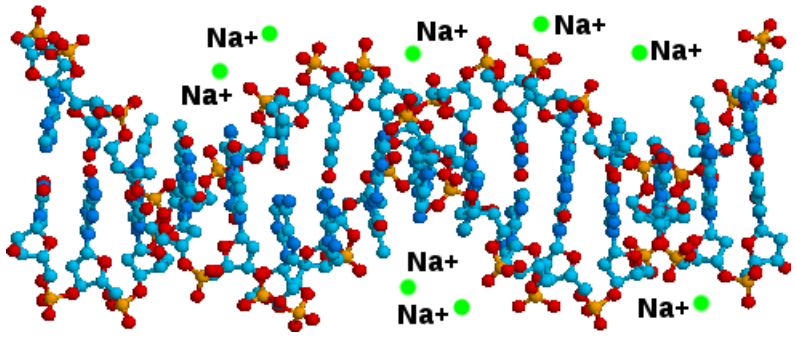
Presenter: Borries Demeler

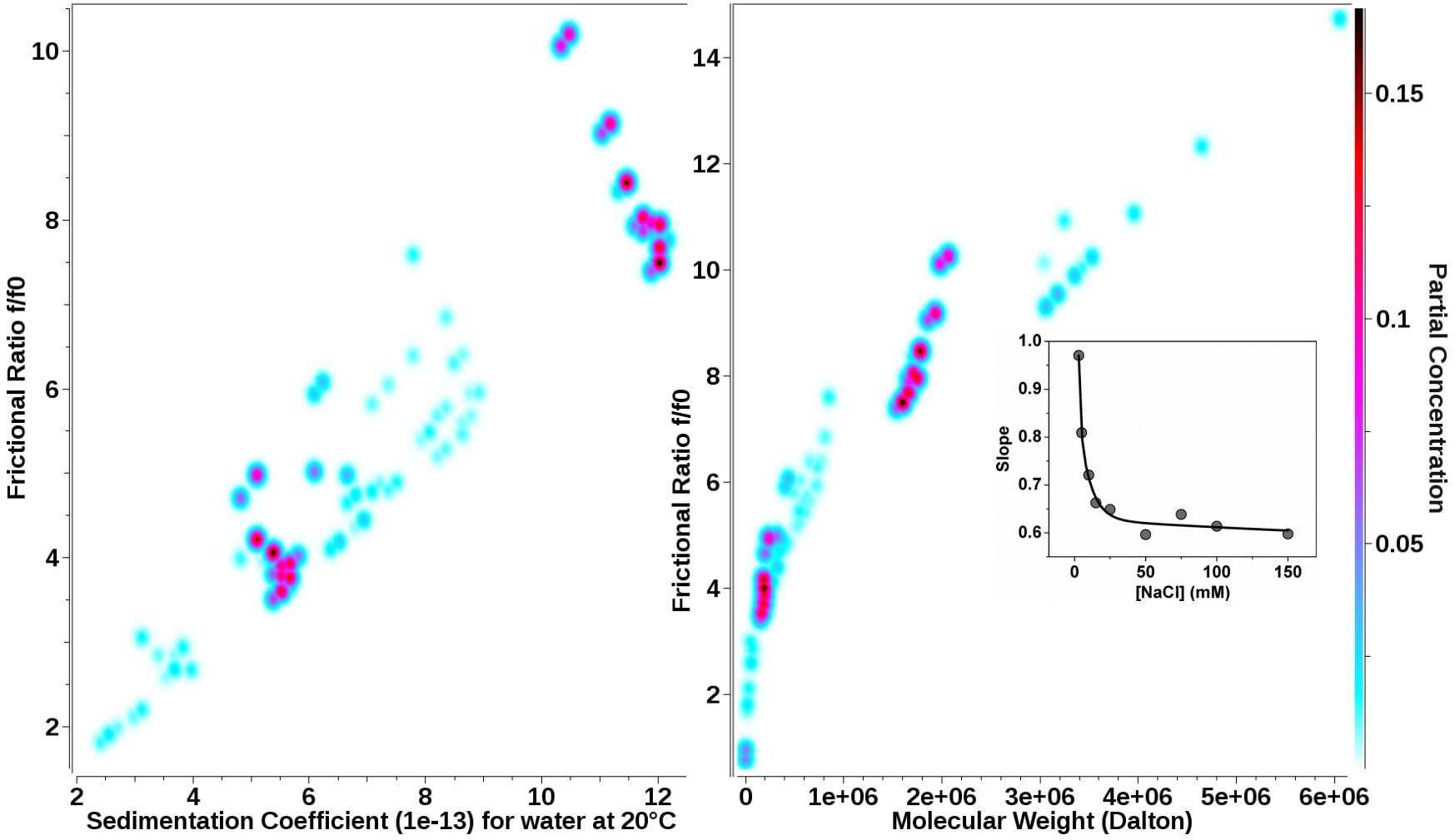
Topic:
Sedimentation Transport II

Copy of Lecture at:

https://demeler.uleth.ca/biophysics/archive/Demeler/

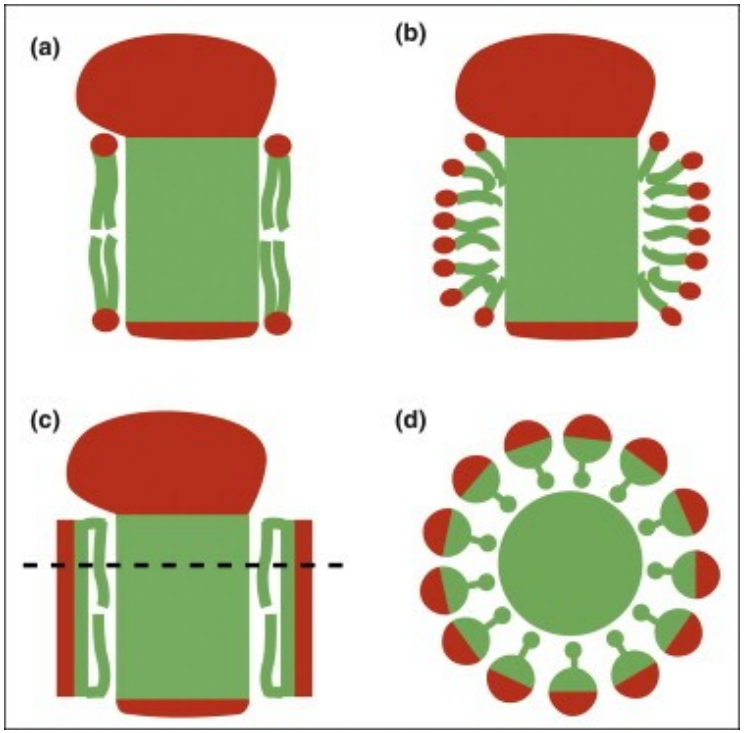
Transport Processes – Partial Specific Volume and Buoyancy





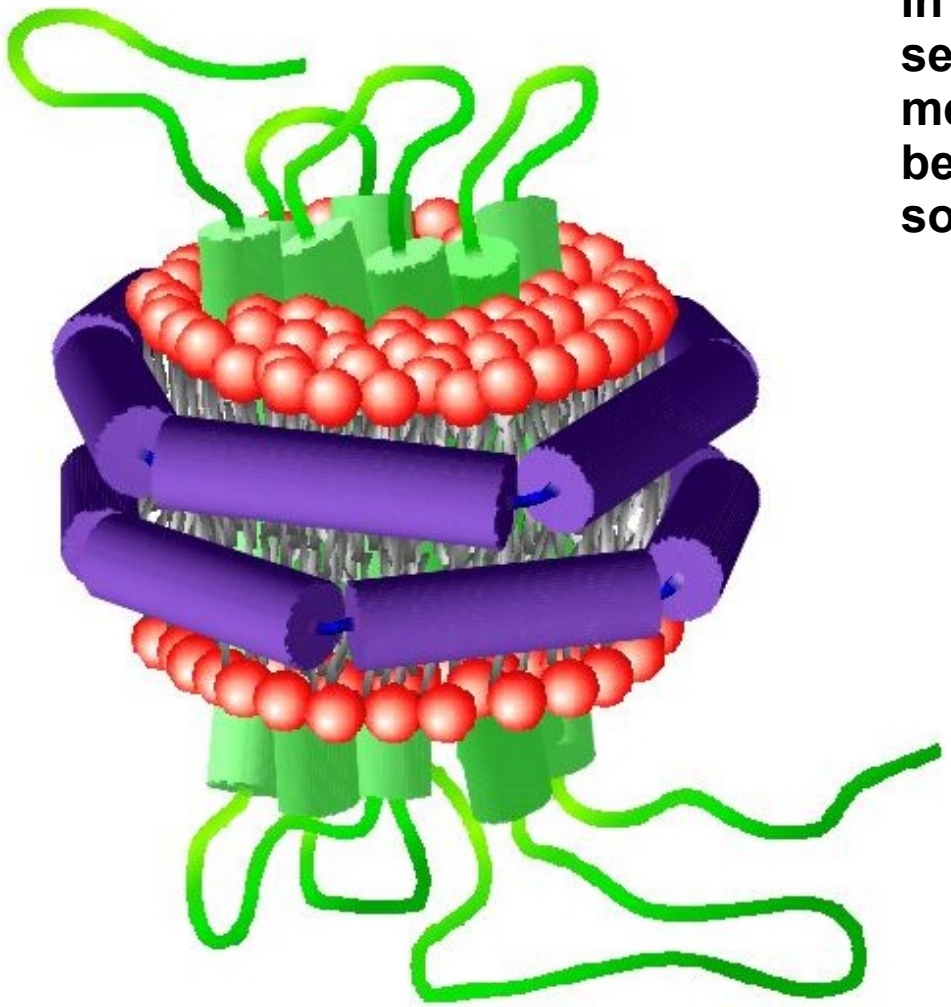
Transport Processes – Partial Specific Volume and Buoyancy

Whenever there is binding, the vbar will correspond to the vbar of the sedimenting particle, and must include all components bound to the molecule of interest. Likewise, the molecular weight will increase due to the binding event.



$$\left(1-\Phi\rho\right)_{sp} = \left(1-\bar{\nu}_{prot}\,\rho\right) + \delta_{det}\left(1-\bar{\nu}_{det}\,\rho\right) + \ldots$$

Transport Processes – Partial Specific Volume and Buoyancy



In this nanodisk assembly, the sedimenting particle consists of a membrane protein, phospholipids, belt protein and any ions and solvent molecules attached to them.

Transport Processes – Partial Specific Volume and Buoyancy

Strategies for measuring MW of proteins when detergent is bound:

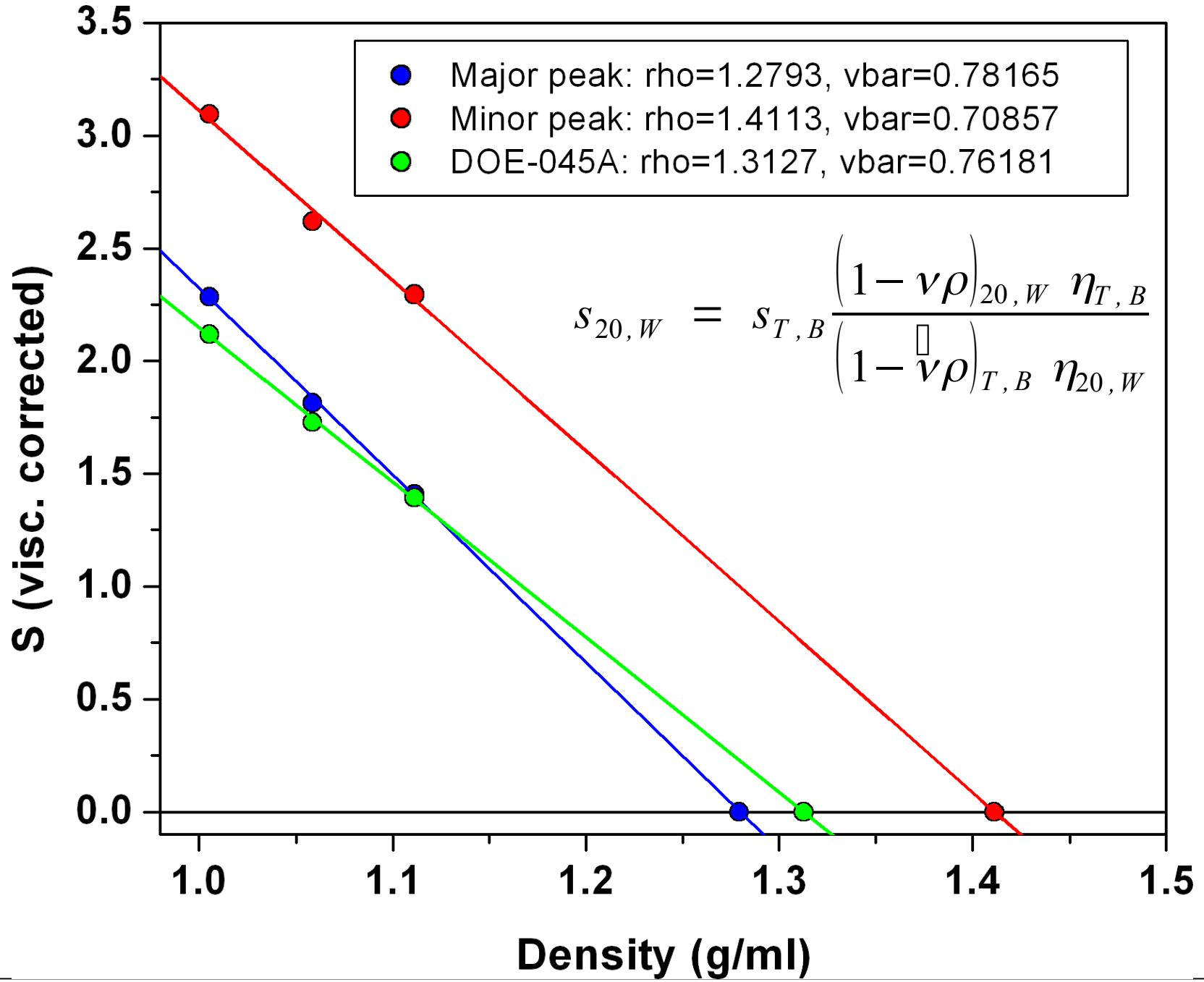
1. **Change the solvent density so that the solvent density is equivalent to the detergent density. Both detergent density and solvent density need to be independently validated. If both are the same, the bound detergent will not contribute to the buoyancy of the molecule, and:**

$$\rho = 1/\bar{\nu}_{det} \text{ where: } \delta_{det}\left(1-\bar{\nu}_{det}\,\rho\right) = 0 \text{ and}$$
$$M\left(1-\Phi\rho\right) = M\left(1-\bar{\nu}_{prot}\rho\right)$$

Then the detergent will not contribute.

2. **If the density of the detergent cannot be matched by any heavy water concentration, the same principle can be used by making multiple measurements in different concentrations of D_2O or H_2O_{18} and evaluating the buoyancy term. Plot the buoyancy term as a function of solvent density and extrapolate this function to the density of the detergent. At this point the buoyancy term only reflects the protein, and the molecular weight reflects the entire sedimenting particle.**

Transport Processes – Partial Specific Volume and Buoyancy



Relationship between M, f, φ, s, and D

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

Concentration **Sedimentation** **Diffusion**

$$f = \frac{RT}{N D}$$

$$M = \frac{s N f}{1-\bar{\nu}\rho}$$

$$V = \frac{M \bar{\nu}}{N}$$

$$r_0 = \left(\frac{3V}{4\pi}\right)^{1/3}$$

$$f_0 = 6\pi\eta r_0$$

$$\varphi = \frac{f}{f_0}$$

Relationship between M, f, φ, s, and D

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Concentration **Sedimentation** **Diffusion**

Shape Dependence

$$f = \frac{RT}{N D}$$

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Concentration **Sedimentation** **Diffusion**

Size

$$f = \frac{RT}{N D}$$

$$M = \frac{s N f}{1 - \bar{\nu}\rho}$$

$$V = \frac{M \bar{\nu}}{N}$$

$$r_0 = \left(\frac{3V}{4\pi}\right)^{1/3}$$

$$f_0 = 6\pi\eta r_0$$

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Relationship between M, f, φ, s, and D

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Concentration **Sedimentation** **Diffusion**

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$$M = \frac{s\,N\,f}{1-\bar{\nu}\rho}$$

$$V = \frac{M\,\bar{\nu}}{N}$$

$$r_0 = \left(\frac{3\,V}{4\pi}\right)^{1/3}$$

$$f_0 = 6\pi\eta r_0$$

Anisotropy — $\varphi = \frac{f}{f_0}$

Relationship between M, f, φ, s, and D

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

Concentration **Sedimentation** **Diffusion**

$$f = \frac{RT}{N D}$$

$$M = \frac{s N f}{1 - \bar{\nu}\rho}$$

$$V = \frac{M\bar{\nu}}{N}$$

Partial specific volume and density

$$r_0 = \left(\frac{3V}{4\pi}\right)^{1/3}$$

$$f_0 = 6\pi\eta r_0$$

$$\varphi = \frac{f}{f_0}$$

Background – Analytical Ultracentrifugation

Information Available from Sedimentation Velocity Experiments:

- Sedimentation coefficient
- Diffusion coefficient (translational)
- Molecular mass (PSV must be known)
- Frictional coefficient
- Frictional ratio (MW or PSV must be known)
- Stokes radius
- Density or partial specific volume
- Partial concentration of a solute
- Equilibrium constants (Kd)
- Rate constants (slow reactions)

Background – Analytical Ultracentrifugation

What is Analytical Ultracentrifugation (AUC)?

A technique to separate macromolecules dissolved in *solution* in a centrifugal force field. AUC can "watch" the molecules *while* they are separating and identify and differentiate them by their hydrodynamic properties and partial concentration. AUC is a "First Principle" method, which does NOT require standards.

How does AUC work?

By applying a centrifugal force, molecules are separated based on their buoyant mass and their frictional properties. The molecules are observed using different optical systems that detect different properties of the molecules, such as refractive index, UV or visible absorbance, or fluorescent light emission.

What molecules can be studied?

Virtually any molecule, colloid or particle that can be dissolved in a liquid can be measured by AUC, as long as it does not sediment by gravity alone (nucleic acids, proteins, carbohydrates, peptides, nanoparticles, synthetic polymers, small molecules, lipids, viruses). The molecule or particle can be as small as salt ion, or as large as an entire virus particle → very large size range (10^2 – 10^8 Dalton).

The ultracentrifuge can spin at 60,000 rpm, generating forces as high as 250,000 x g. Even small molecules will sediment in such a force.

Background – Analytical Ultracentrifugation

What can be learned from AUC?

- **Excellent method for characterizing any molecule or molecular interaction in the solution environment – small sample requirement, up to 14 samples can be analyzed. Analysis is based on first principles - *No standards are required***
- **Molecules can be studied in a physiological environment – solution conditions can be adjusted (concentration dependency, effect of pH, ionic strength, buffer type, ligands, oxidation state, temperature, etc.)**
- **Very large size range (10^2 – 10^8 Dalton)**
- **Dynamics - measure oligomerization states of reversible self- or hetero-associations, ligand binding, slow kinetics and Kd**
- **Composition analysis – number of components, their partial concentration, molecular weight, and anisotropy**
- **Conformational analysis - folding/melting studies of biopolymers, conformational changes based on changes in solution properties**

Background – Analytical Ultracentrifugation

Available Optical Systems:

Multiple detectors extend the range of AUC applications:

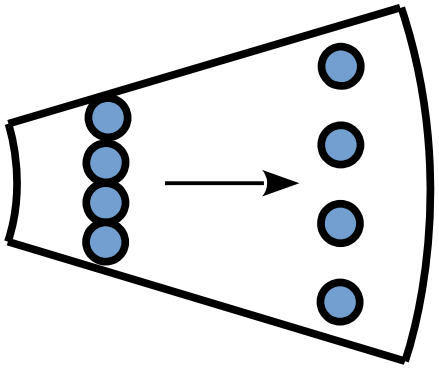
- **UV/vis absorbance (single wavelength):** all-purpose detector for biopolymers and materials absorbing in the visible. Slower detection, good for low protein concentration – measure intensity of transmitted light.

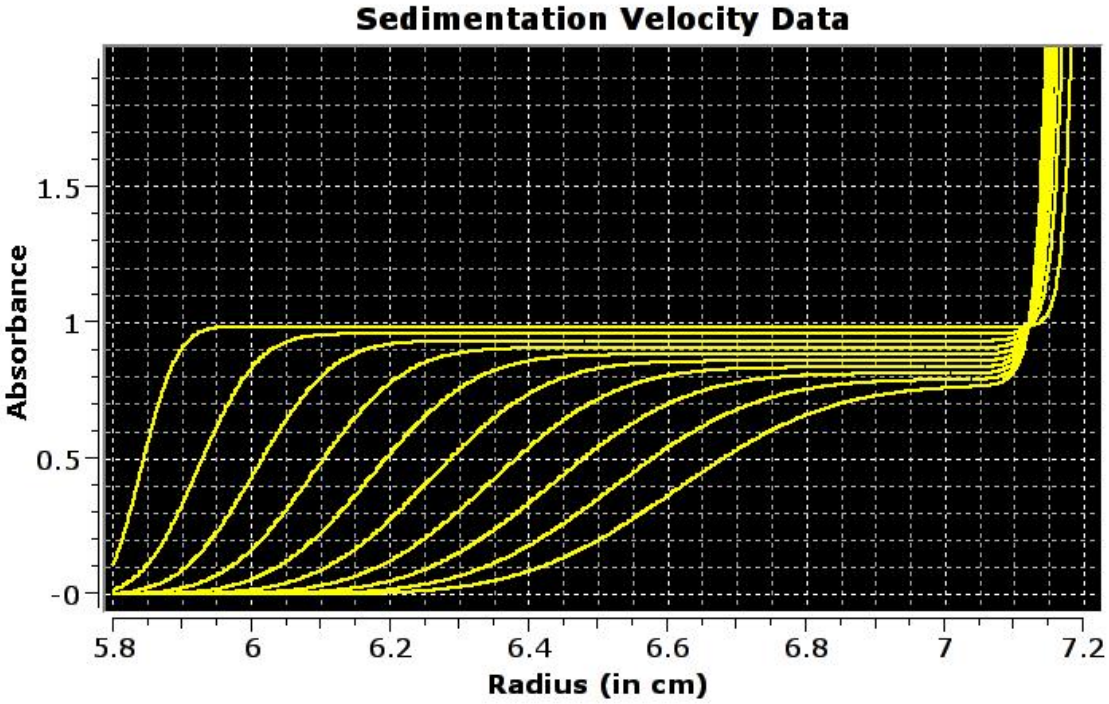
- **Fluorescence:** Study molecules with intrinsic fluorophores or eGFP fusions in impure cell extracts, exquisite selectivity for binding experiments, add fluorescently labeled antibodies to an impure cell extract, study the order of assembly in a multi-domain protein complex – measure intensity of fluorescence emission with a confocal microscope setup (488 nm excitation)

- **Interference:** fast data acquisition and measurement of non-absorbing molecules, carbohydrates, high-concentration studies – measure refractive index differences at 675 nm

- **Multi-wavelength UV/vis:** obtain additional spectral dimension in addition to hydrodynamic separation for independent characterization of molecular properties – measures intensity of transmission of multiple wavelengths

Radial Dilution



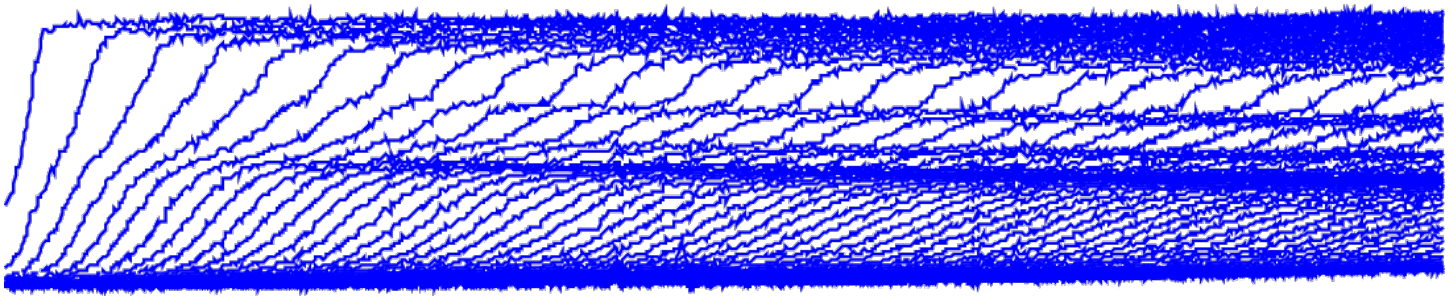


Radial Dilution occurs because of the cell's sector shape. Molecules sedimenting towards the outside of the cell will dilute as they sediment.

All molecules - no matter at what position they are - will dilute at the same rate, causing a reduction in the observed optical density. At any given time, this dilution is the same at each point in the cell.

Radial Dilution can be observed through a reduction in the plateau absorbance in successive scans

Calculation of the Sedimentation Coefficient:

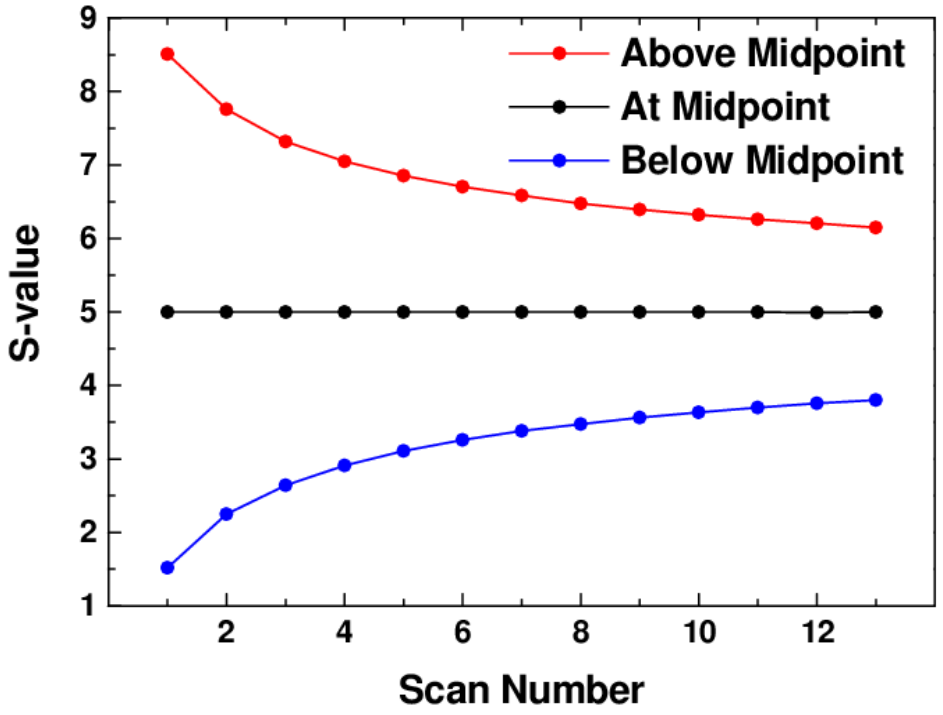


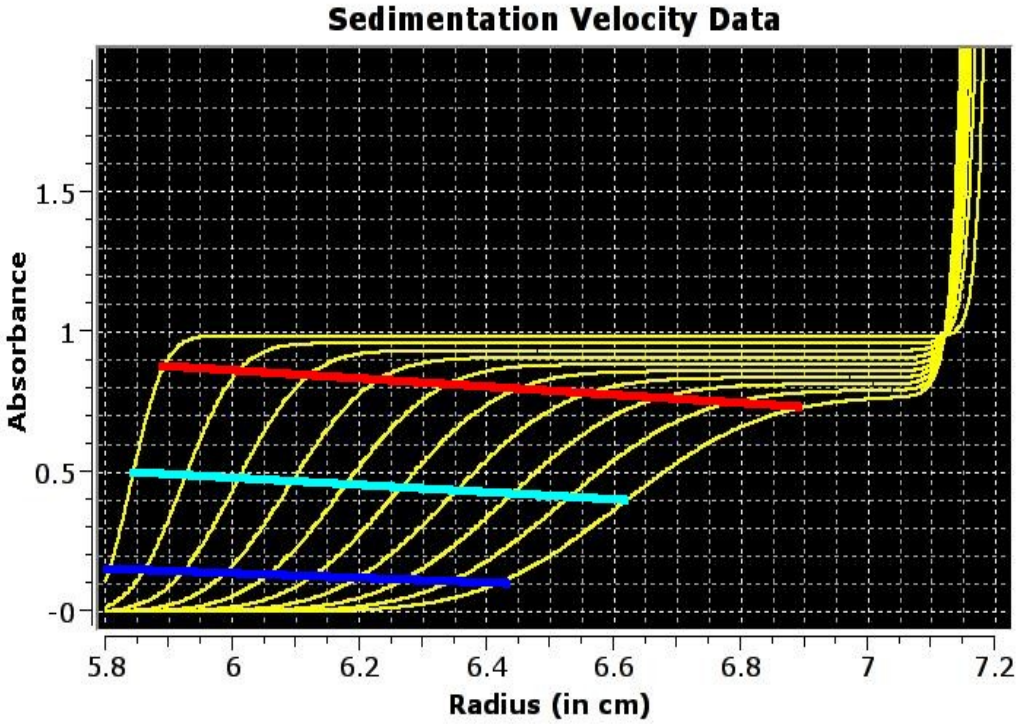
$$\frac{M\left(1-\bar{v}\rho\right)}{N\ f} = \frac{v}{\omega^2 r} = s \qquad v = \frac{dr}{dt}$$

$$\frac{dr}{r} = \omega^2\ s\ dt \qquad \int_{r=m}^{r=b} \frac{1}{r} dr = \int_{t=0}^{t=scan} s\,\omega^2 dt$$

$$\hat{s}_b = \ln\left(\frac{r_b(t)}{r_m(t_0)}\right)\left(\omega^2\left(t-t_0\right)\right)^{-1}$$

Calculation of the Sedimentation Coefficient





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

$$v = \frac{dr}{dt} \qquad \frac{dr}{r} = \omega^2\ s\ dt$$

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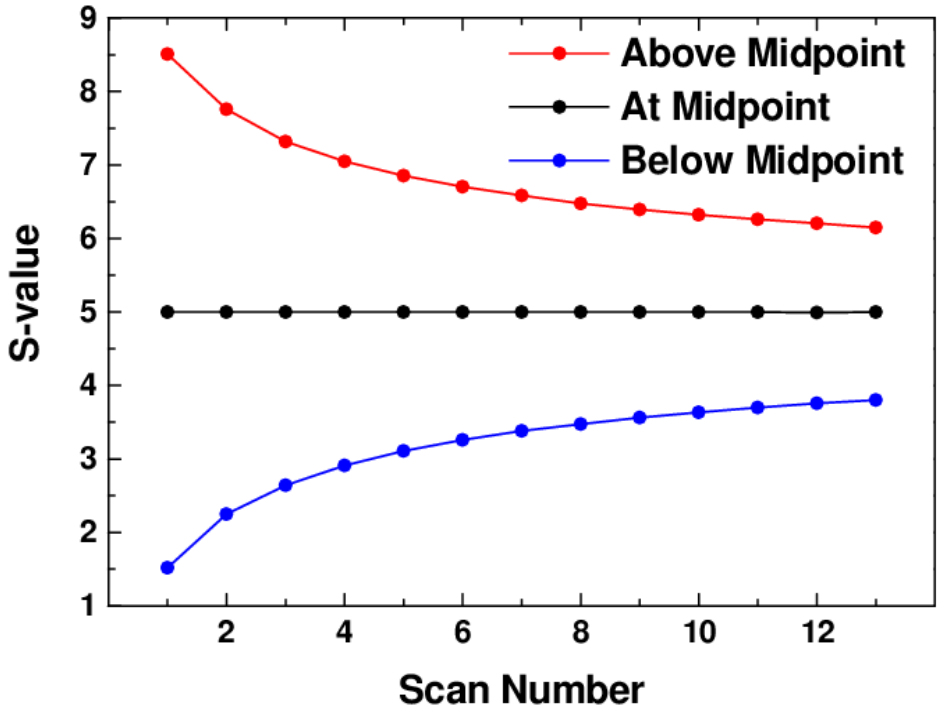
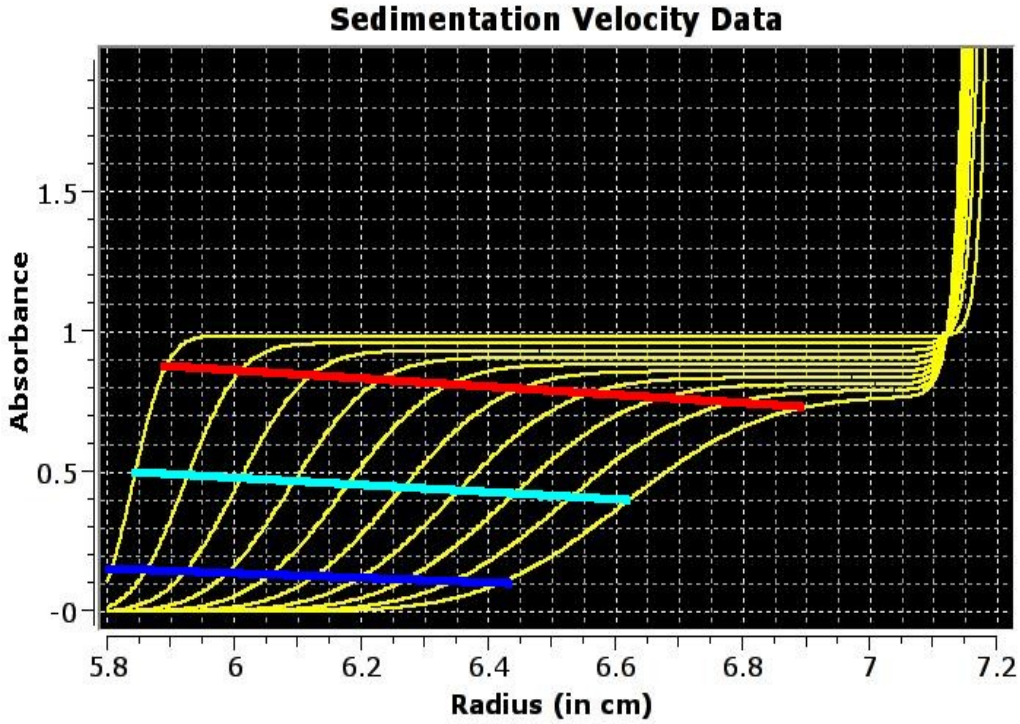
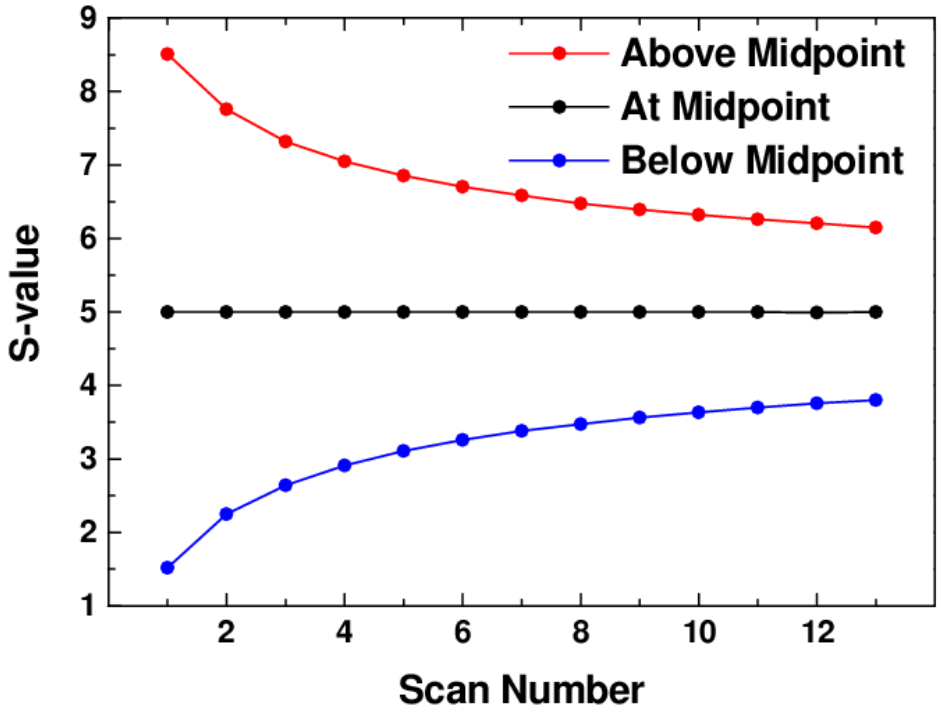
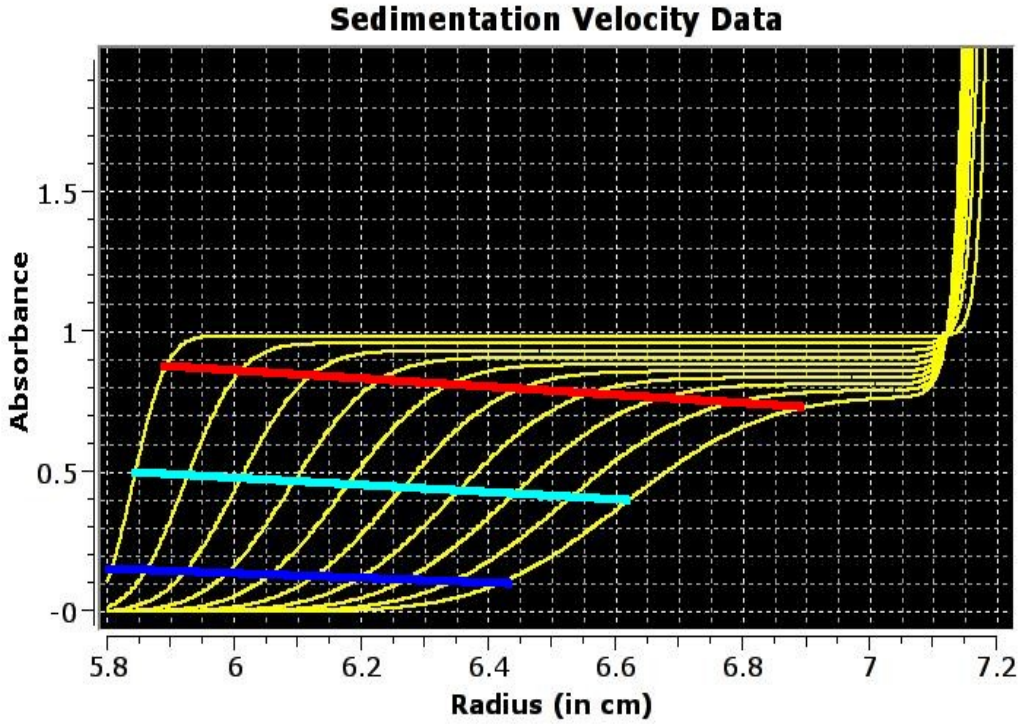




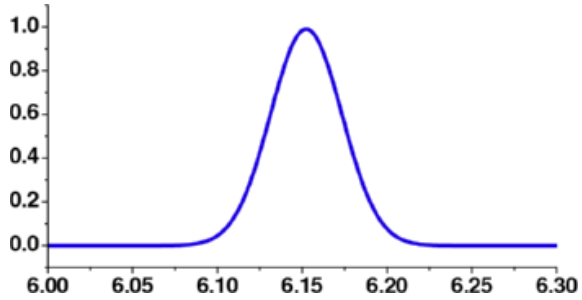
The s-value from non-midpoint boundary positions from different scans are different, because diffusion contributes to the transport in those boundary regions.

Calculation of the Sedimentation Coefficient



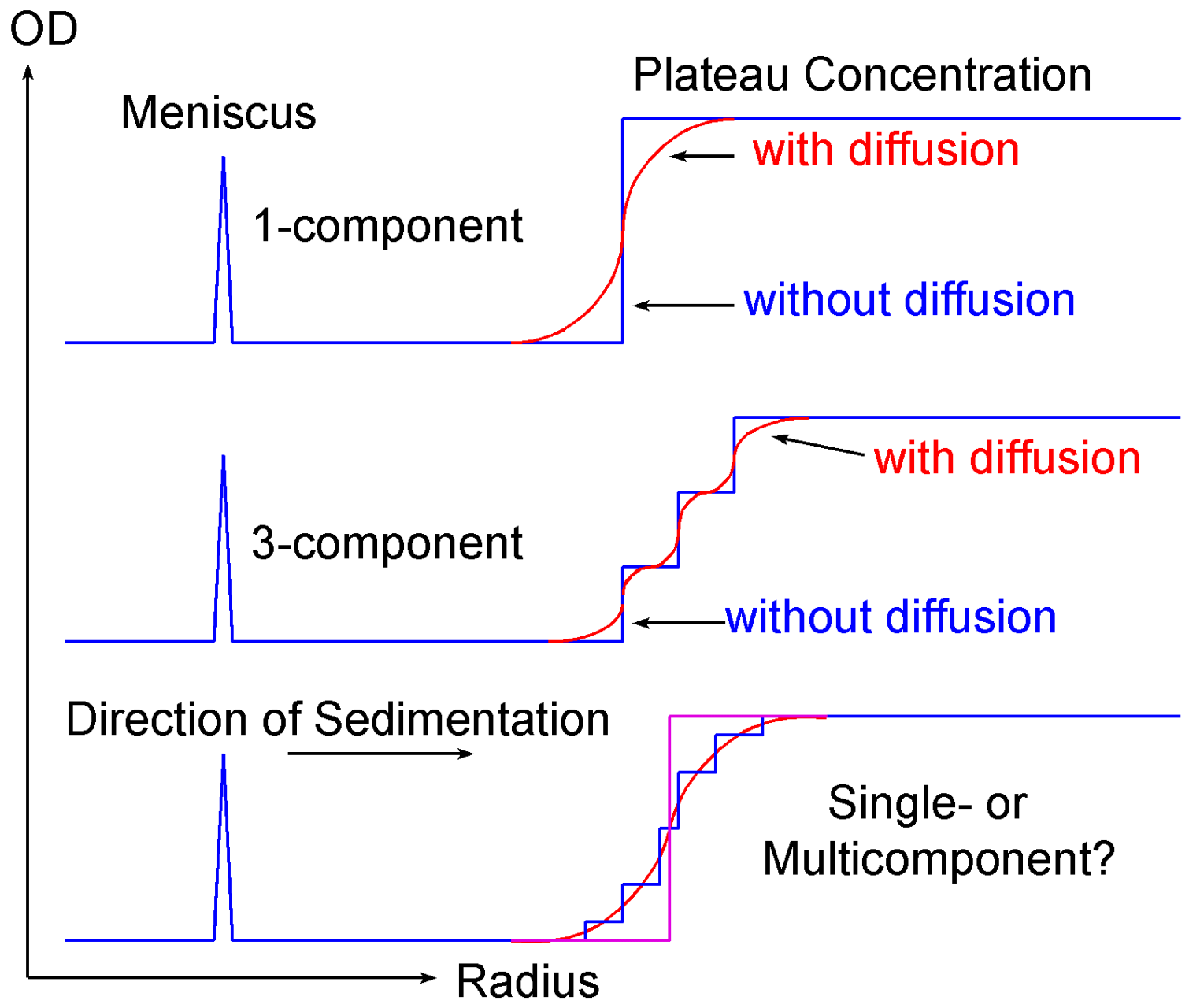


The flow due to diffusion is proportional to the concentration gradient and the diffusion coefficient (Fick's first law):



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

What about Diffusion and Heterogeneity?



van Holde – Weischet Method

Description of the Problem:

How do we distinguish between transport due to diffusion and transport due to sedimentation?

How do we distinguish boundary spreading due to heterogeneity from boundary spreading due to diffusion?

For unknown samples, can we analyze the sample in a model-independent way?

van Holde – Weischet Method

Key Observation by van Holde et al.:

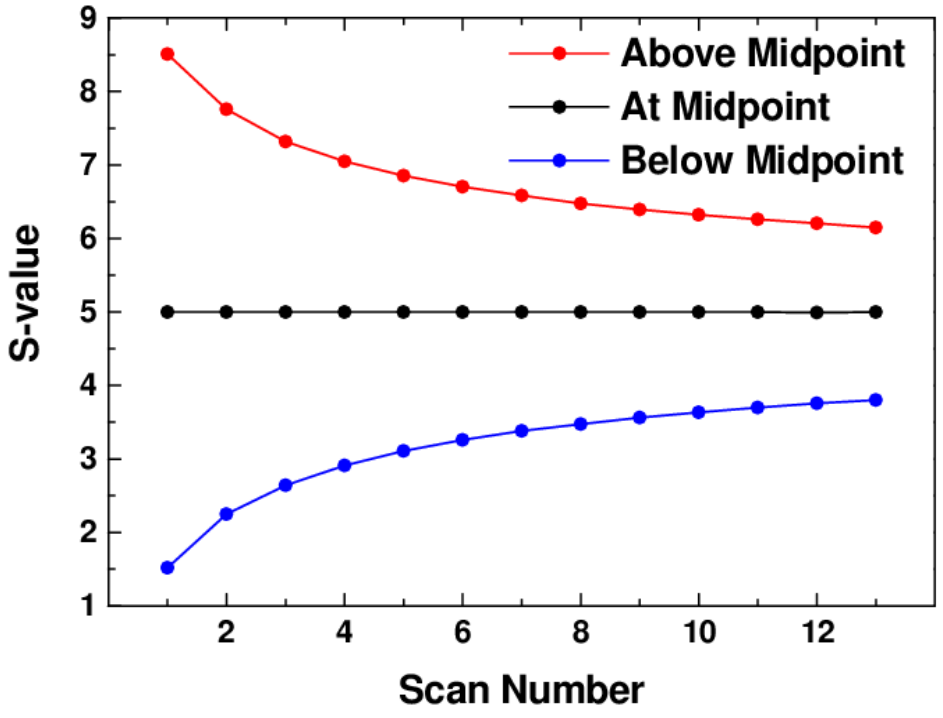
Sedimentation is a transport process proportional to the first power of time, while Diffusion is a transport process which is proportional to the square-root power of time.

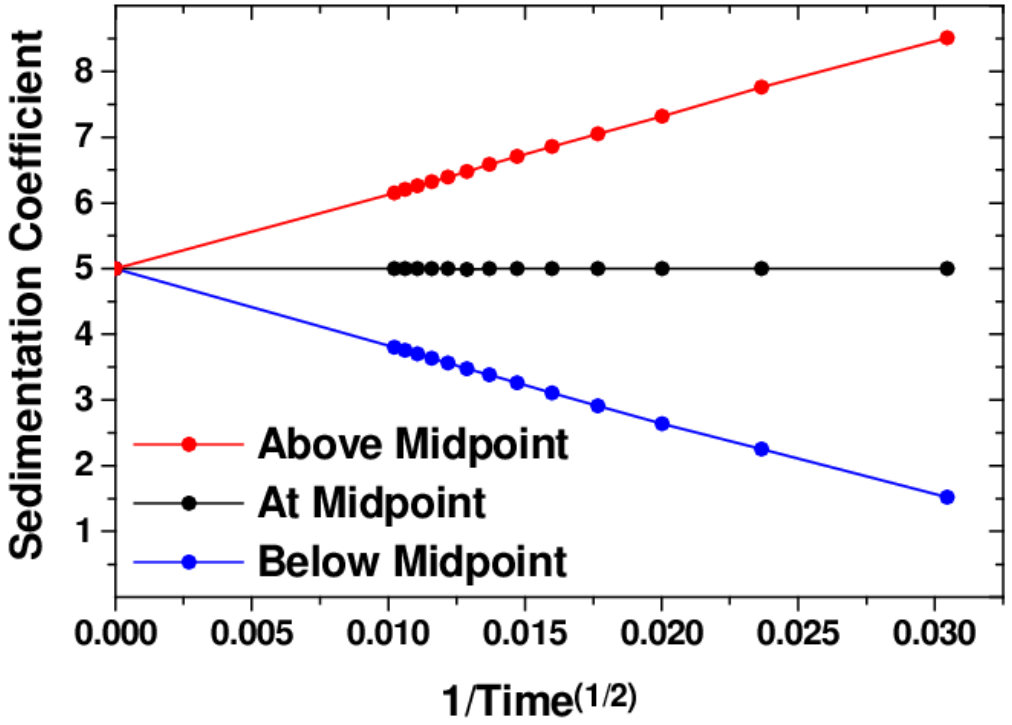
At infinity transport due to diffusion will be negligible compared to transport due to sedimentation - i.e., all components will separate out if the rotorspeed is fast enough.

van Holde, K. E. and W. O. Weischet. (1978). Boundary Analysis of Sedimentation Velocity Experiments with Monodisperse and Paucidisperse Solutes. Biopolymers, 17:1387-1403

Demeler, B. and K. E. van Holde. Sedimentation velocity analysis of highly heterogeneous systems. (2004). Anal. Biochem. Vol 335(2):279-288

van Holde – Weischet Analysis





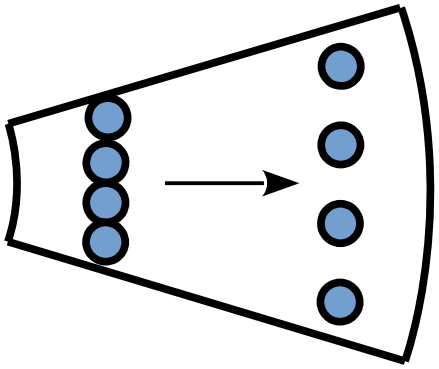
Extrapolation of the same values to infinite time by plotting vs. the inverse square-root of time

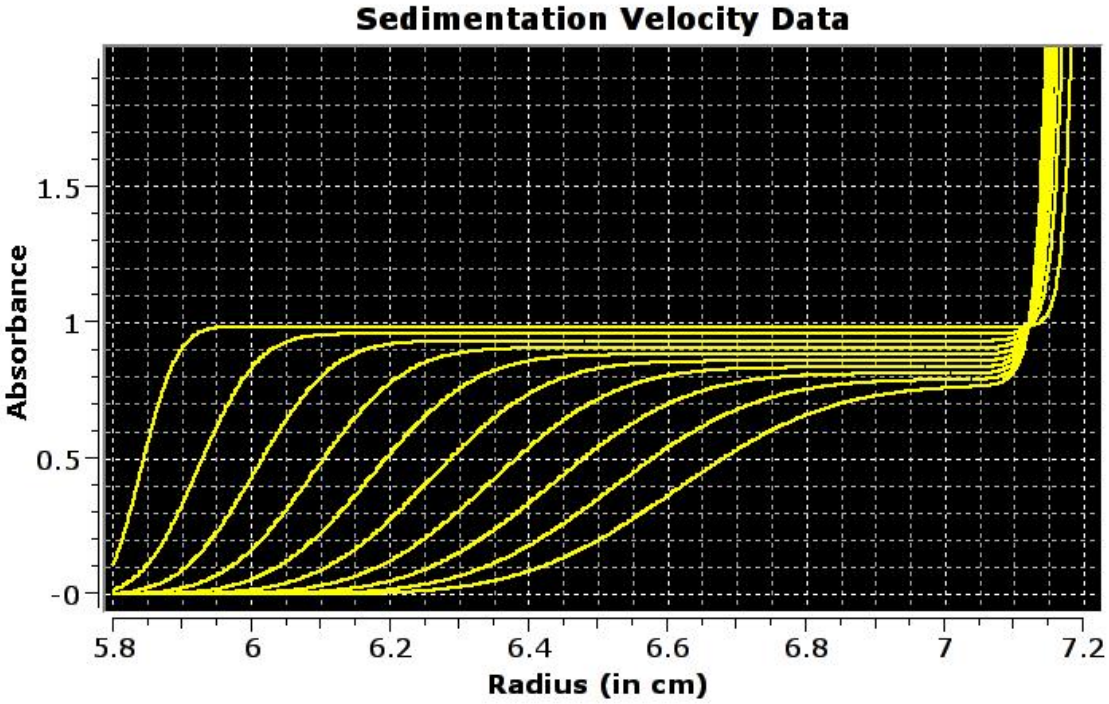
$$\frac{v}{\omega^2 r} = s$$

$$\frac{dr}{r} = \omega^2 s \, dt$$

$$\hat{s}_b = \ln\left(\frac{r_b(t)}{r_a(t_0)}\right)\left[\omega^2 (t - t_0)\right]^{-1}$$

Radial Dilution





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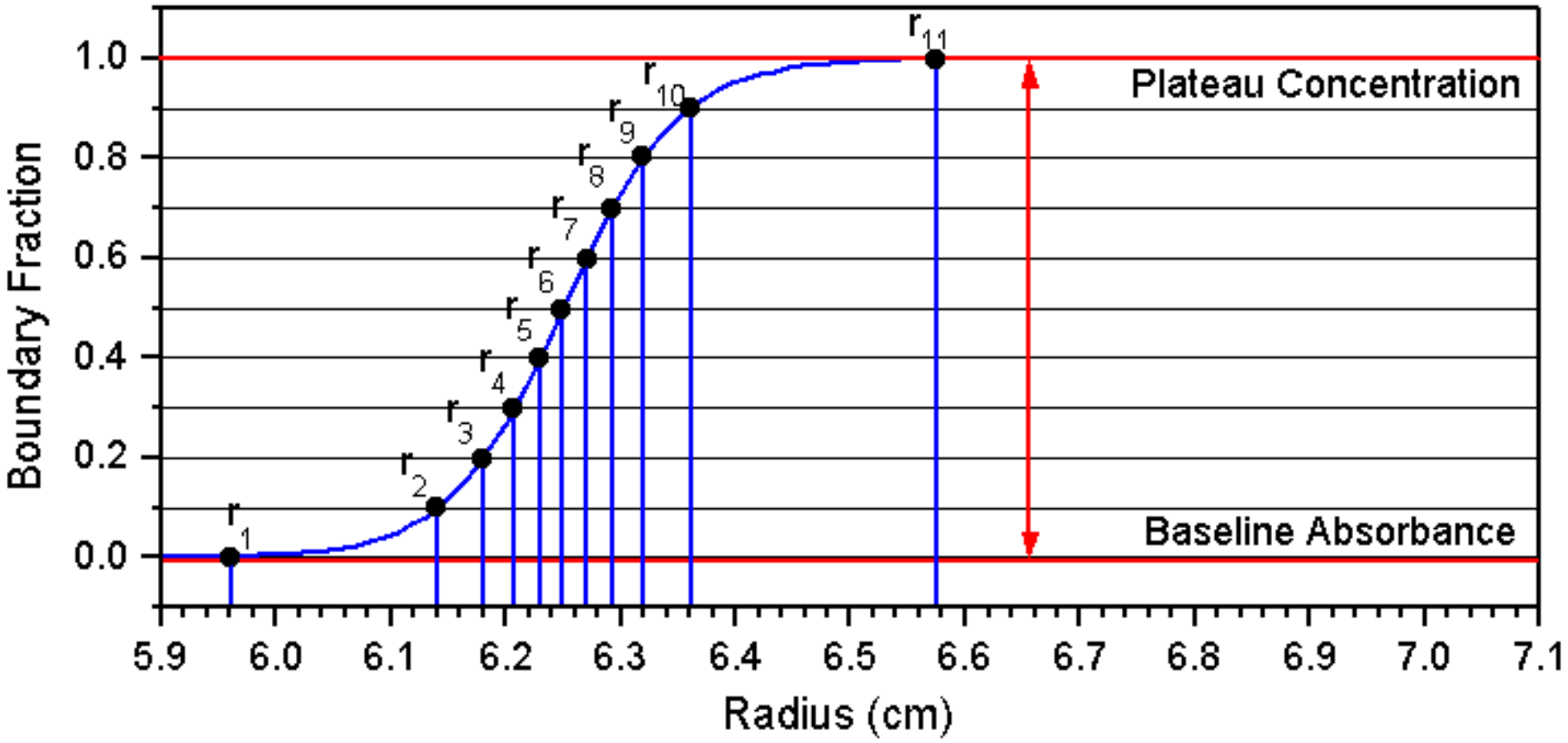
van Holde – Weischet Analysis



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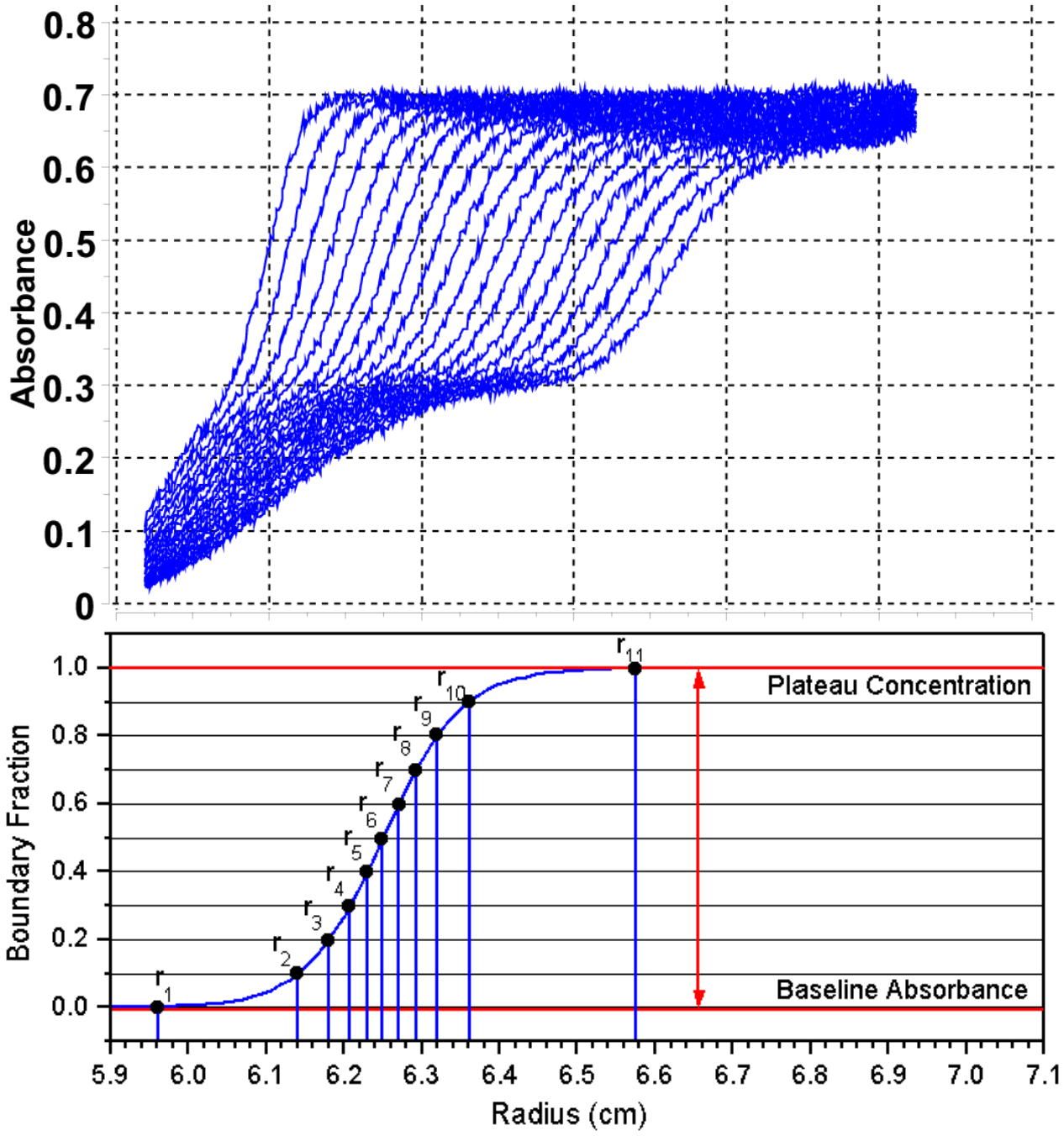
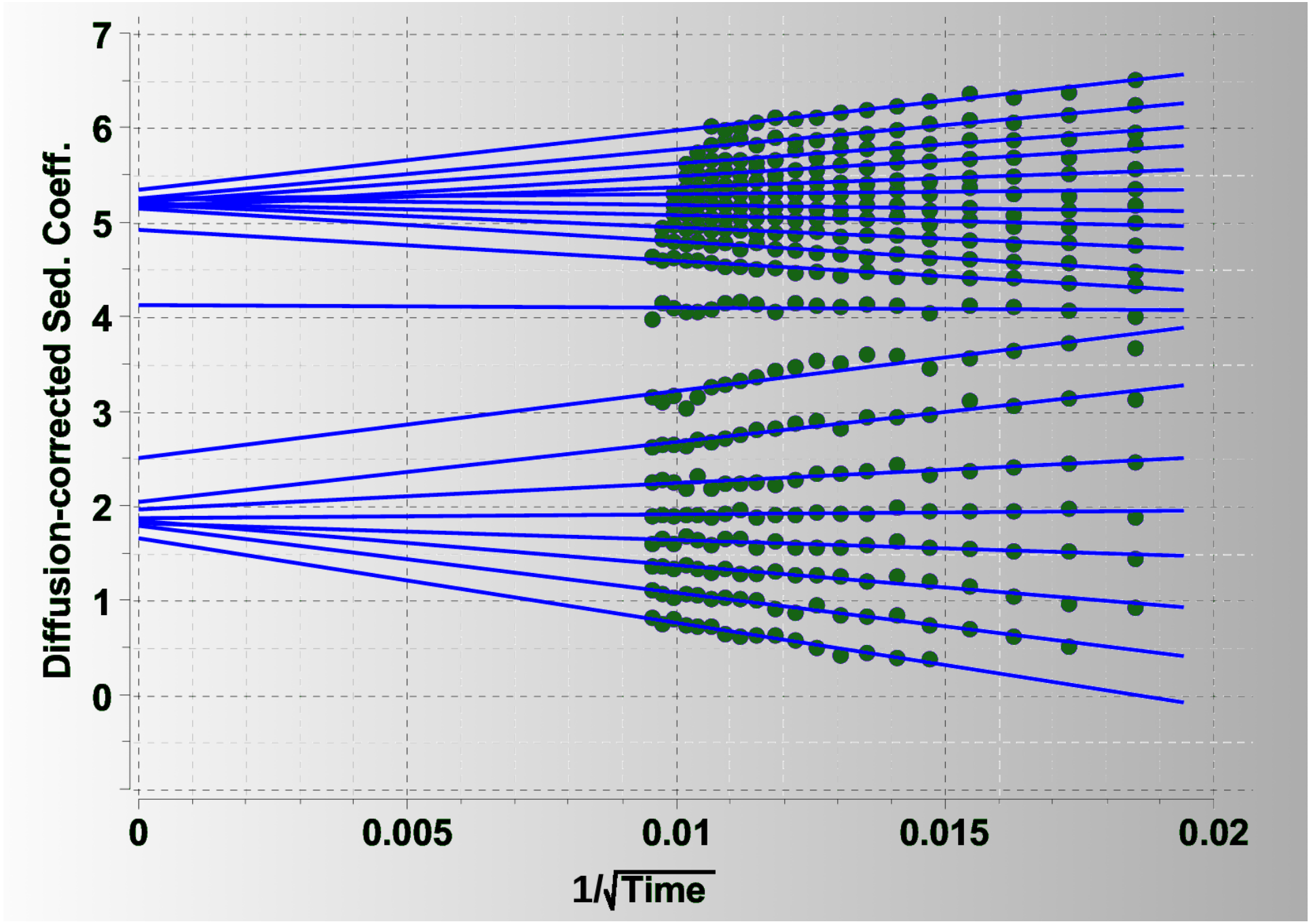


van Holde – Weischet Extrapolation Plot:



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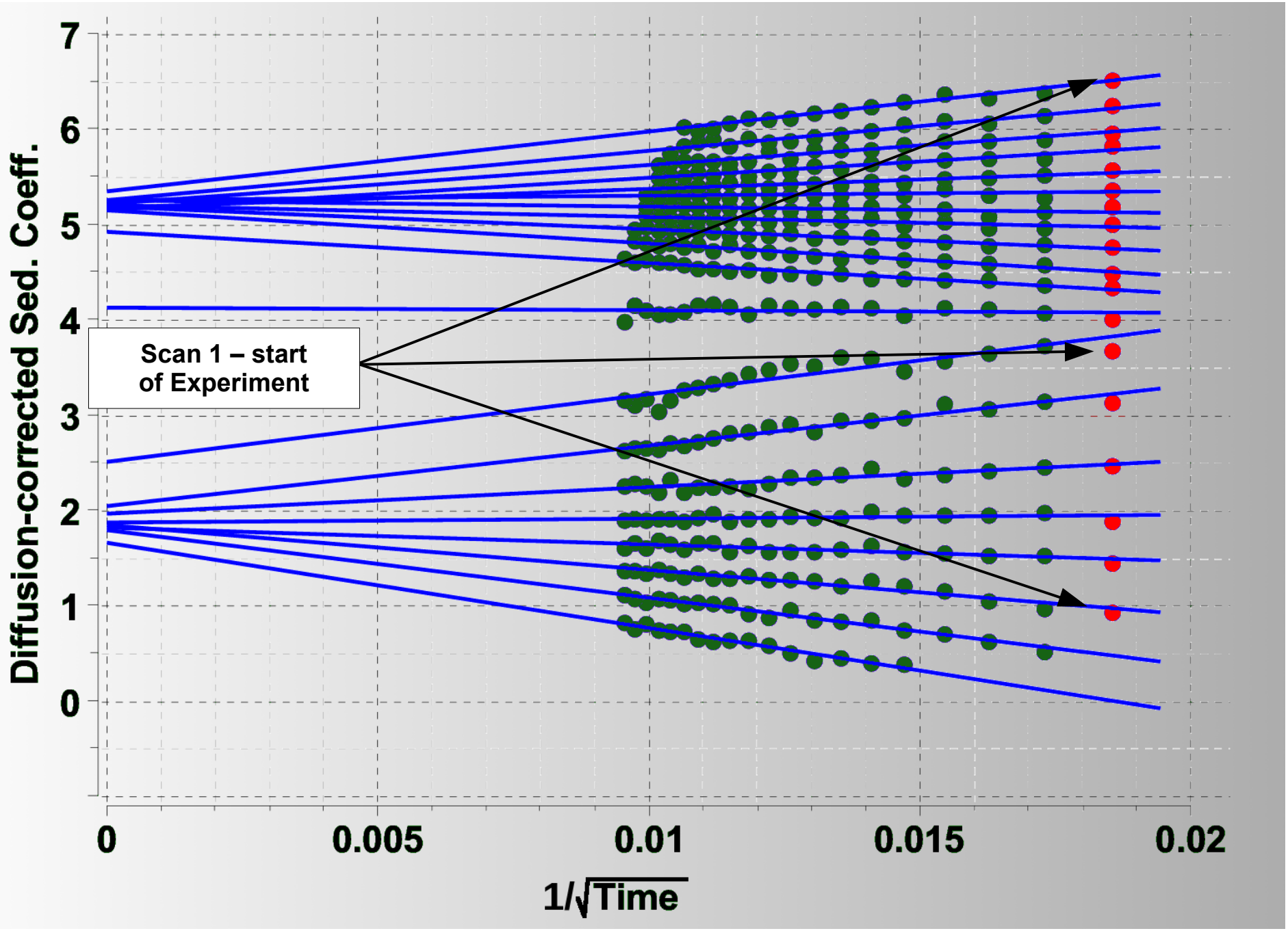
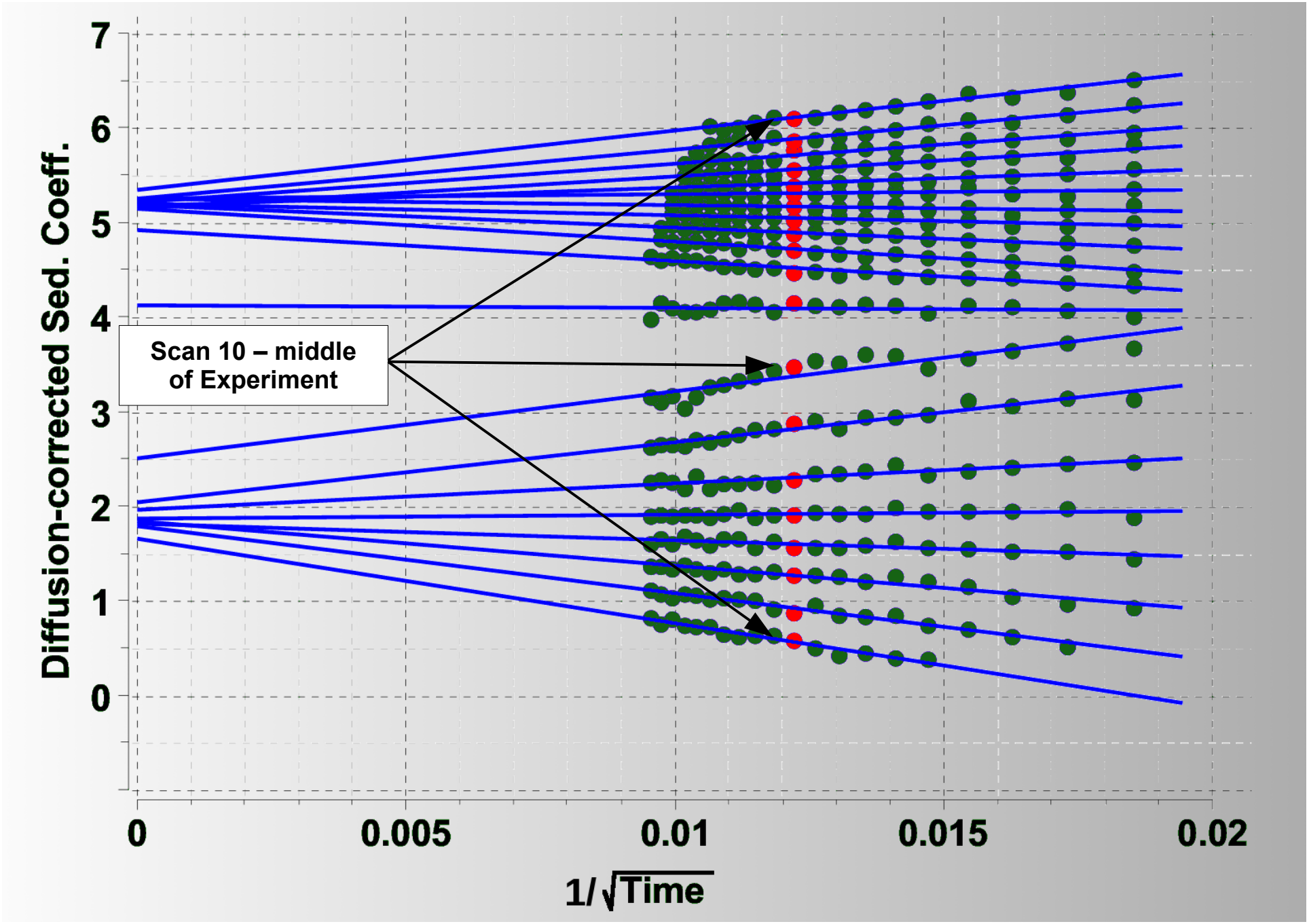


van Holde – Weischet Method



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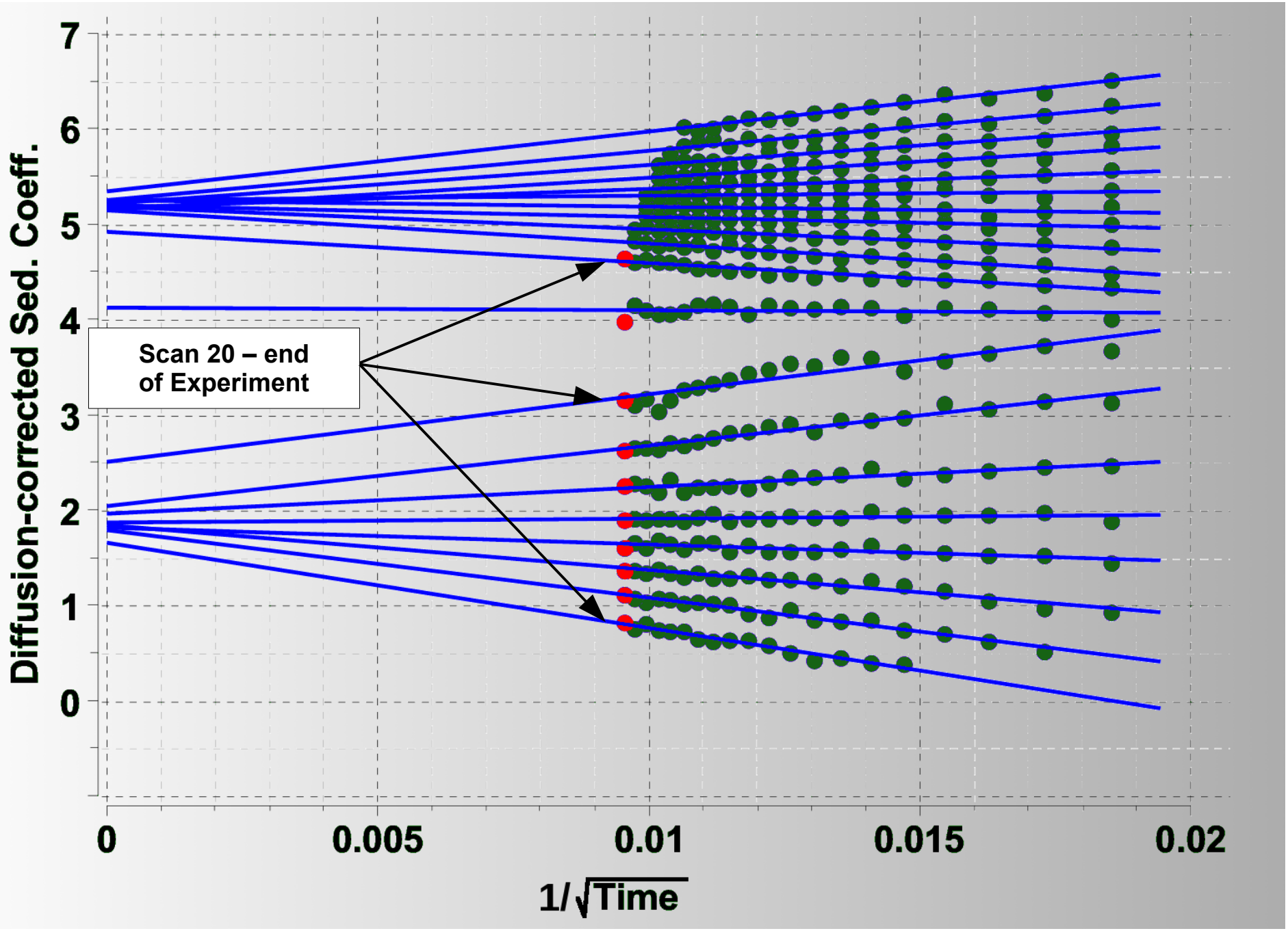

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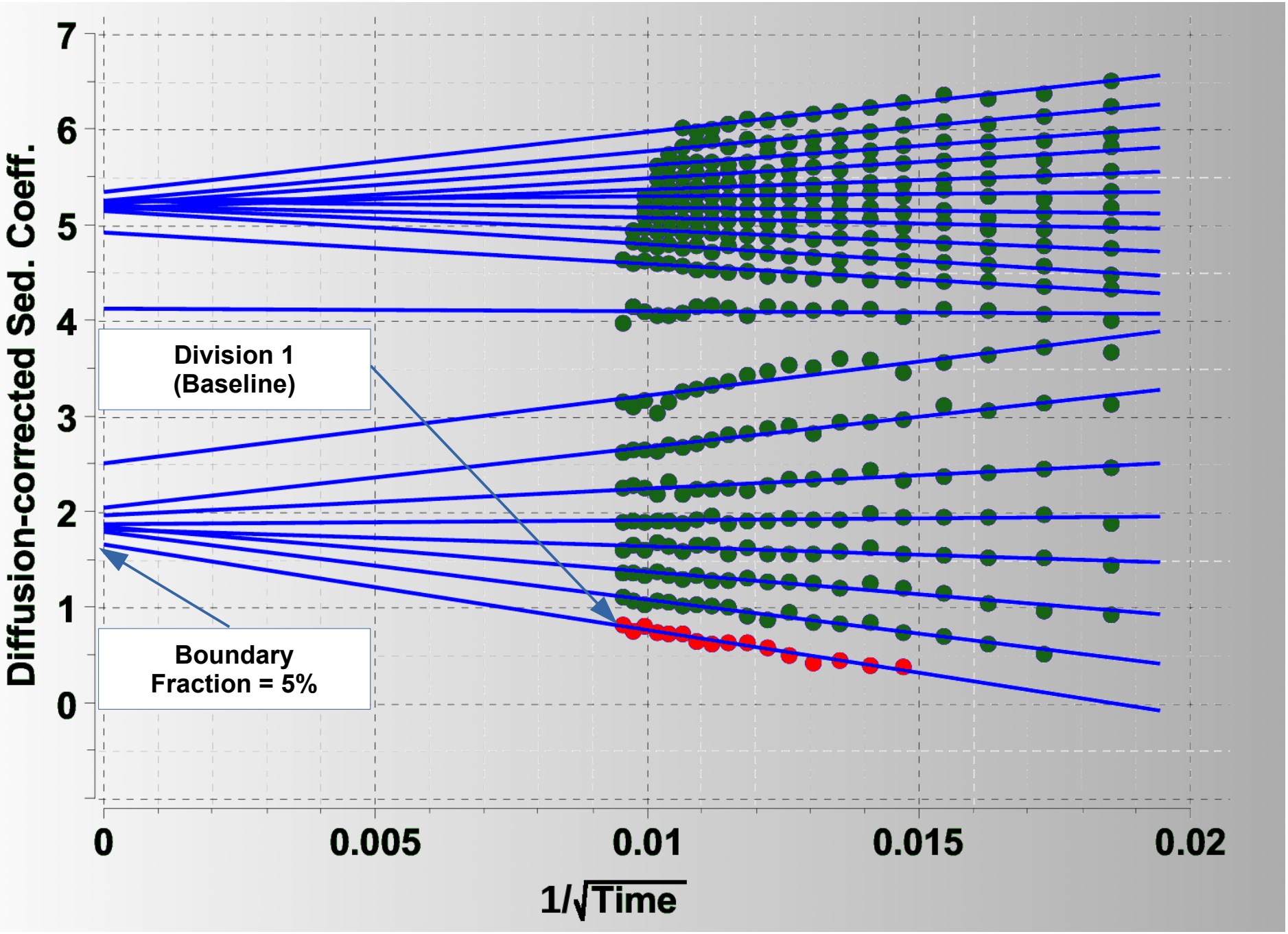

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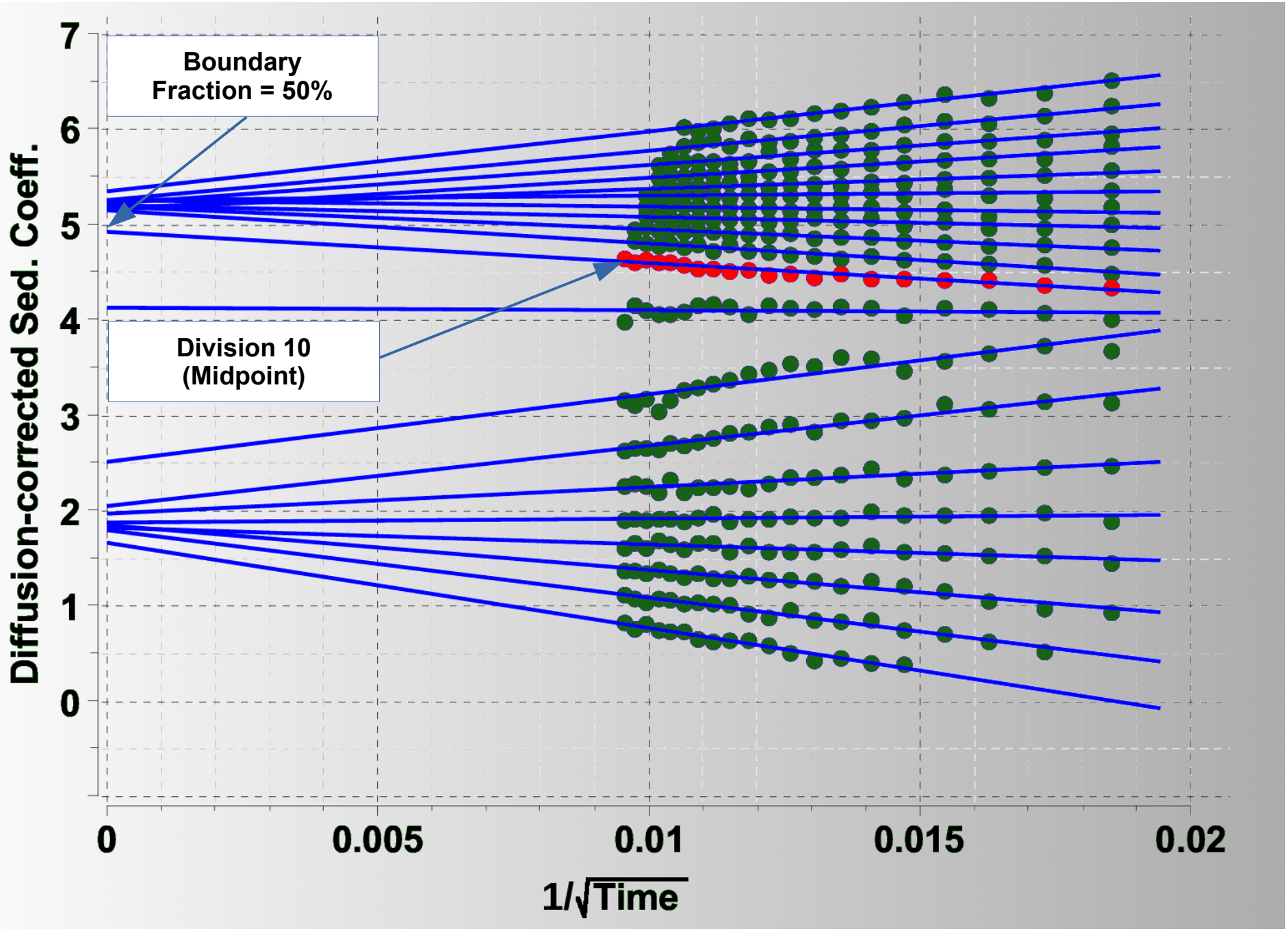

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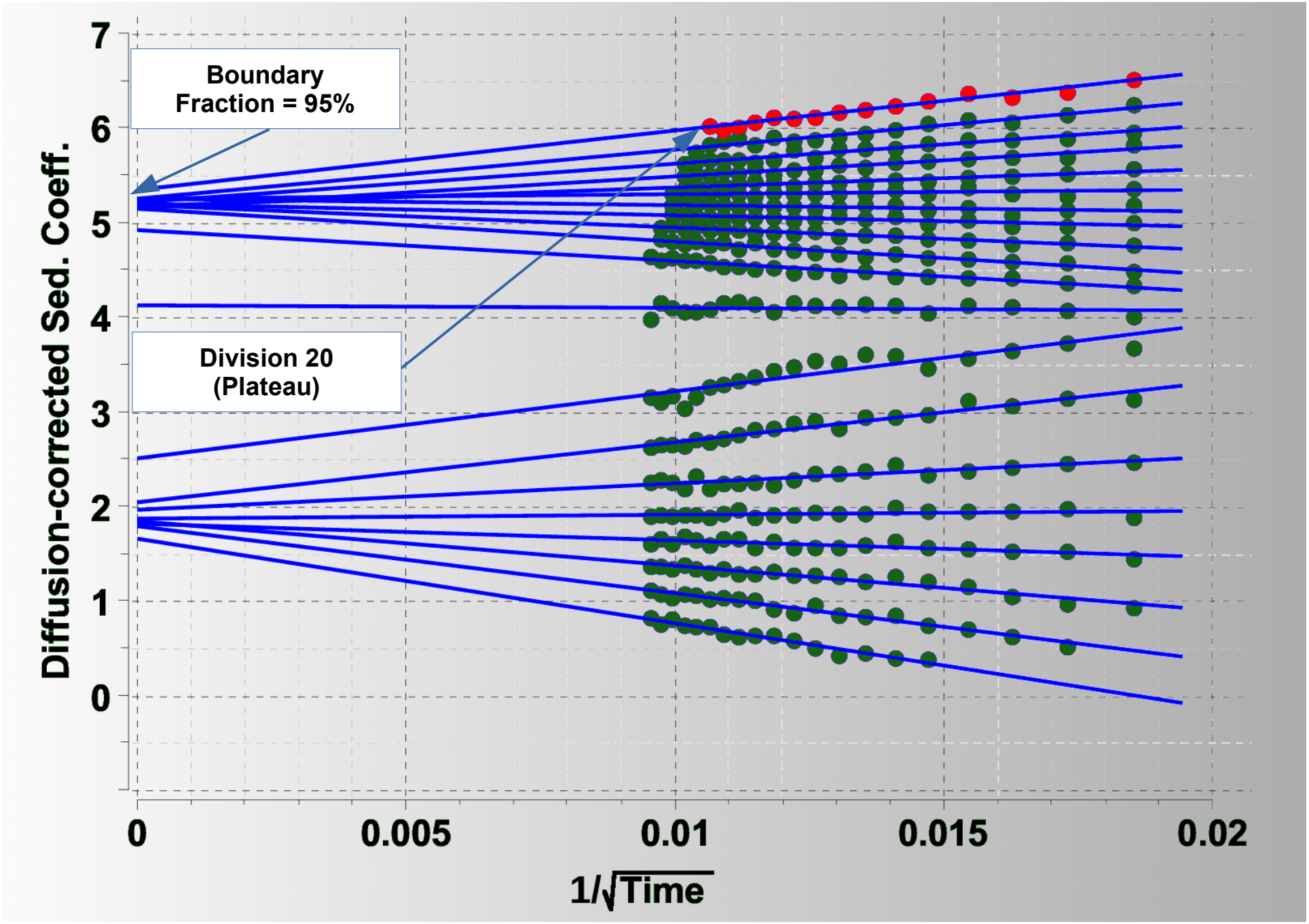

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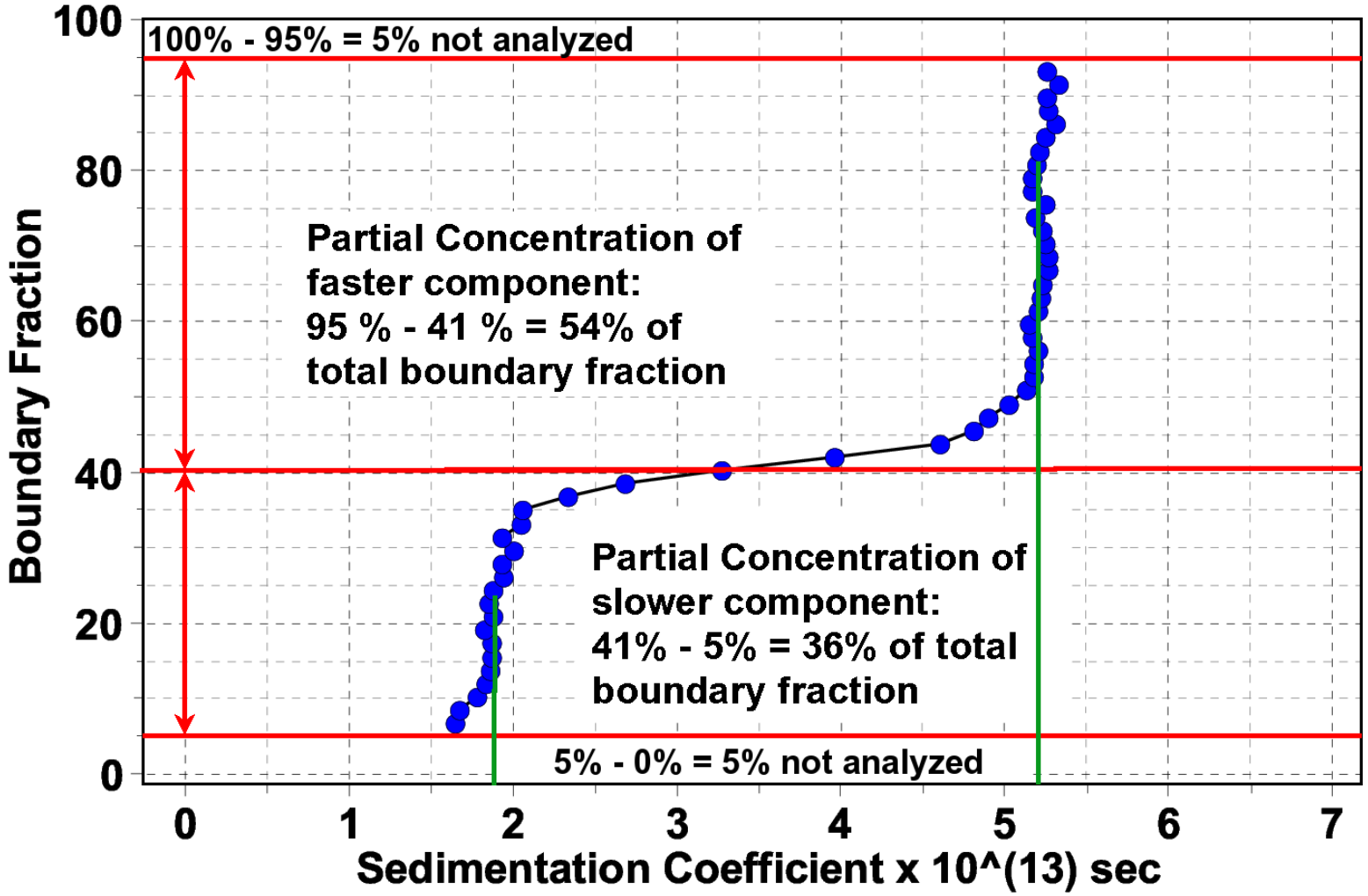
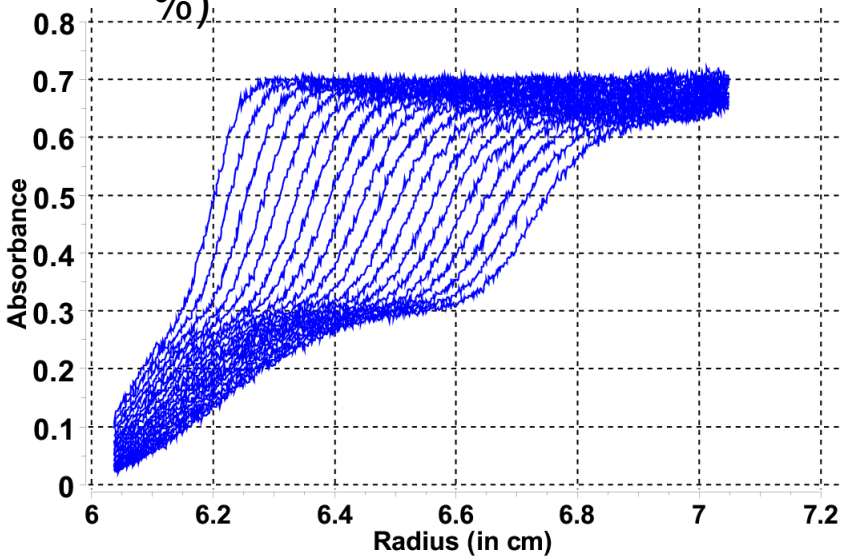


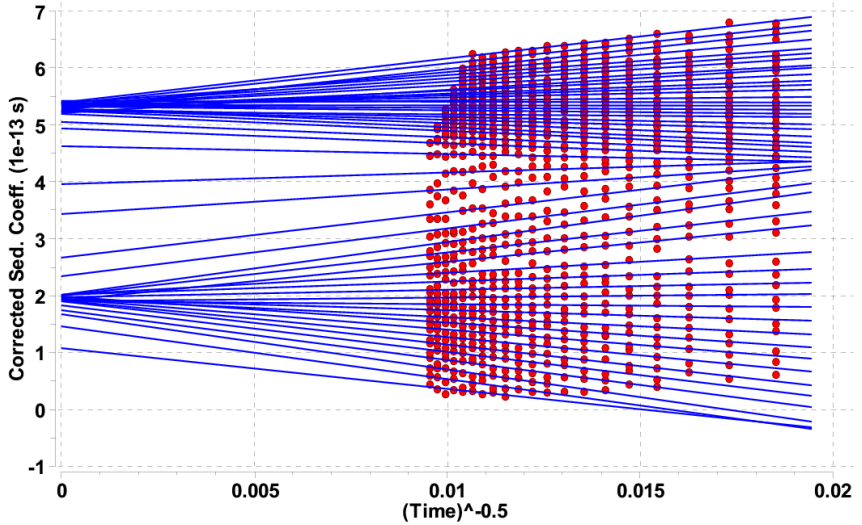
van Holde – Weischet Method

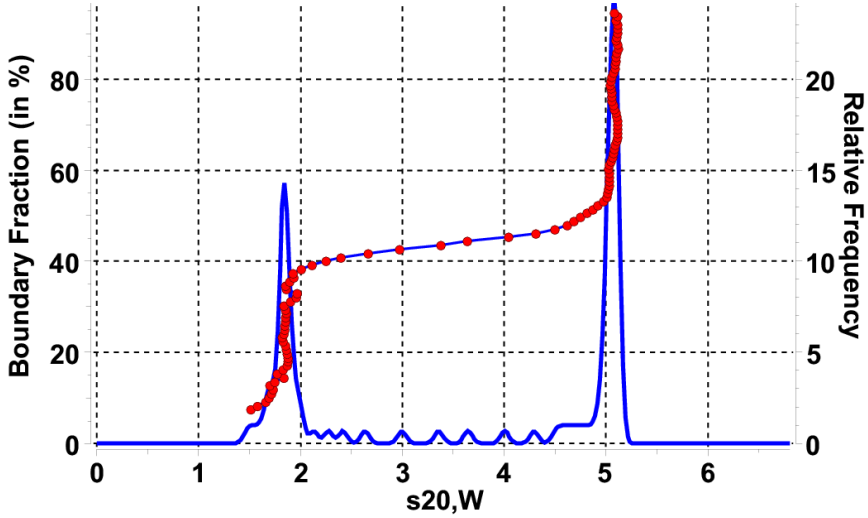
van Holde – Weischet method:

s_1: 5.35 x 10^{-13} (52 %)

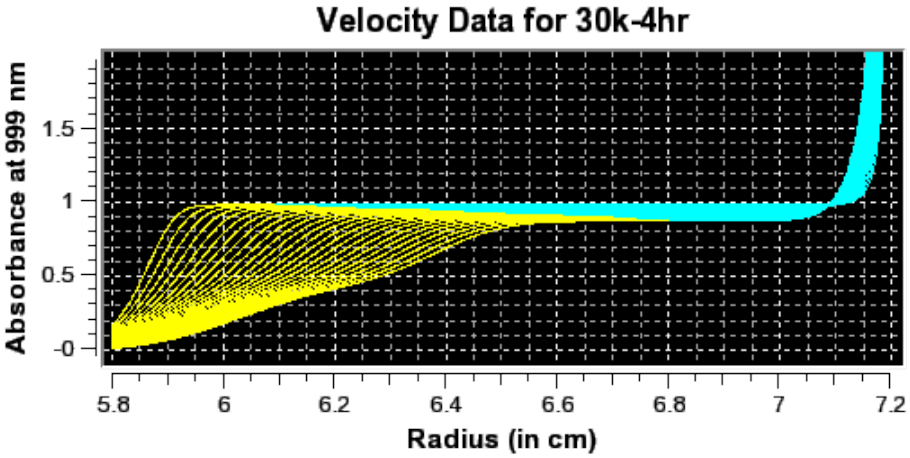
s_2: 1.87 x 10^{-13} (39 %)

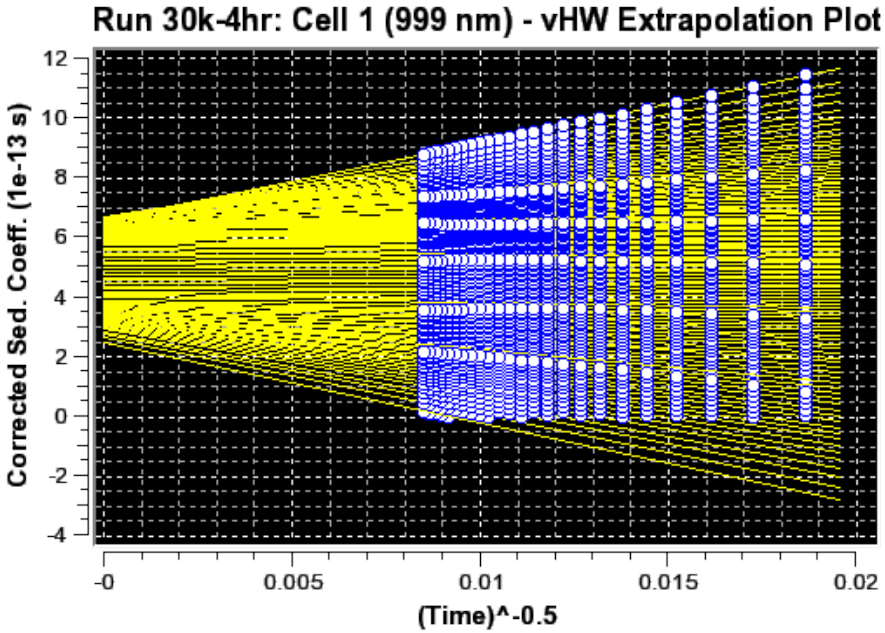


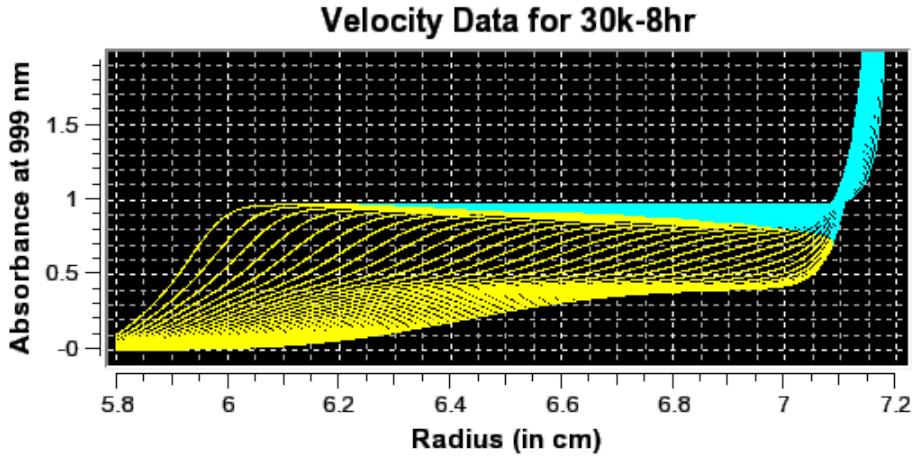


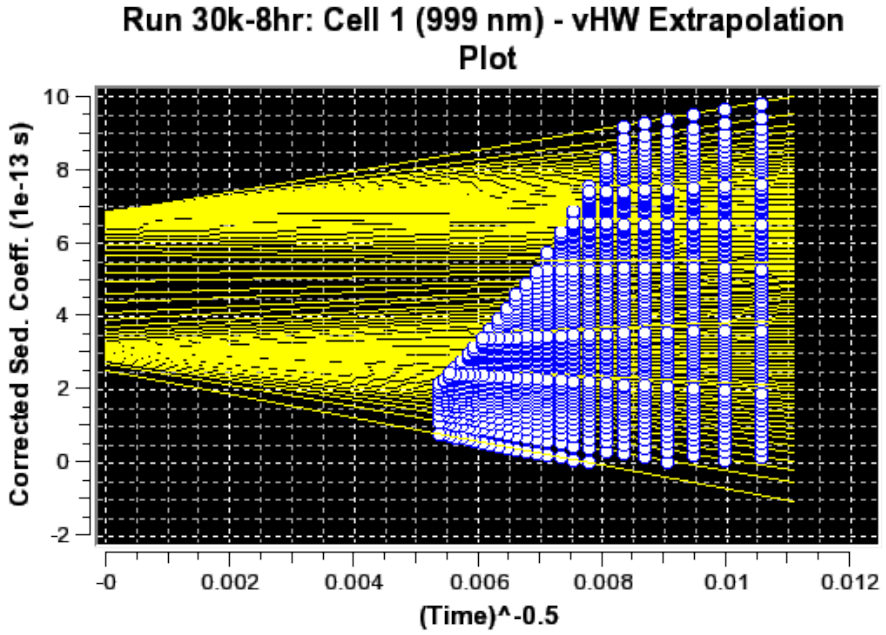


Effect of Experiment Duration on Resolution

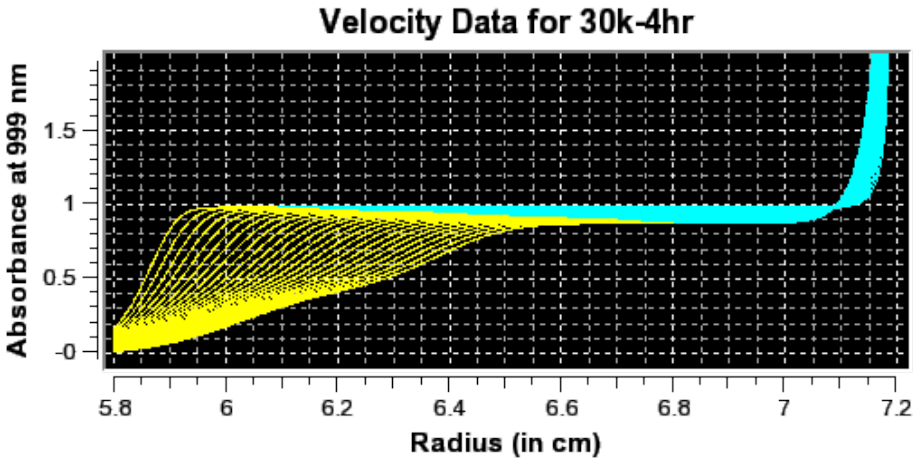


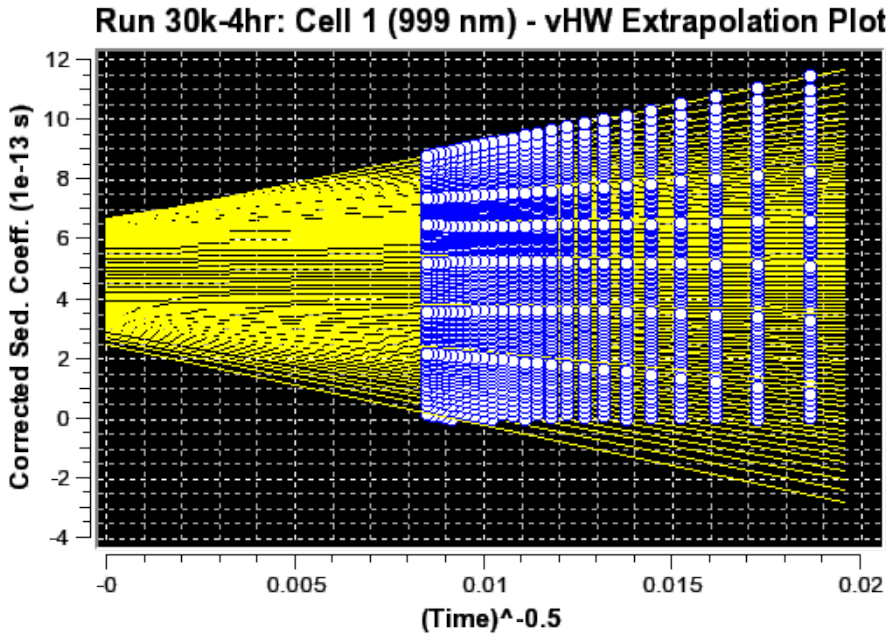


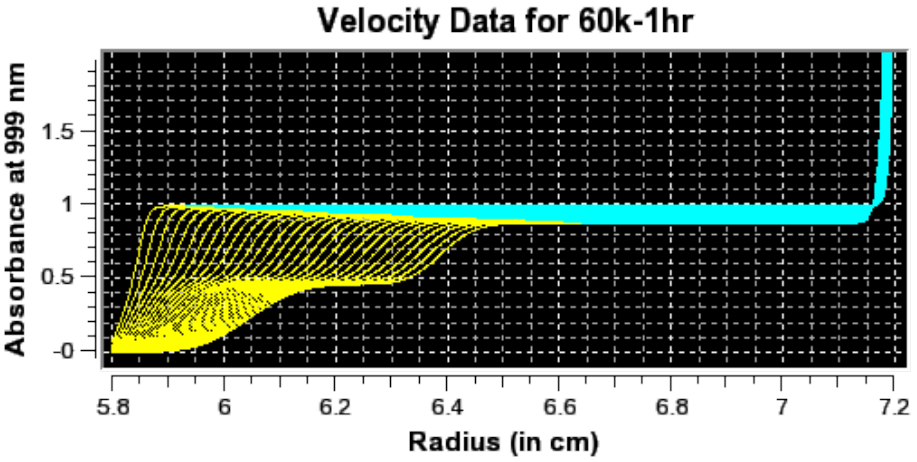


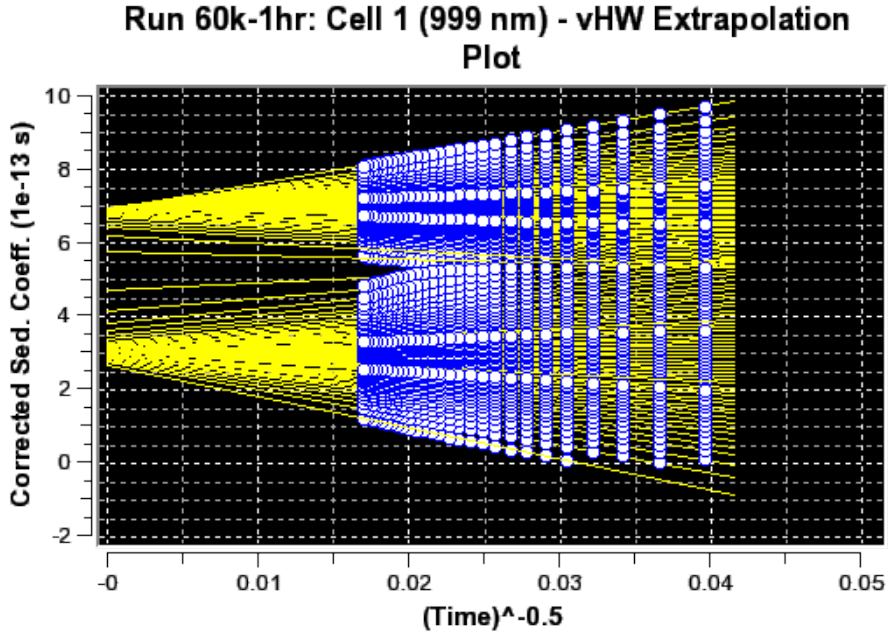


Effect of Rotorspeed with constant $\omega^2 t$ on Resolution:

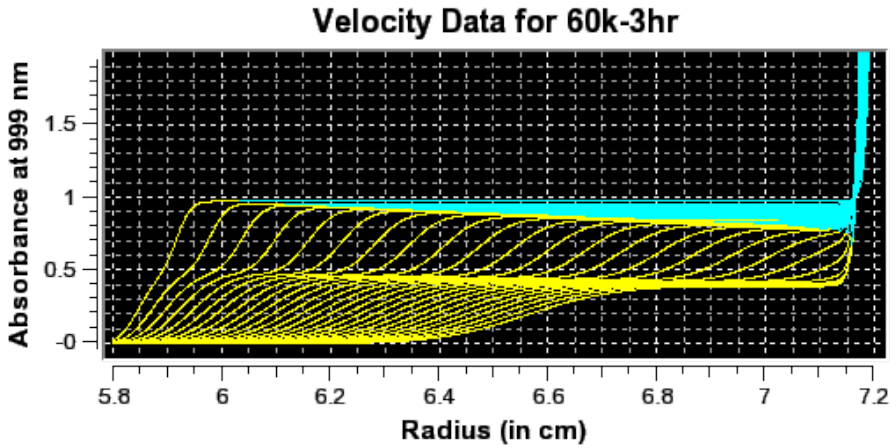


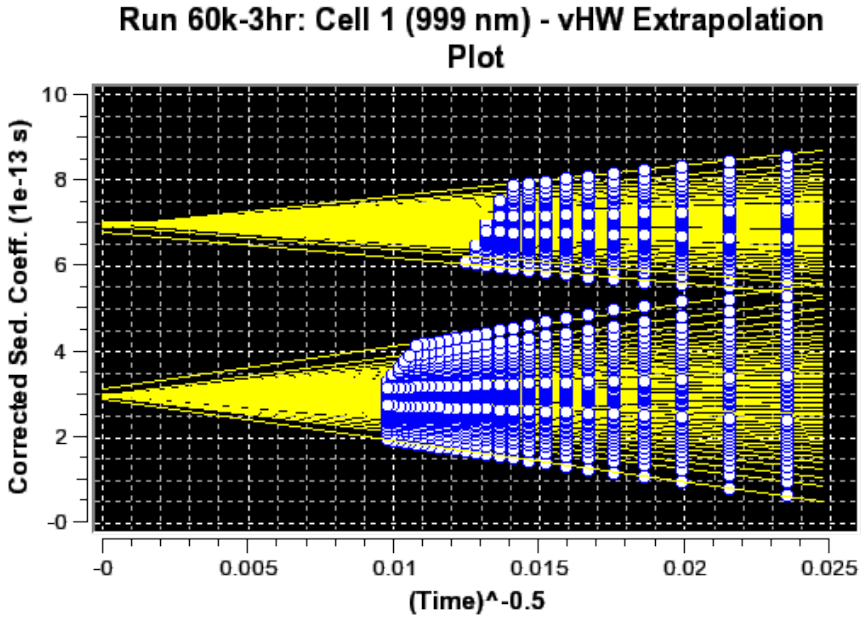


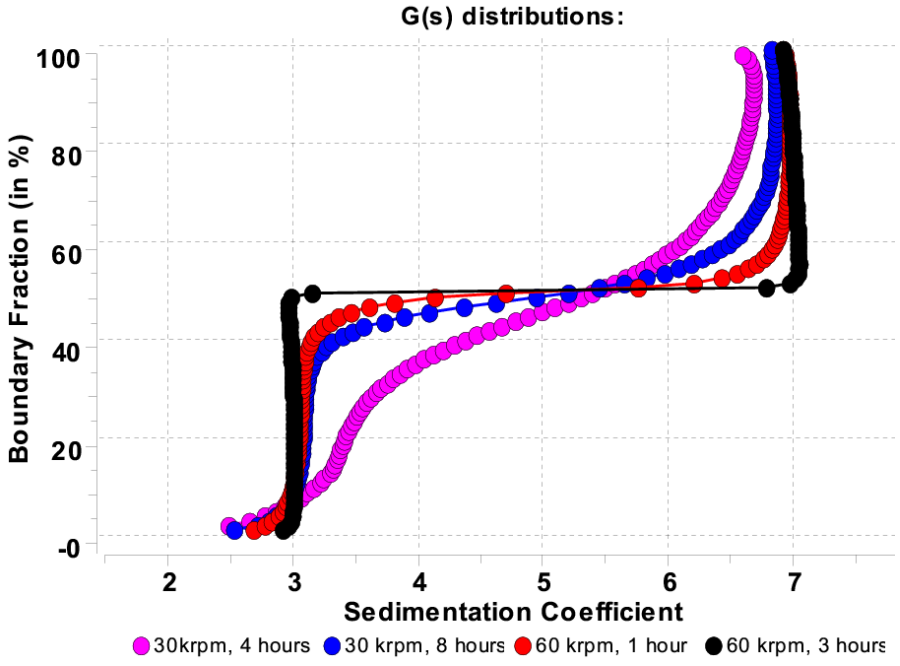


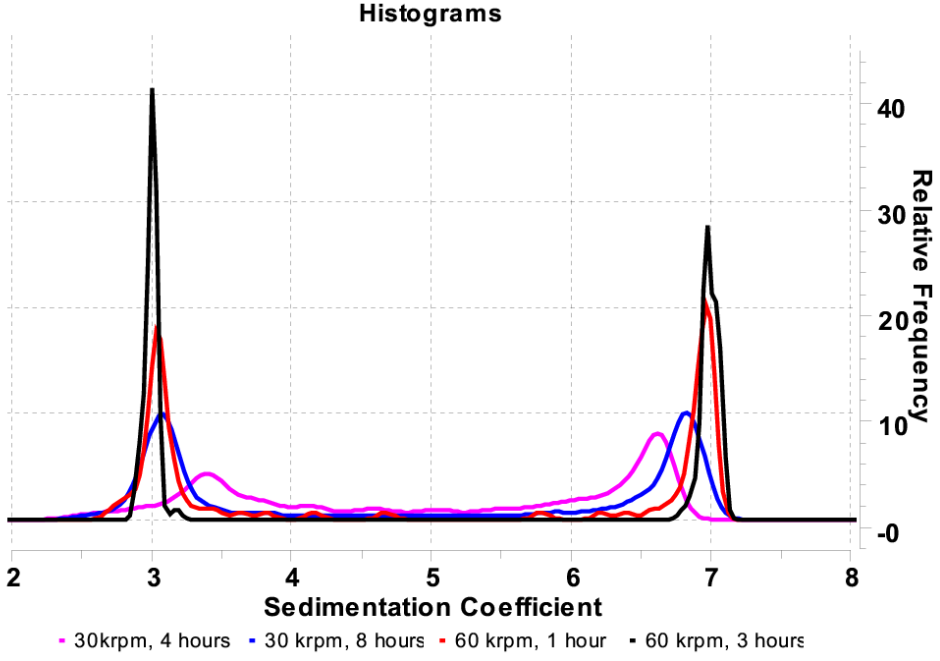


Resolution Comparison:







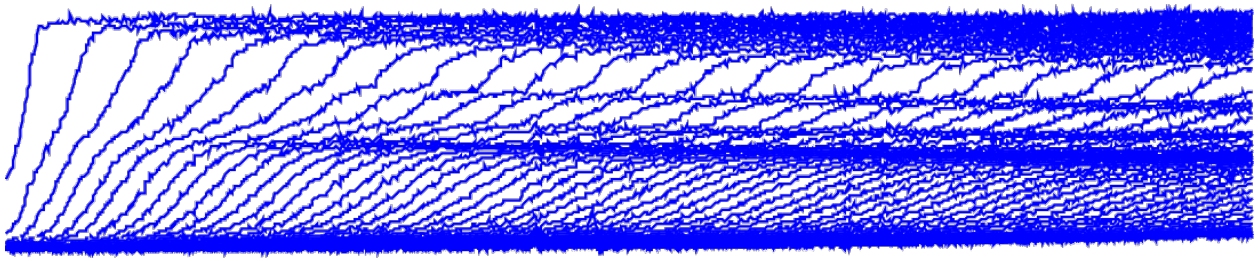


Resolution Comparison:

van Holde – Weischet Analysis Application Examples:

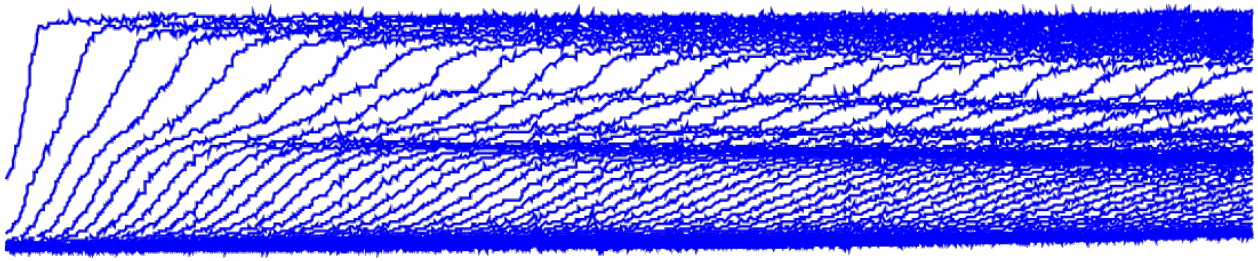
- Concentration dependent nonideality of *s*.
- Aggregation and irreversible self-association
- Composition Analysis
- Reversibly Self-Associating Systems vs. non-interacting systems
- Stoichiometry of Association
- Relative quantification of individual components
- Conformational information

Sedimentation Velocity:



Sedimentation velocity profile of a mixture of macromolecules over time

Sedimentation Velocity:

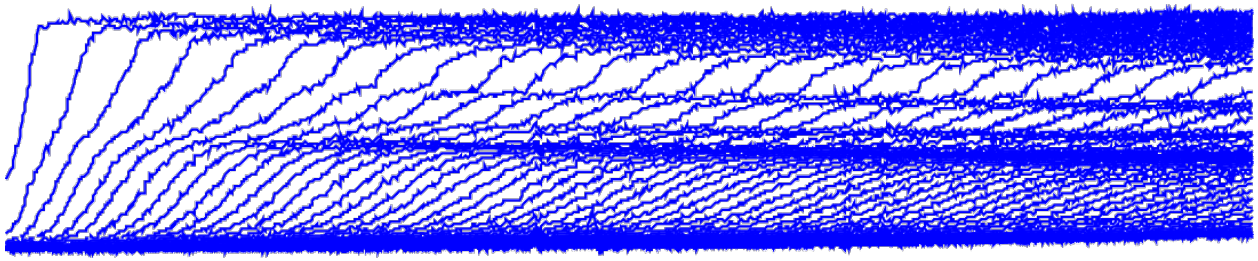


Composition Analysis

We can answer these questions:

How many components?

Sedimentation Velocity:



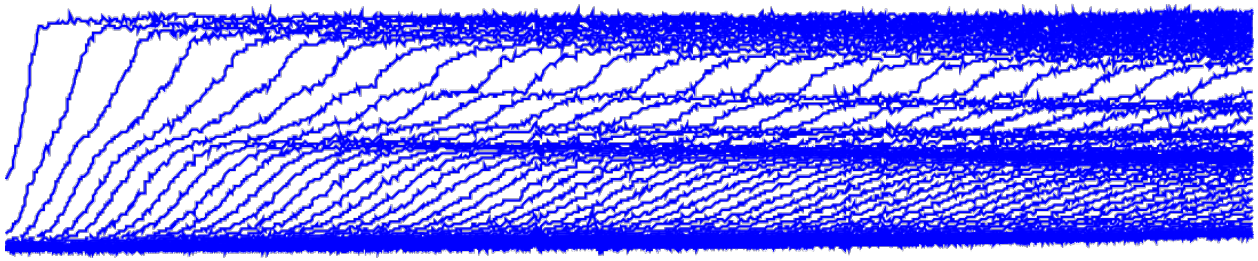
Composition Analysis

We can answer these questions:

How many components?

What are their molecular weights?

Sedimentation Velocity:



Composition Analysis

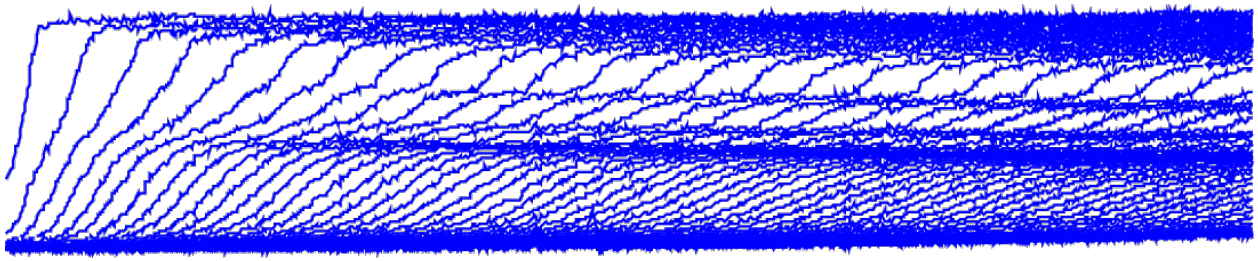
We can answer these questions:

How many components?

What are their molecular weights?

What are their shapes?

Sedimentation Velocity:



Composition Analysis

We can answer these questions:

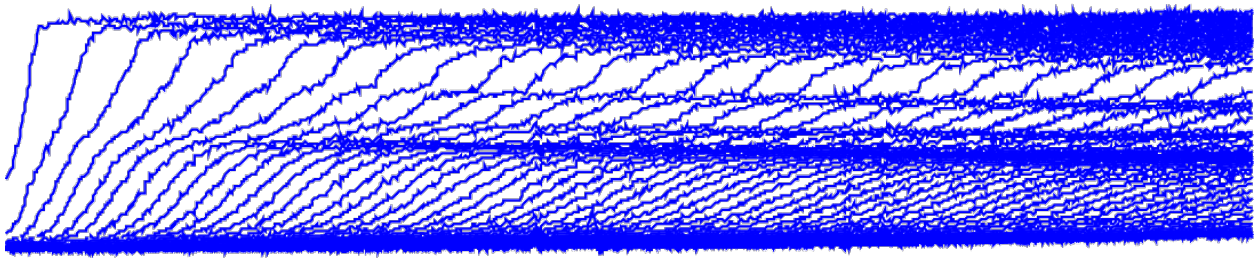
How many components?

What are their molecular weights?

What are their shapes?

What is the partial concentration of each component?

Sedimentation Velocity:



Composition Analysis

We can answer these questions:

How many components?

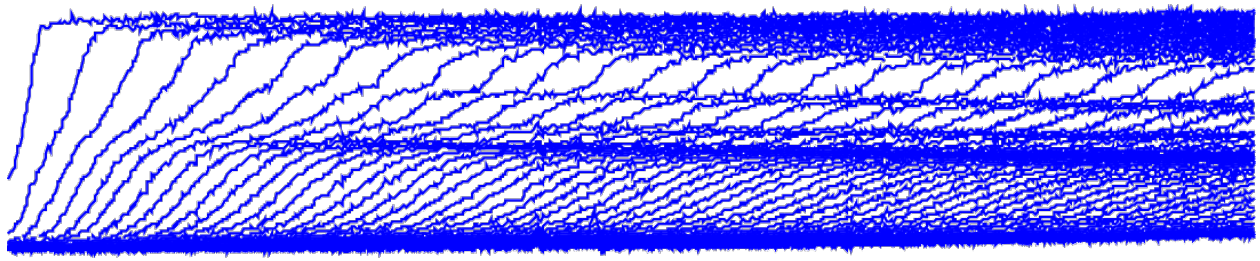
What are their molecular weights?

What are their shapes?

What is the partial concentration of each component?

Do the components interact (how fast, strong)?

Sedimentation Velocity:



Composition Analysis

We can answer these questions:

How many components?

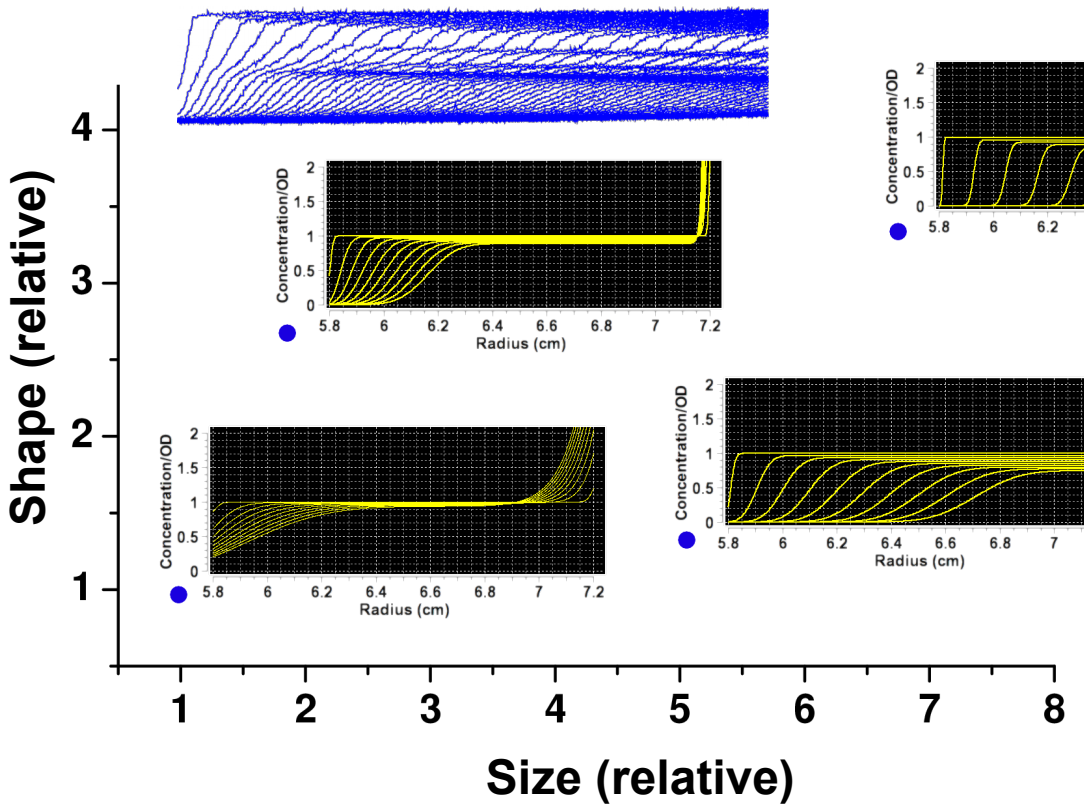
What are their molecular weights?

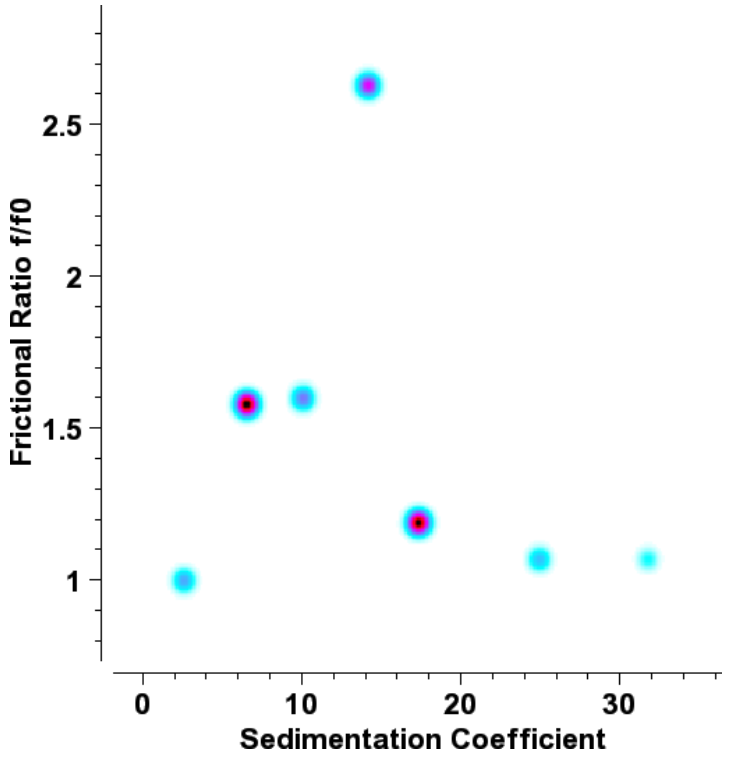
What are their shapes?

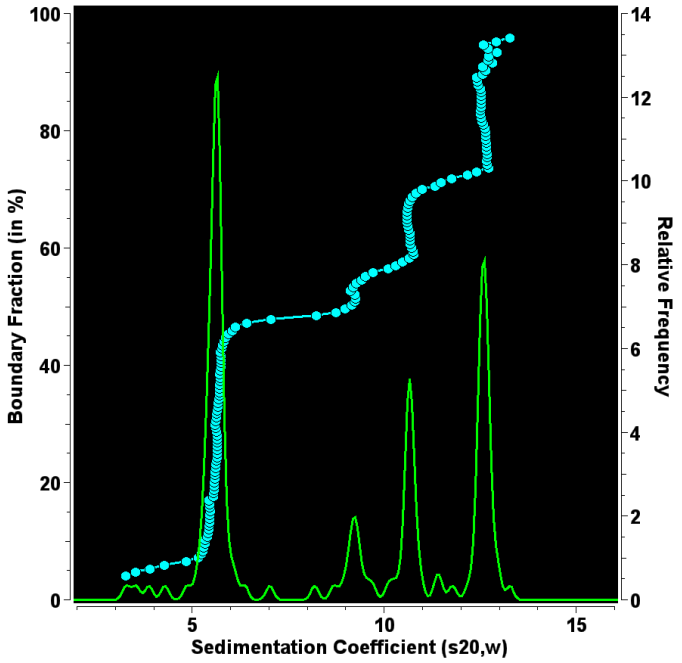
What is the partial concentration of each component?

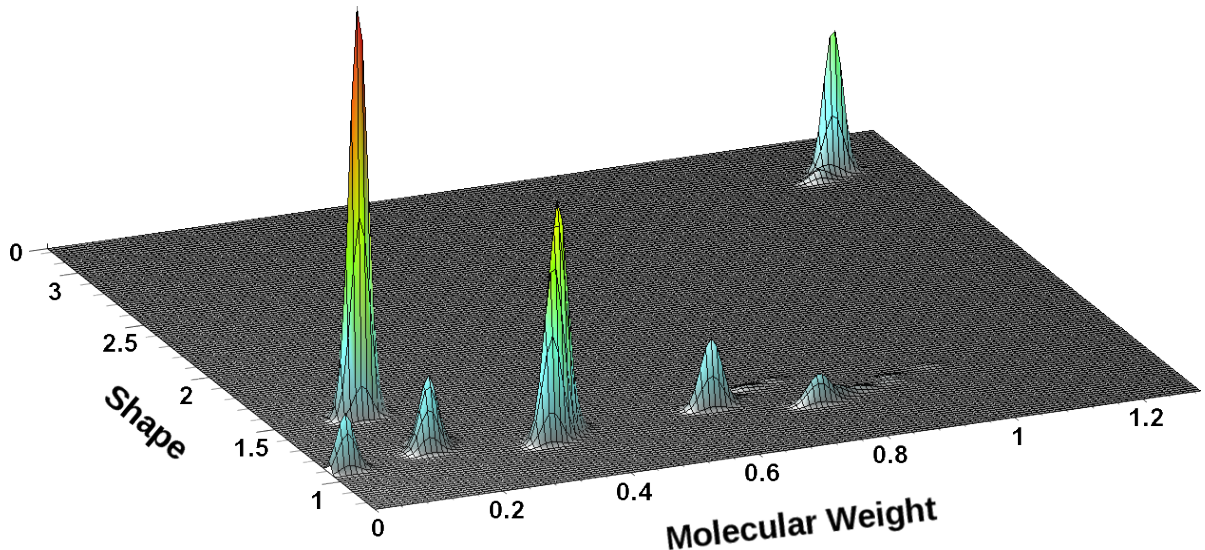
Do the components interact (how fast, strong)?

What is the reliability of our measurement?


Shape (relative)
Size (relative)
Concentration/OD
Radius (cm)


Frictional Ratio f/f0
Sedimentation Coefficient


Boundary Fraction (in %)
Relative Frequency
Sedimentation Coefficient (s20,w)


Shape
Molecular Weight

Sedimentation Velocity:

Optimization Methods:

2-dimensional Spectrum Analysis (2DSA):

Provides degenerate, linear fit to experimental data over a finite domain, identifying regions with signal in the mass/shape domain, used to remove systematic noise contributions

Genetic Algorithms (GA):

Provides parsimonious regularization of 2DSA spectrum. Satisfies Occam's razor. Also used for fitting of discrete, non-linear models (reversible association, non-ideality, co-sedimenting solutes)

Monte Carlo Analysis (MC)

Used to measure the effect of noise on the fitted parameters, yields parameter distribution statistics

Parametrically Constrained Spectrum Analysis (PCSA)

Used to regularize 2-dimensional spectrum analysis. Enforce a unique mapping of one molar mass/sedimentation coefficient per frictional ratio.