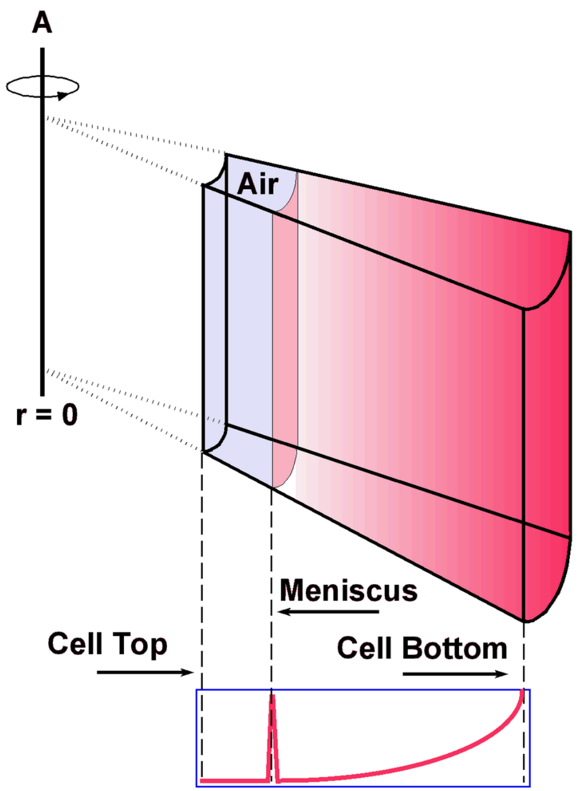
$$J = s\omega^2 r C - D\frac{\partial C}{\partial r} = 0$$

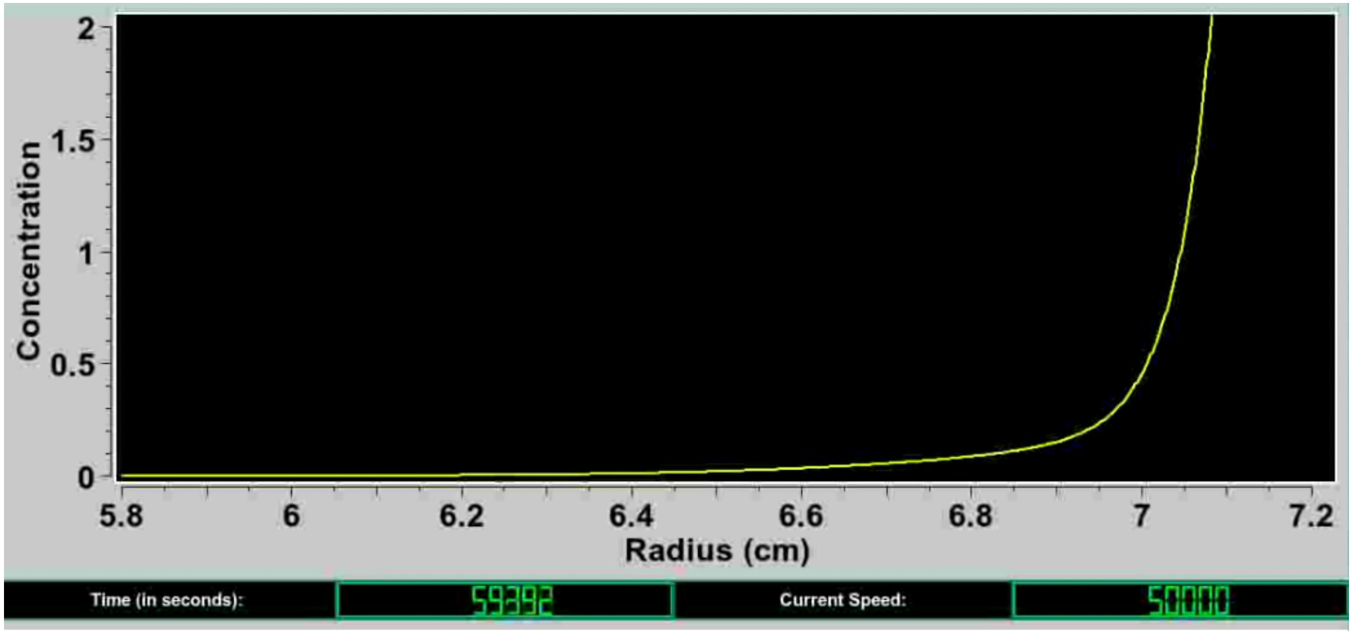






Transport Processes – Sedimentation and Diffusion



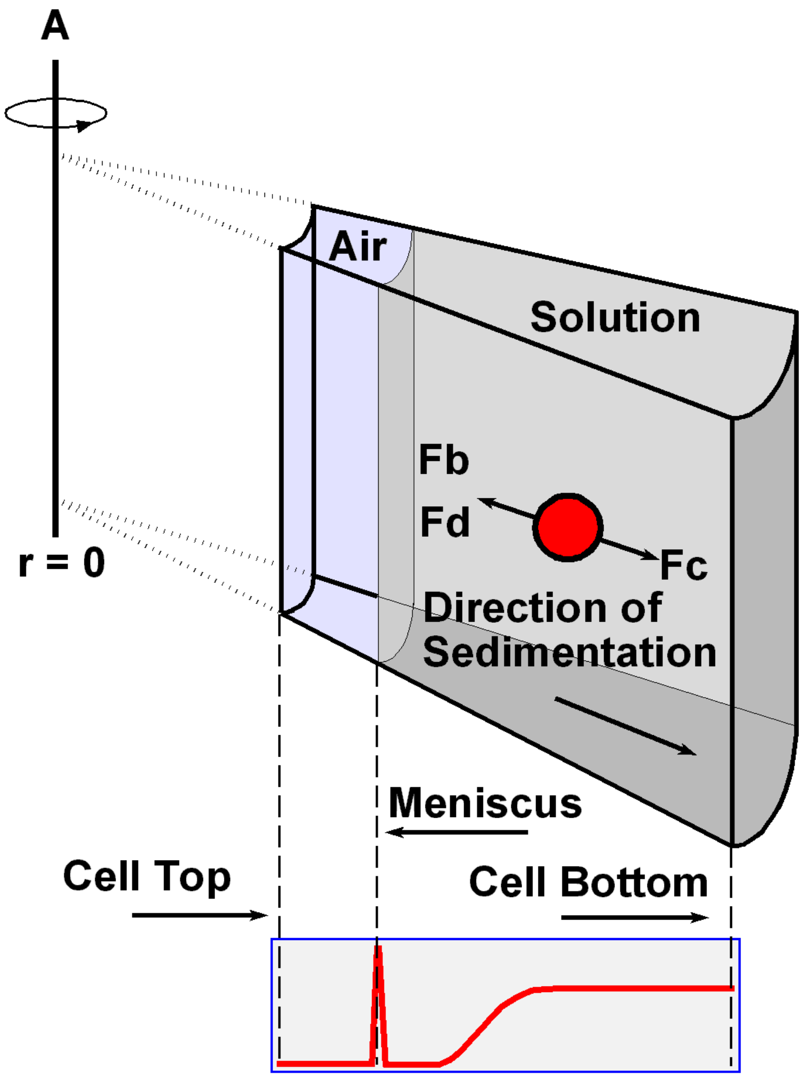


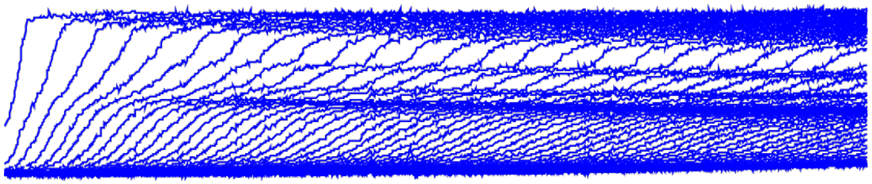
$$J = s\omega^2 r C - D\frac{\partial C}{\partial r} = 0$$

Sedimentation Equilibrium
Duration is > 1 day
low rotorspeed

$$C = C_0\, e^{\left[\sigma\left(r^2 - r_m^2\right)\right]}$$

Transport Processes – Sedimentation





Sedimentation:

Forces at Equilibrium:

Fc - Fb - Fd = 0

***Fb* (buoyancy)** = $\omega^2 r m_s$

***Fd* (viscous drag)** = $f v$

***Fc* (centrifugal force)** = $\omega^2 r m$

$\omega = rpm * (\pi/30)$

Explanation:

Fb is the buoyancy force - the force required to displace the buffer surrounding the solute, and m_s is the mass of the displaced solvent.

Transport Processes – Sedimentation

Fb (buoyancy) = $\omega^2 r\, m_s$
Fd (viscous drag) = $f\, v$
Fc (centrifugal force) = $\omega^2 r\, m$

Substitute the mass of the solvent, m_s, with the mass of the solute, m

$$m_s = m\bar{v}\rho\,, \quad Fb = \omega^2 r m \bar{v} \rho$$

Rearrange the force equation: $Fc - Fb - Fd = 0$ and substitute

$$\omega^2 r m - \omega^2 r m \bar{v} \rho = f v$$

Place molecular parameter on one side and experimental parameters on the other

$$\frac{m\left(1 - \bar{v}\rho\right)}{f} = \frac{v}{\omega^2 r}$$

Put into molar units by multiplying with Avogadro's number, N

$$\frac{M\left(1 - \bar{v}\rho\right)}{Nf} = \frac{v}{\omega^2 r} = s$$

Transport Processes – Sedimentation:

$$\frac{M(1-\bar{v}\rho)}{Nf} = \frac{v}{\omega^2 r} = s$$

Definition:

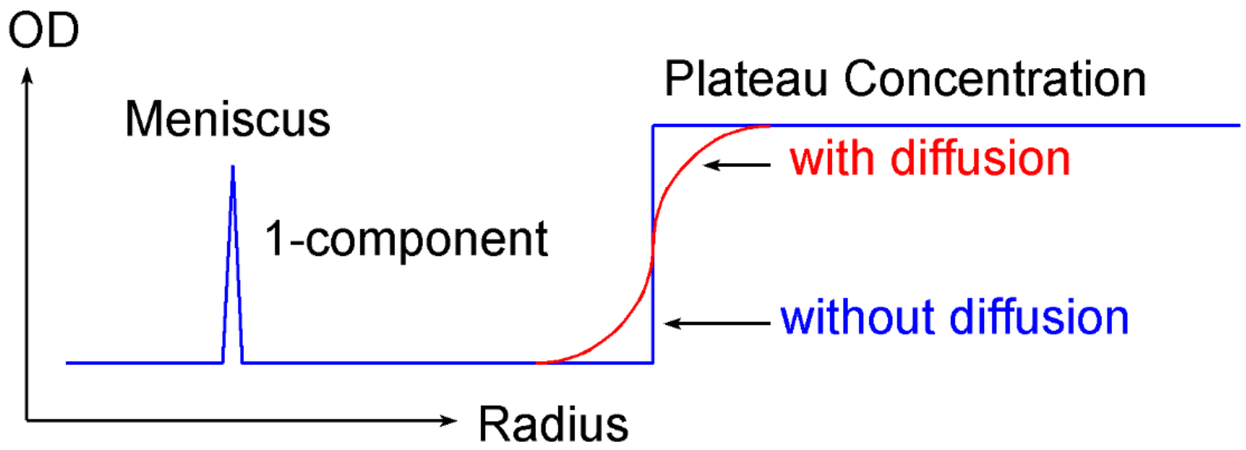
The sedimentation velocity, *v*, divided by the centrifugal field strength, $\omega^2 r$, is equal to the sedimentation coefficient, *s*

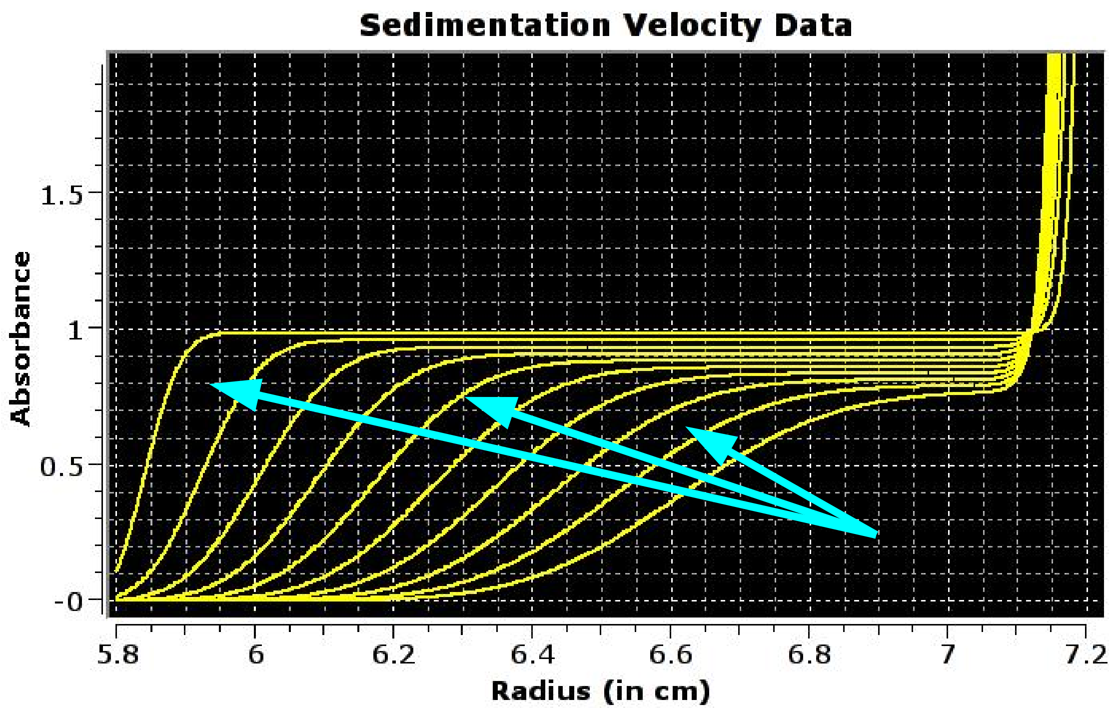
Take-home message:

The sedimentation coefficient is proportional to *M* and inversely proportional to *f*

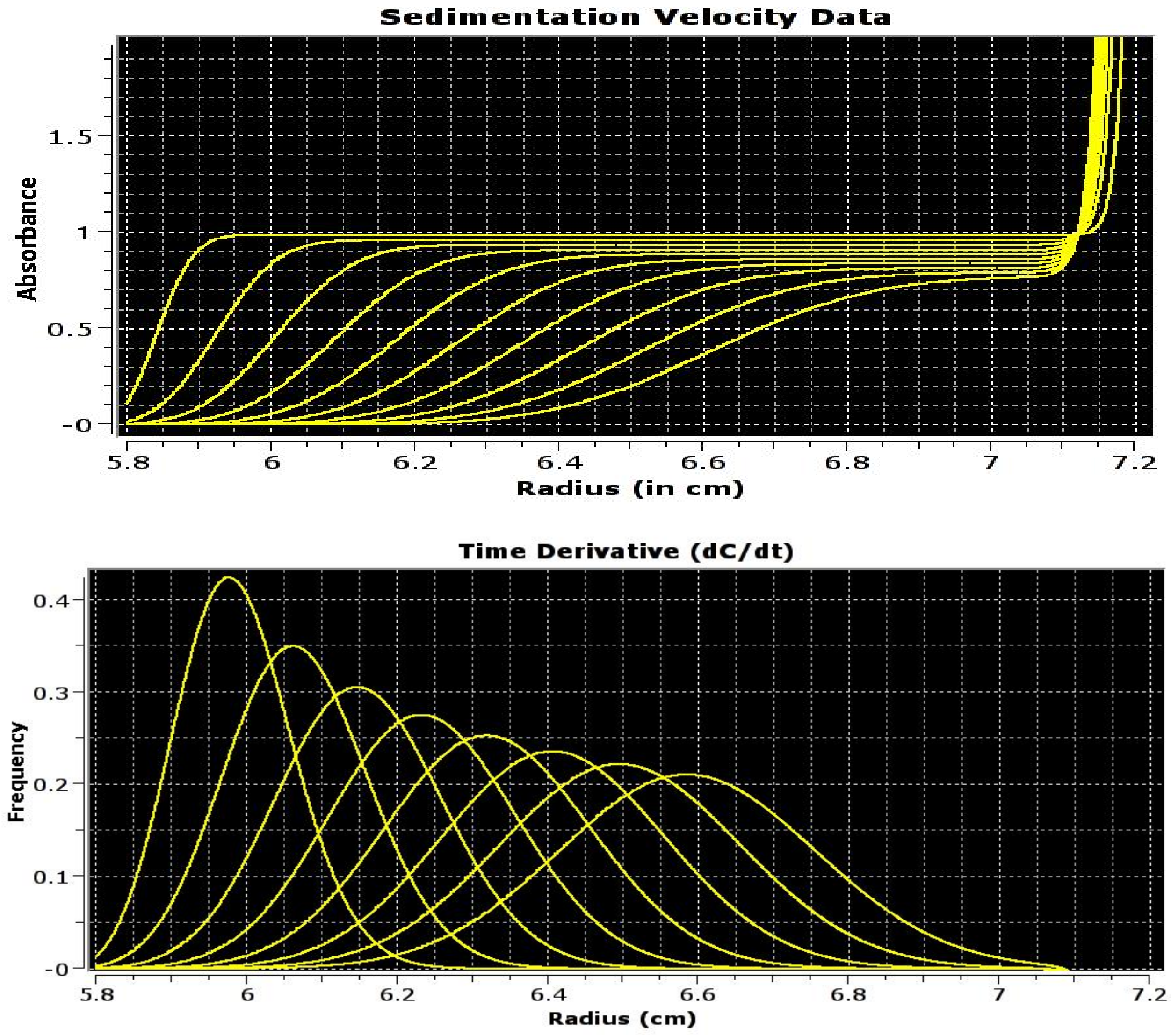
Transport Processes – Diffusion:

Effect of Diffusion on the sedimenting boundary shape:





Transport Processes – Diffusion:



Question: ***When is the concentration gradient the steepest in the sedimentation experiment?***

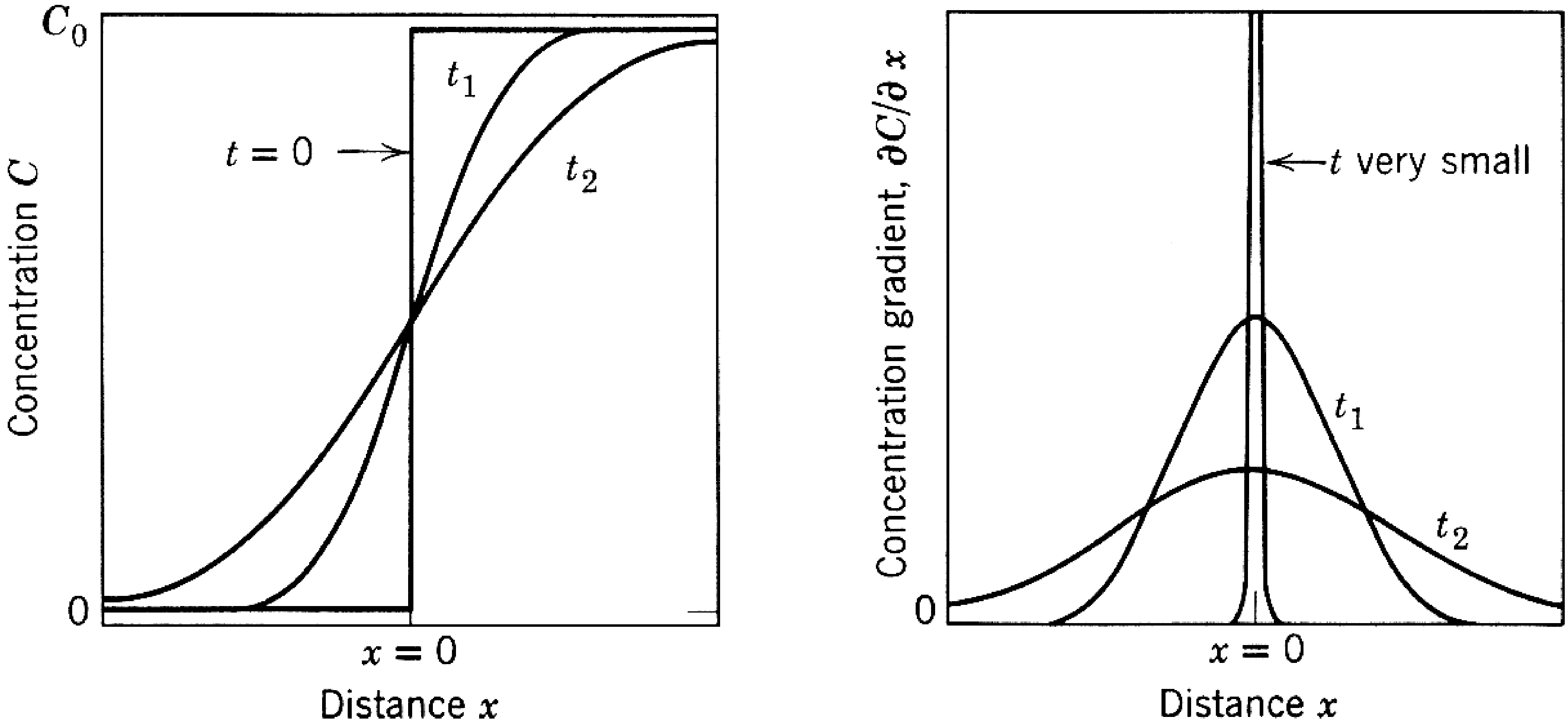
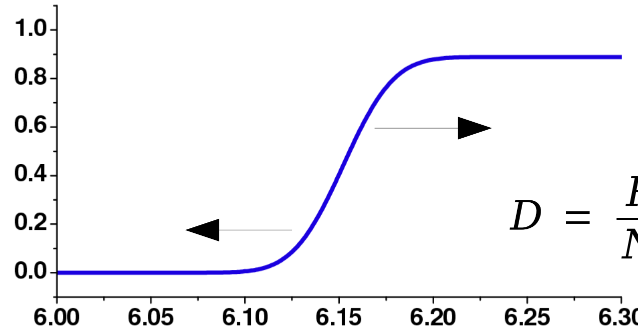


Fig. 21–1. Progress of a diffusion experiment with initially sharp boundary at $x = 0$.

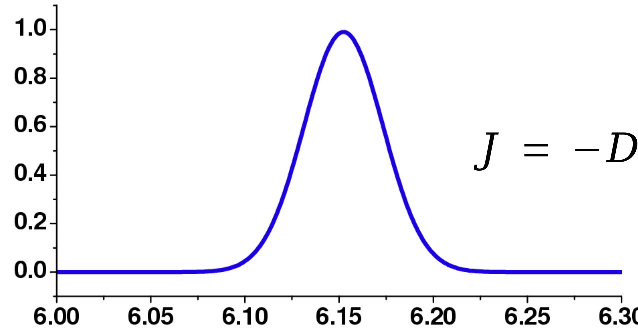
Transport Processes – Diffusion:

Random translation of a particle due to **Brownian motion**



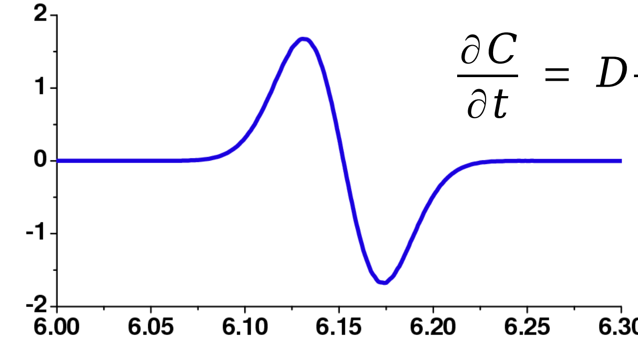
$$D = \frac{RT}{Nf}$$

The flow due to diffusion is proportional to the concentration gradient and the diffusion coefficient (Fick's first law):



$$J = -D\frac{\partial C}{\partial x}$$

The rate of change of concentration is proportional to the change in steepness of the concentration gradient (Fick's second law):



$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$$

Transport Processes – Diffusion:

How do we measure Diffusion?

1. **Boundary method**
2. **Dynamic light scattering**
3. **Fluorescence Correlation Spectroscopy**
4. **Sedimentation Velocity**

Transport Processes – Diffusion:

$$D = \frac{RT}{Nf} \quad and \quad f = 6\pi\eta r$$

$$r = \frac{RT}{N 6\pi\eta D}$$

For a spherical particle, we can predict the frictional coefficient with the Stokes - Einstein relationship.

For any molecule, the measured frictional coefficient can then be used to calculate the corresponding radius. This is called the Stokes radius. This is the radius of a hypothetical sphere that has the same frictional coefficient as the molecule. The Stokes radius has a volume that is larger or equal to the volume of the actual molecule. Most macromolecules are NOT spherical.

If the volume is known, the radius r_0 of a hypothetical minimal sphere can be calculated, as well as its frictional coefficient, f_0.

The ratio of $\varphi = f/f_0$ is called the frictional ratio, and defines the anisotropy of the molecule.