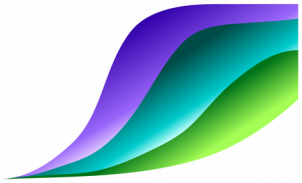




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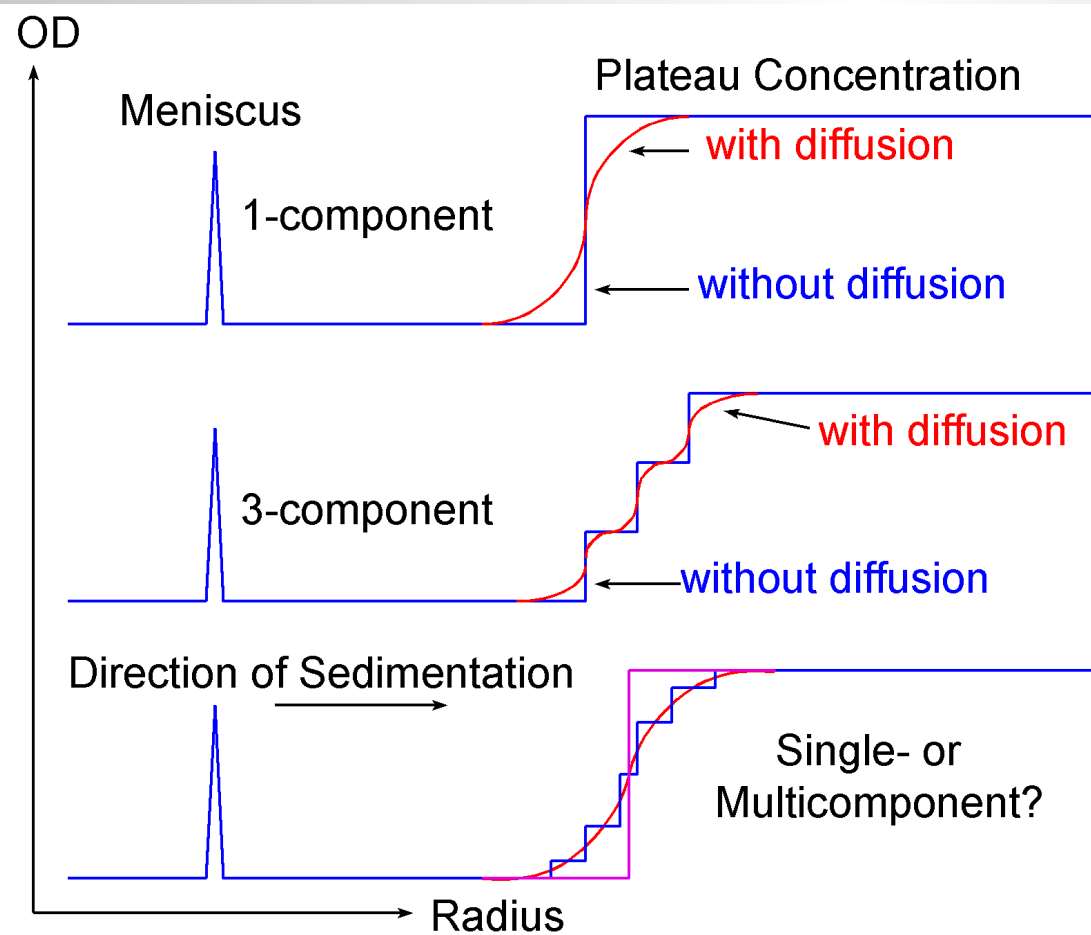


NORTHWEST
BIOPHYSICS
CONSORTIUM

van Holde – Weischet Analysis



What about Diffusion and Heterogeneity?



Enhanced van Holde – Weischedel 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?

Enhanced van Holde – Weischet Method:

...is a graphical transformation of the velocity data:

transport due to Diffusion $\sim \sqrt{t}$

transport due to sedimentation $\sim t$

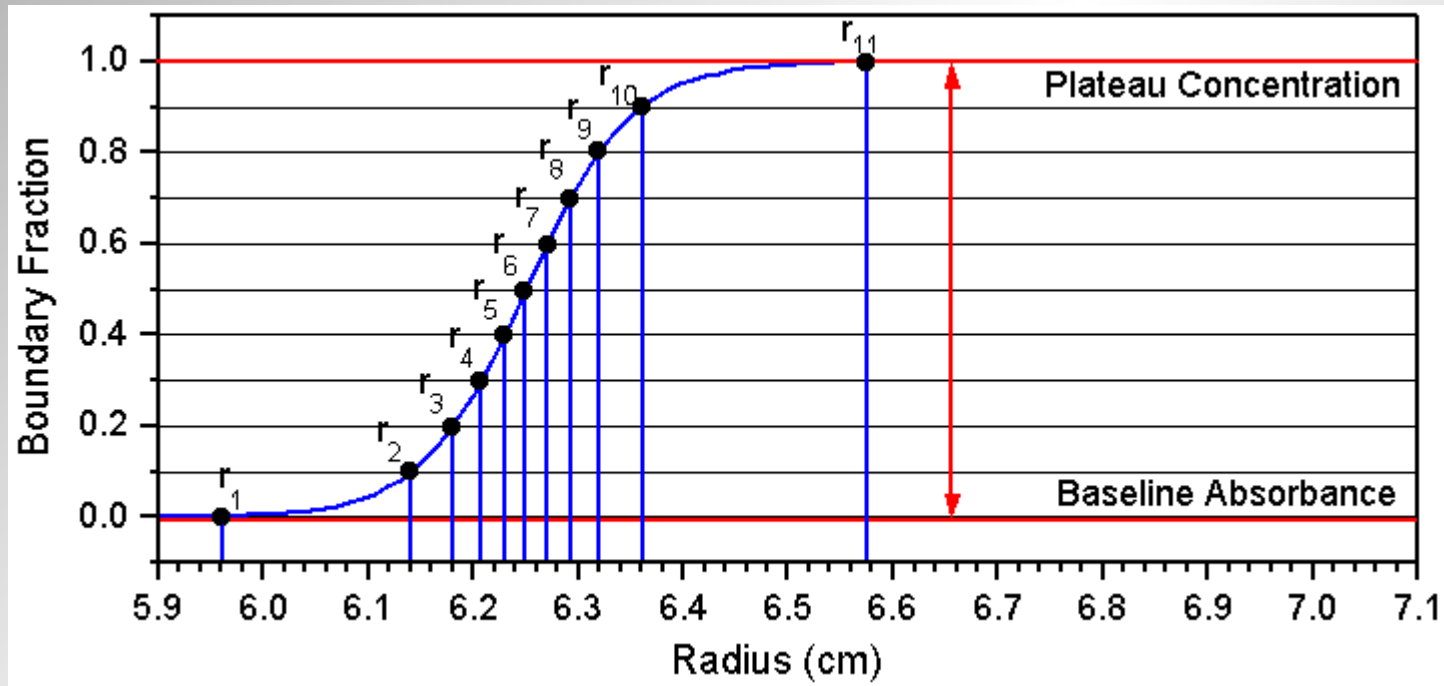
At infinity transport due to diffusion will be negligible compared to transport due to sedimentation - i.e., all components will separate out if the rotorspeed is fast enough.

...yields diffusion corrected sedimentation coefficient distributions

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

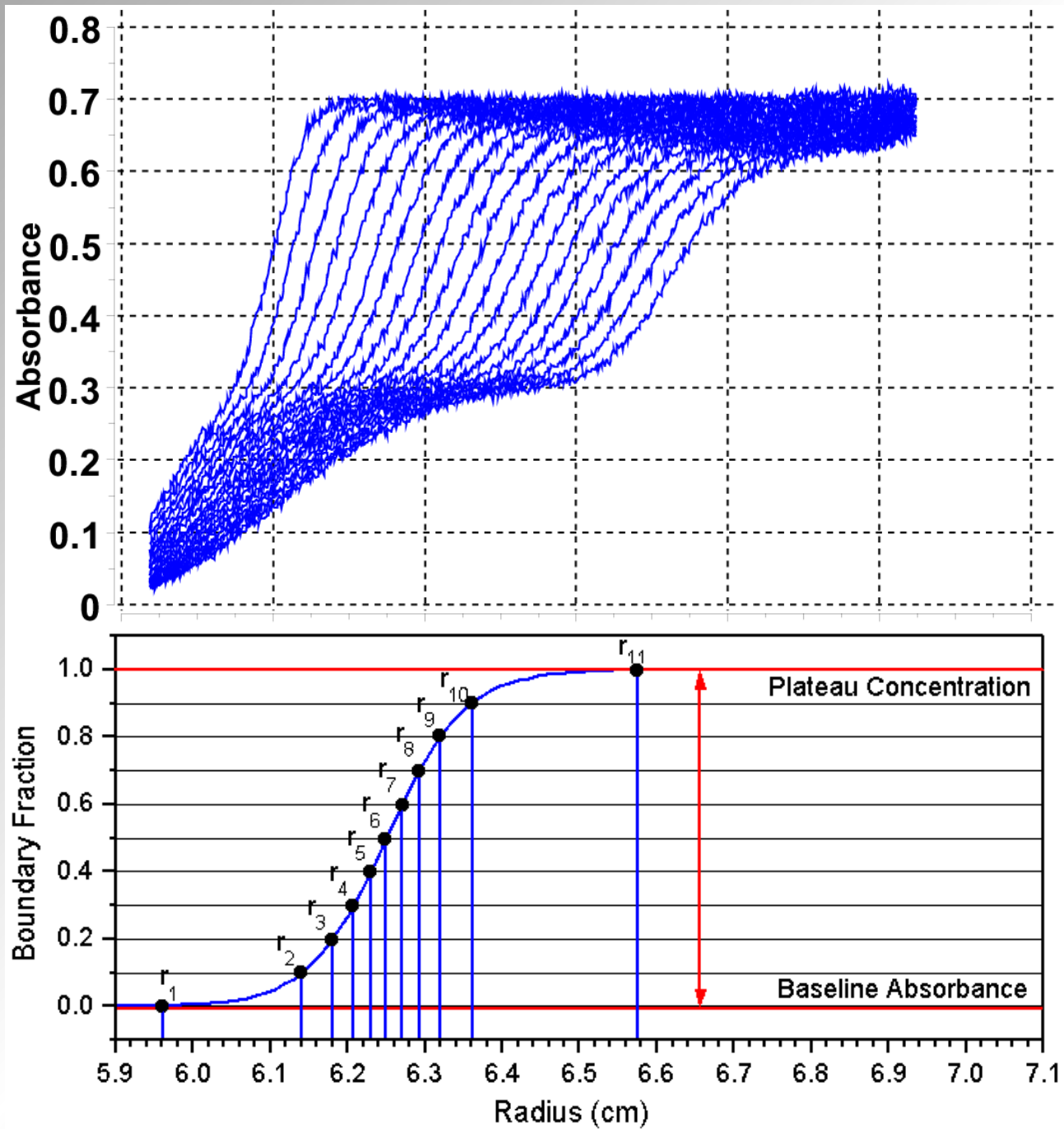
Calculation of apparent Sedimentation Coefficients:



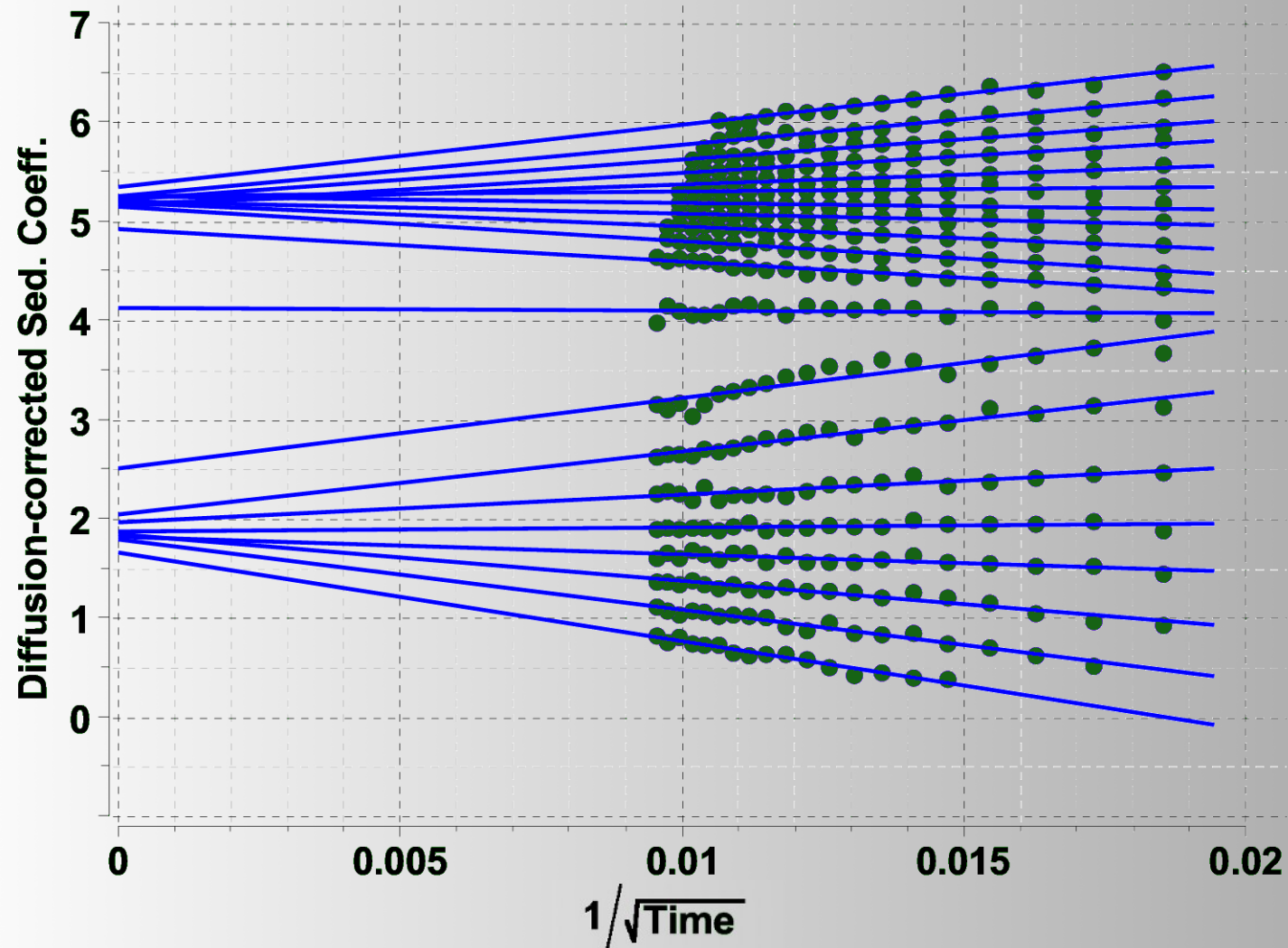
$$\frac{v}{\omega^2 r} = s$$

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

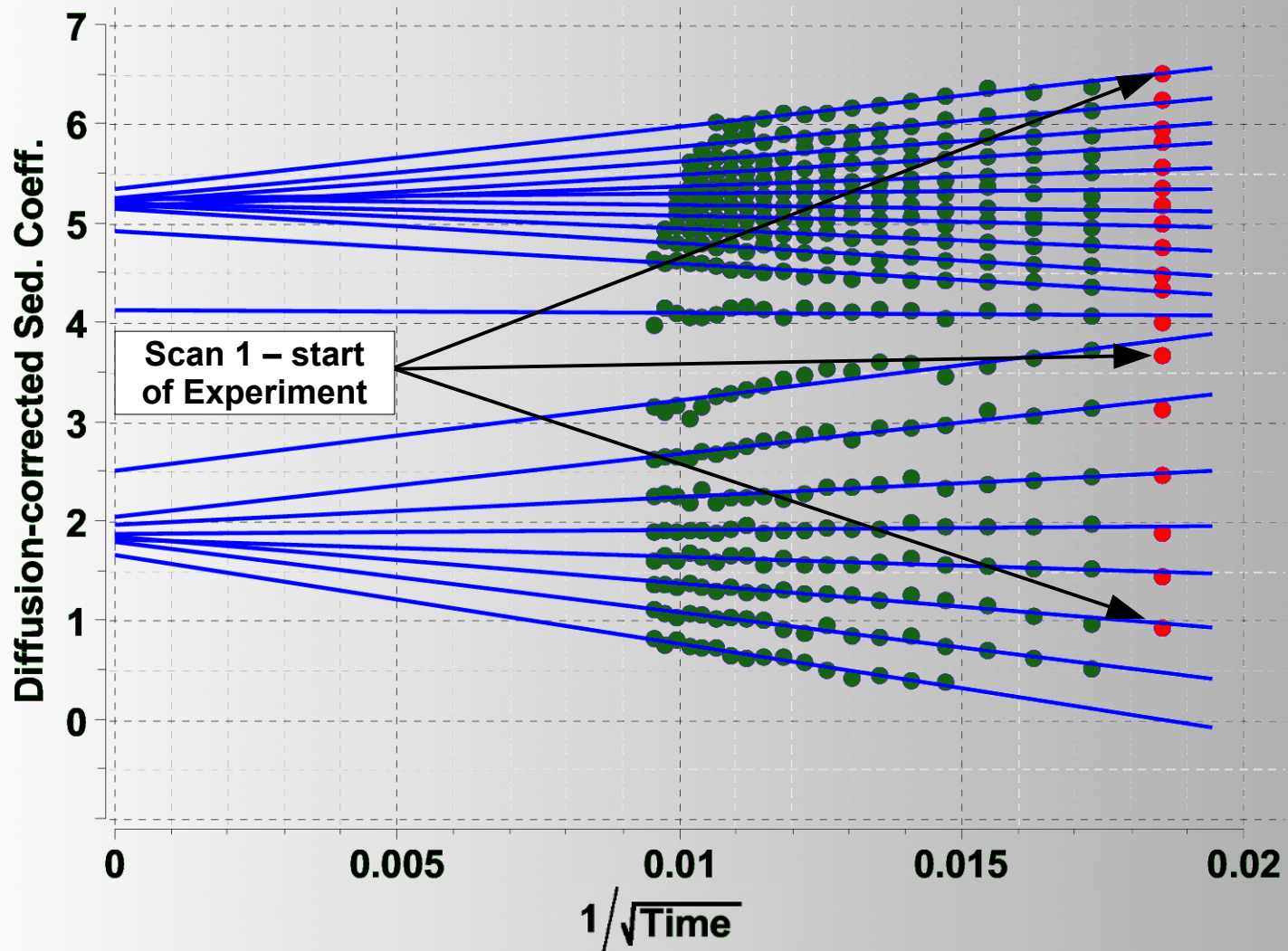
$$\hat{s}_b = \ln\left(\frac{r_b(t)}{r_a(t_0)}\right) [\omega^2 (t - t_0)]^{-1}$$



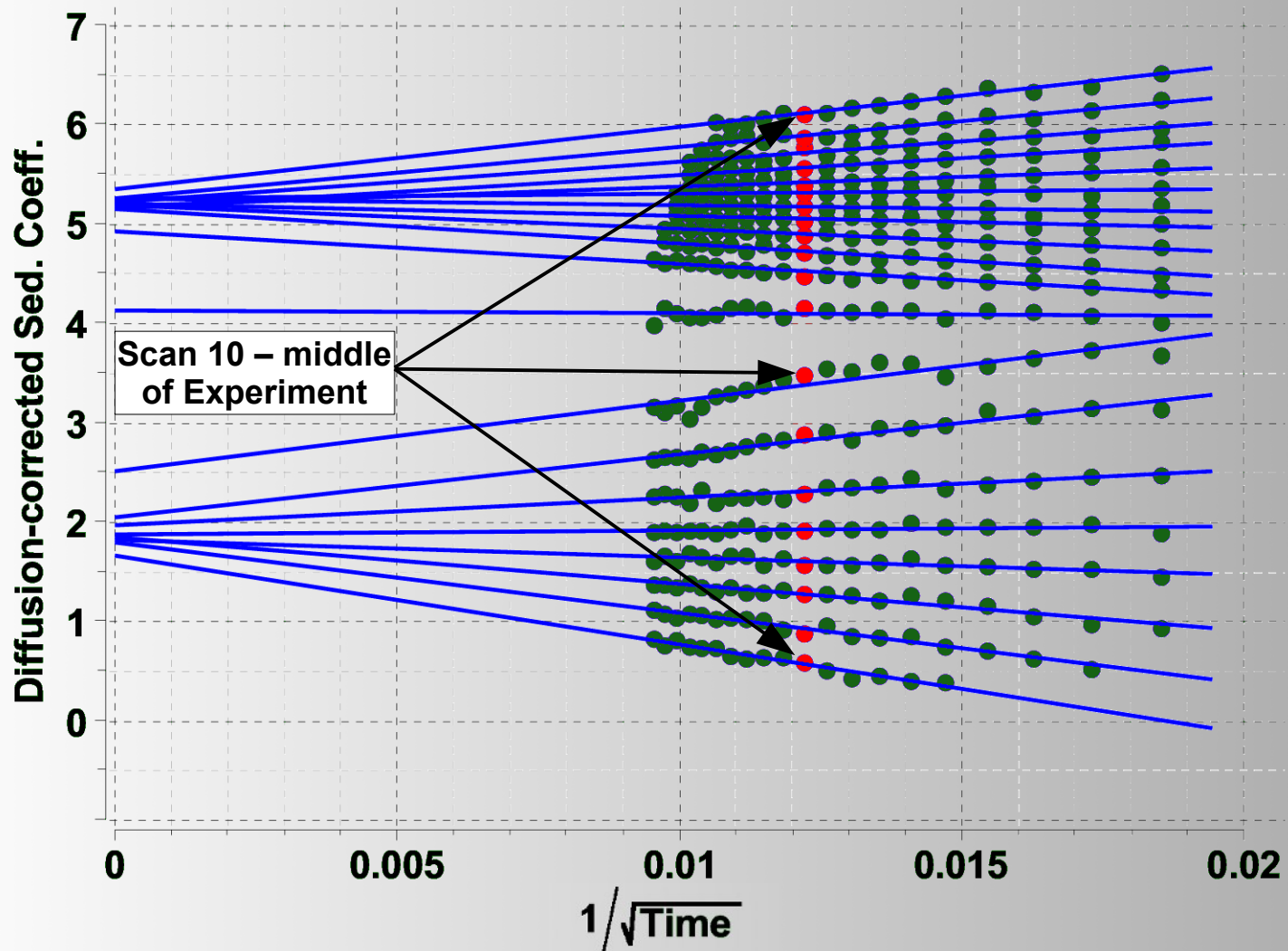
van Holde – Weischet Extrapolation Plot:



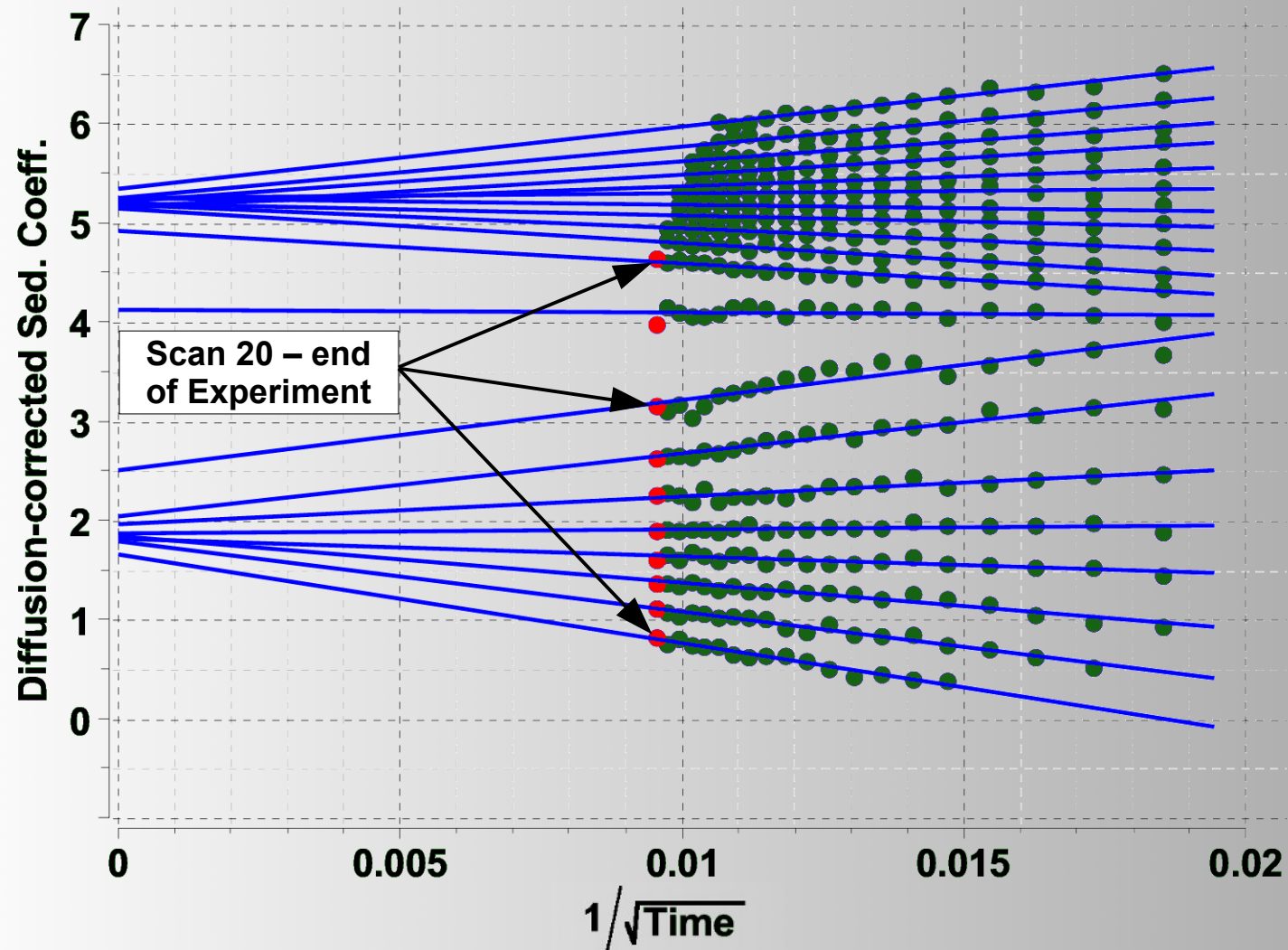
van Holde – Weischet Extrapolation Plot:



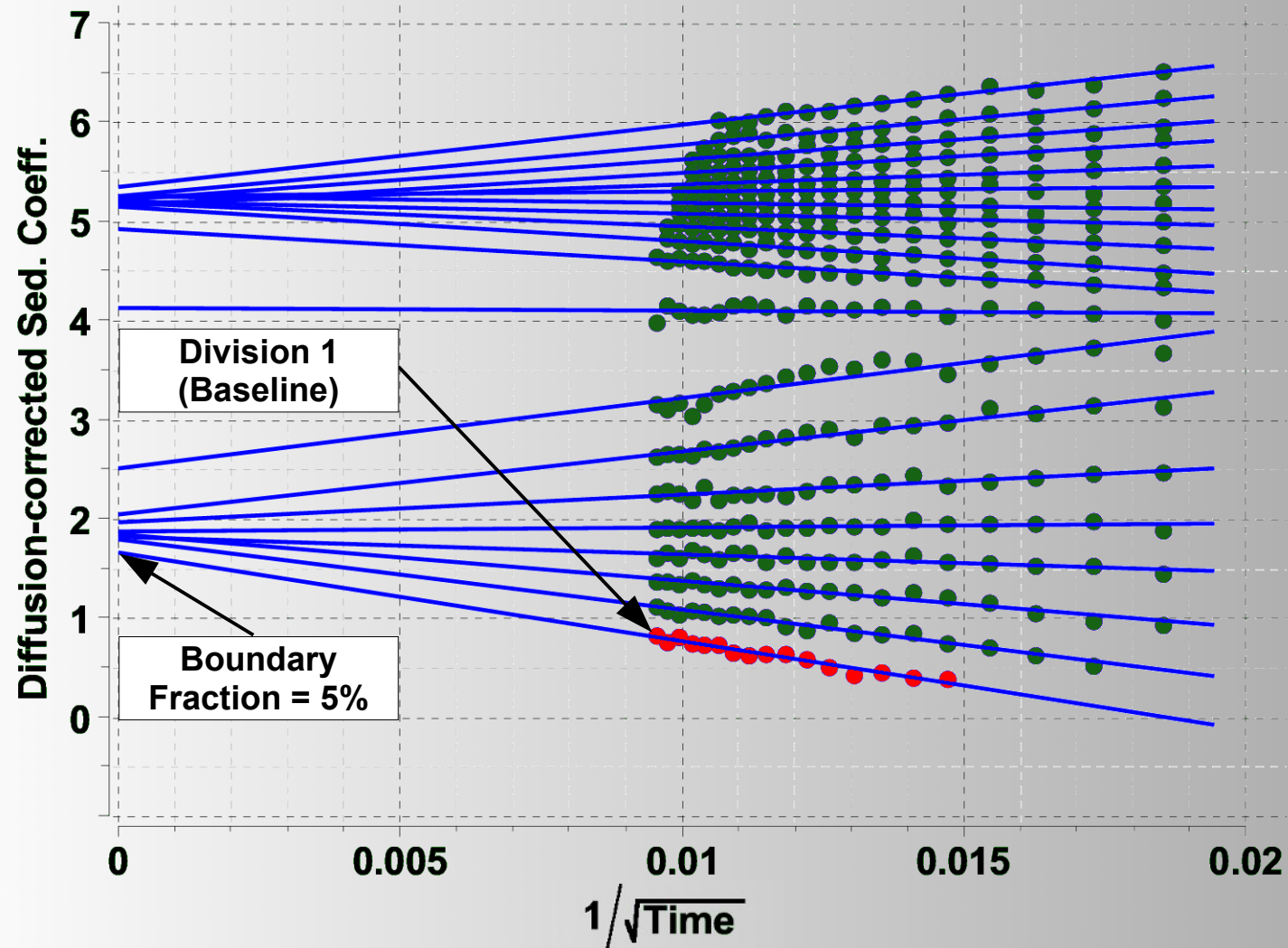
van Holde – Weischet Extrapolation Plot:



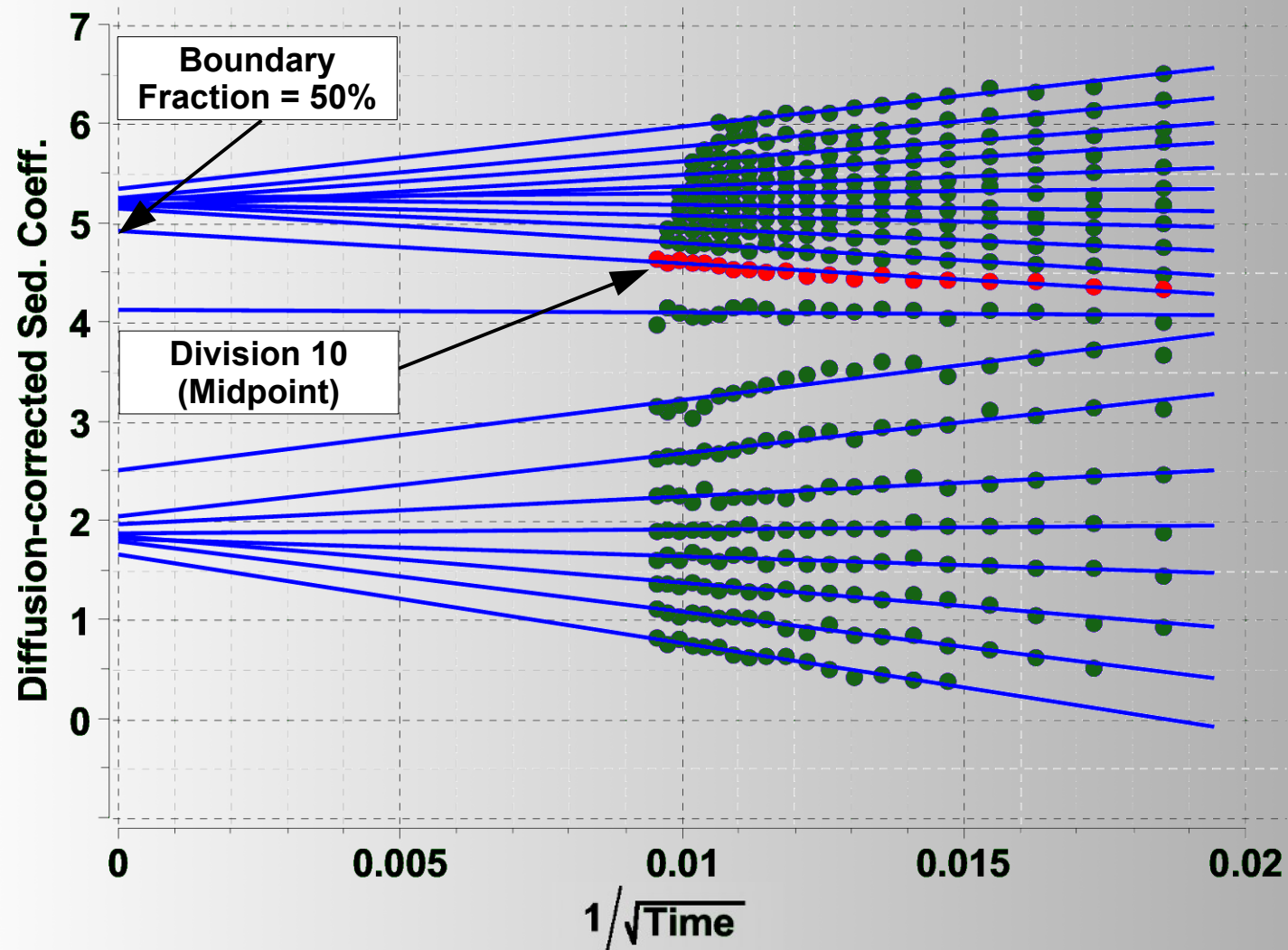
van Holde – Weischet Extrapolation Plot:



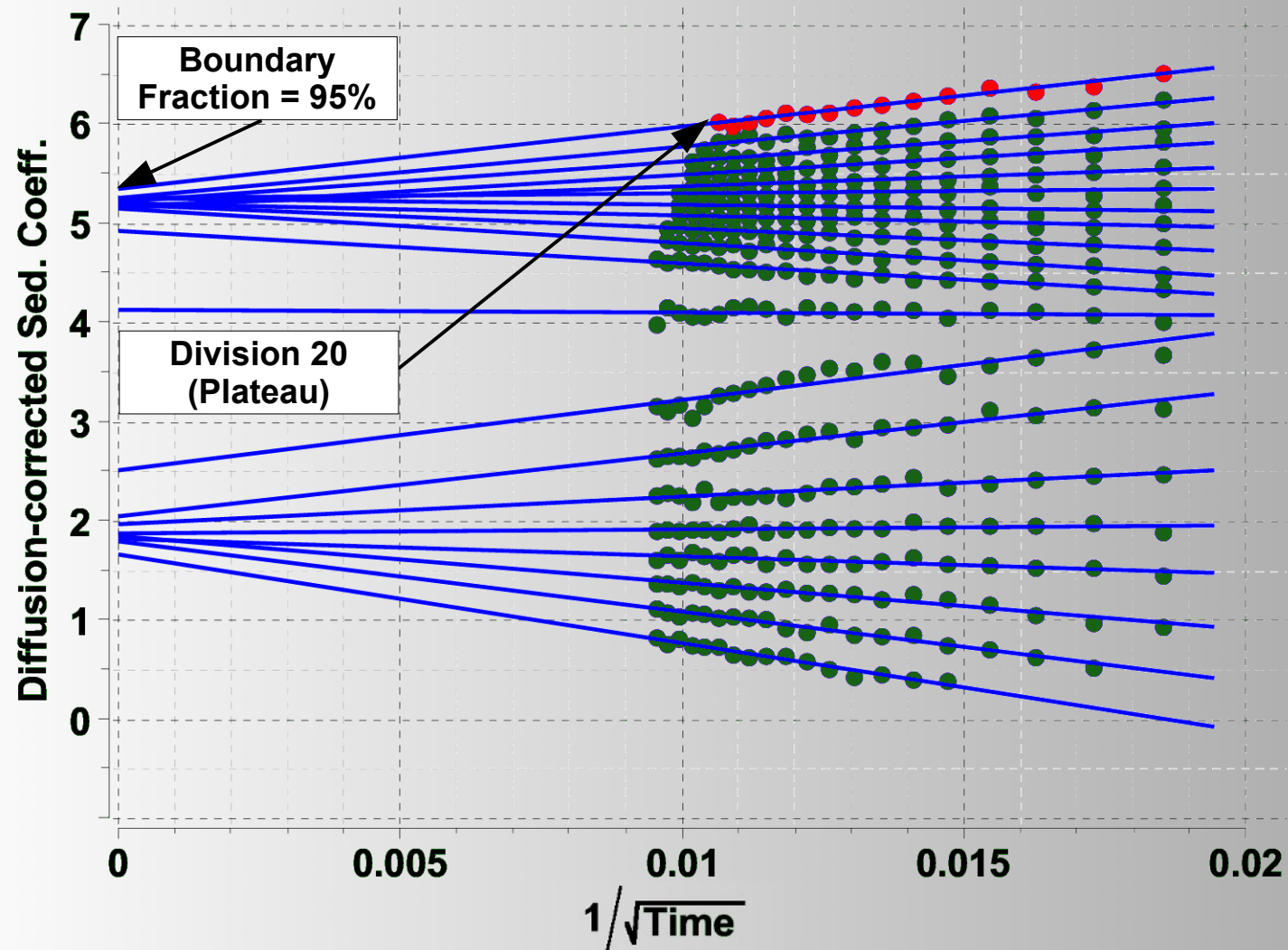
van Holde – Weischet Extrapolation Plot:



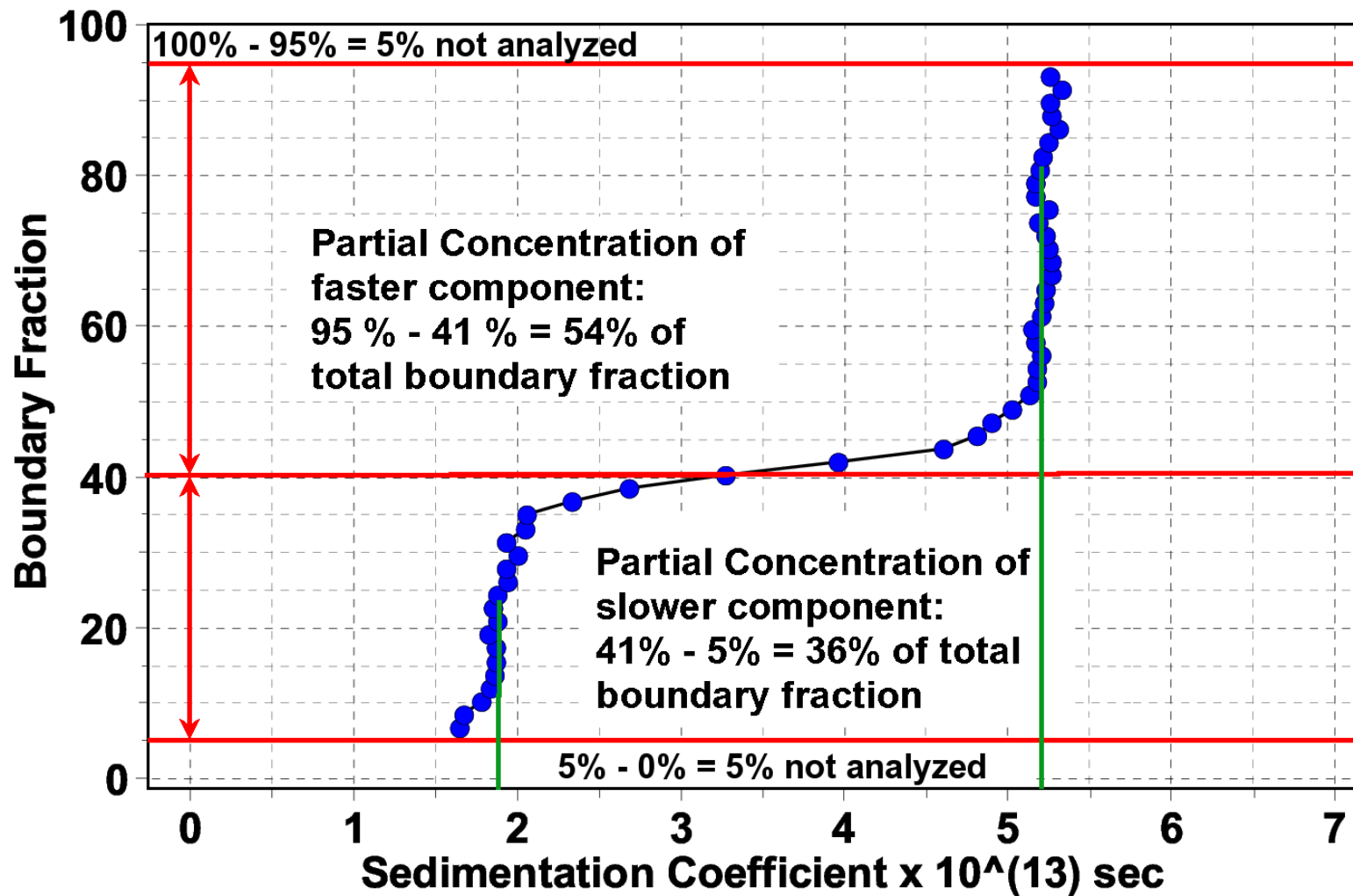
van Holde – Weischet Extrapolation Plot:



van Holde – Weischet Extrapolation Plot:



van Holde – Weischet Integral Distribution Plot (G(s)):

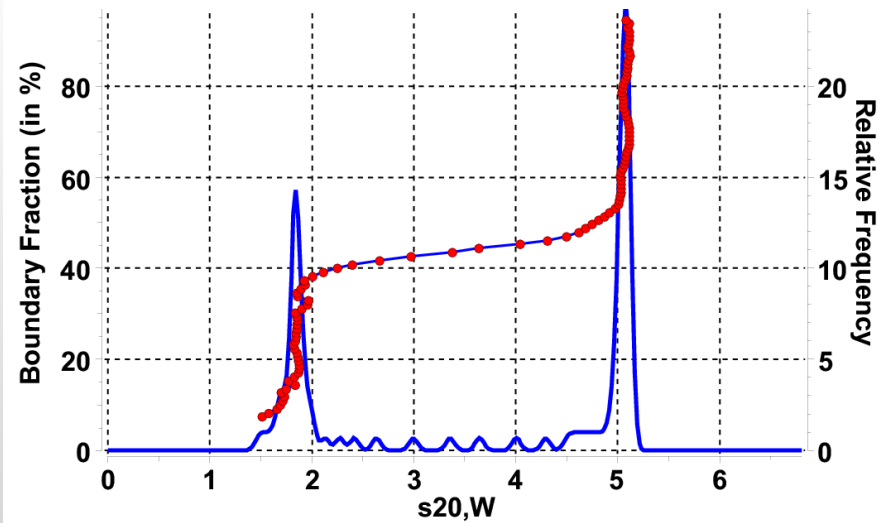
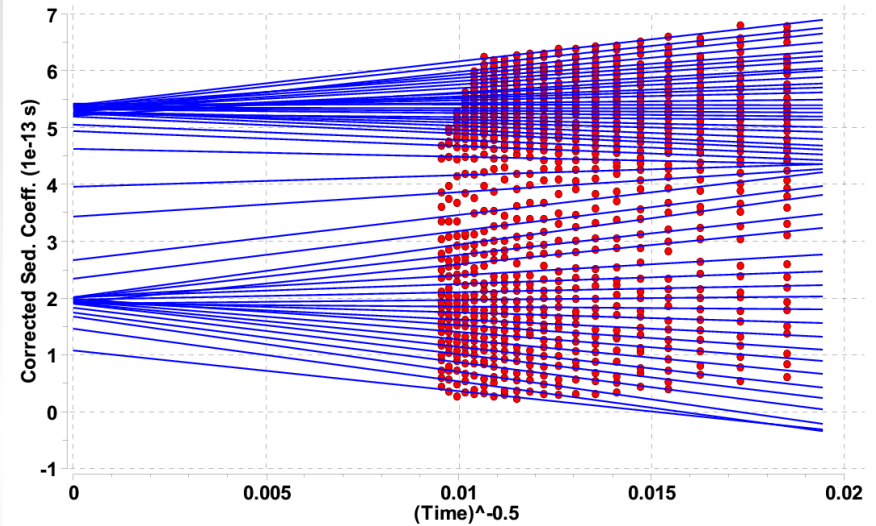
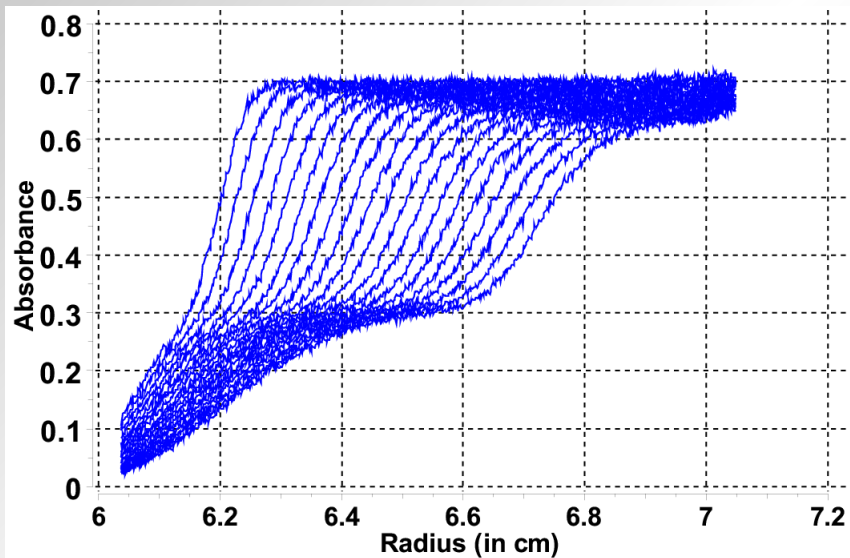


Enhanced van Holde – Weischet Method:

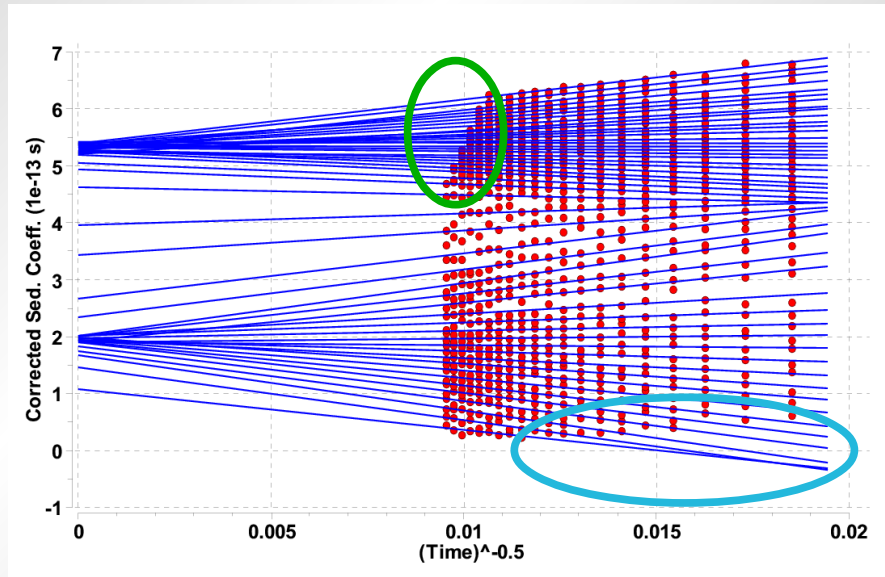
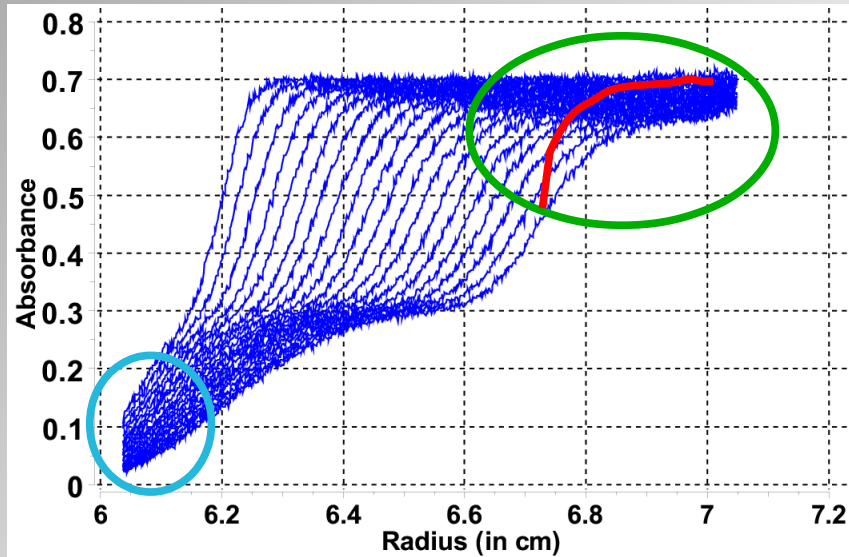
van Holde – Weischet method:

$s_1: 5.35 \times 10^{-13}$ (52 %)

$s_2: 1.87 \times 10^{-13}$ (39 %)



Enhanced van Holde – Weischet Method:



Green: Back diffusion distorts boundary – data points are excluded

Cyan: boundary has not cleared meniscus – data points are excluded

Application Examples:

van Holde – Weischet Analysis Application

Examples:

Concentration dependent nonideality of s.

Aggregation and irreversible self-association

Composition Analysis

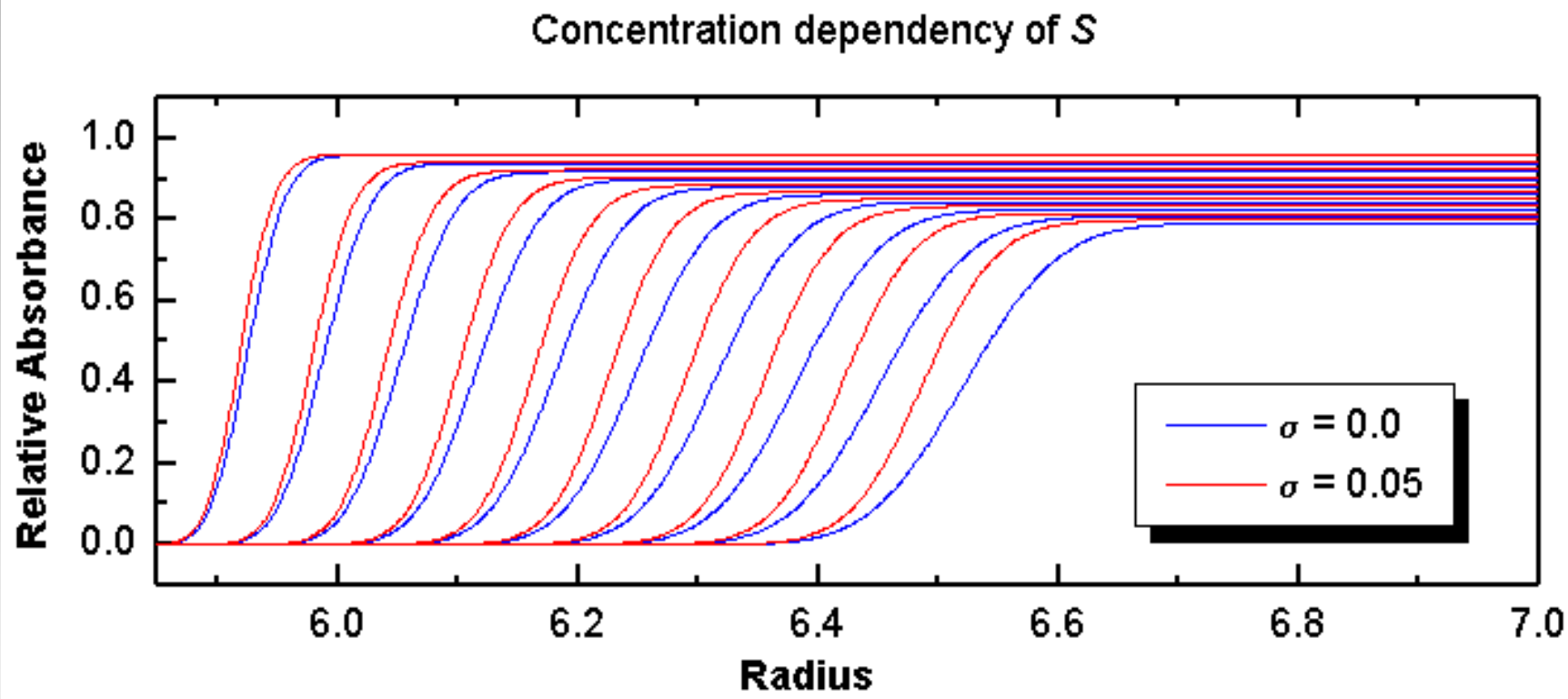
Reversibly Self-Associating Systems vs. non-interacting systems

Stoichiometry of Association

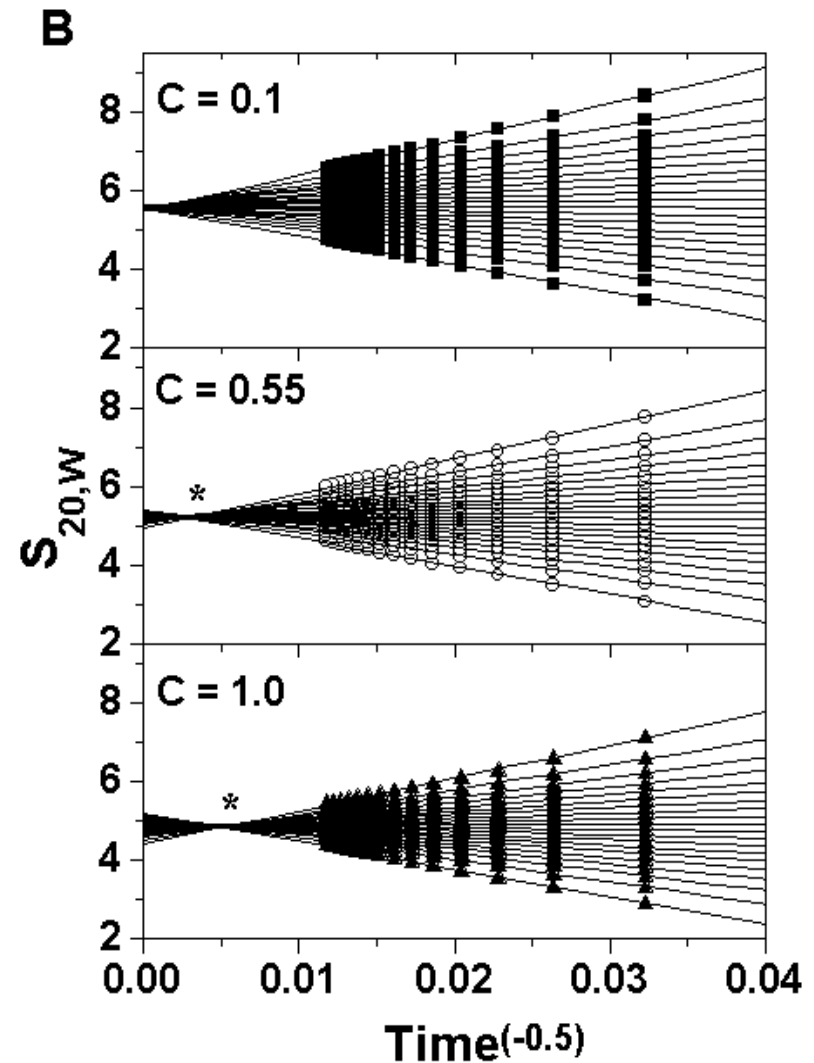
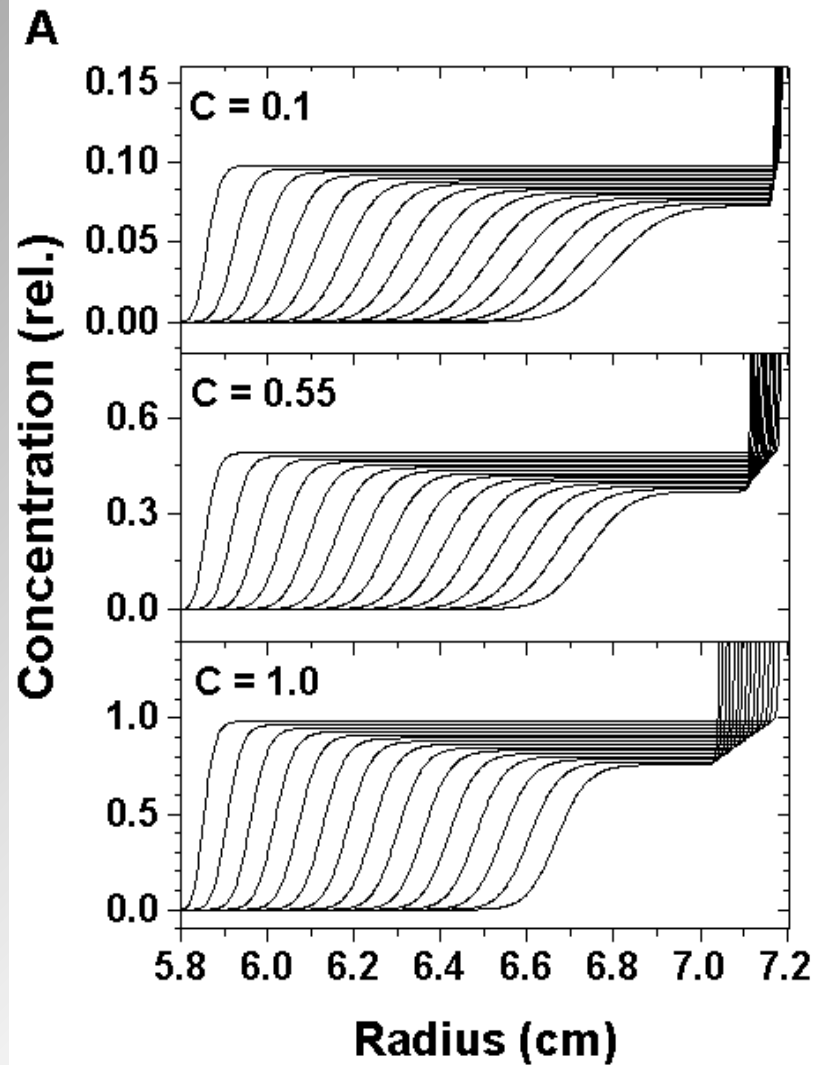
Relative quantification of individual components

Conformational information

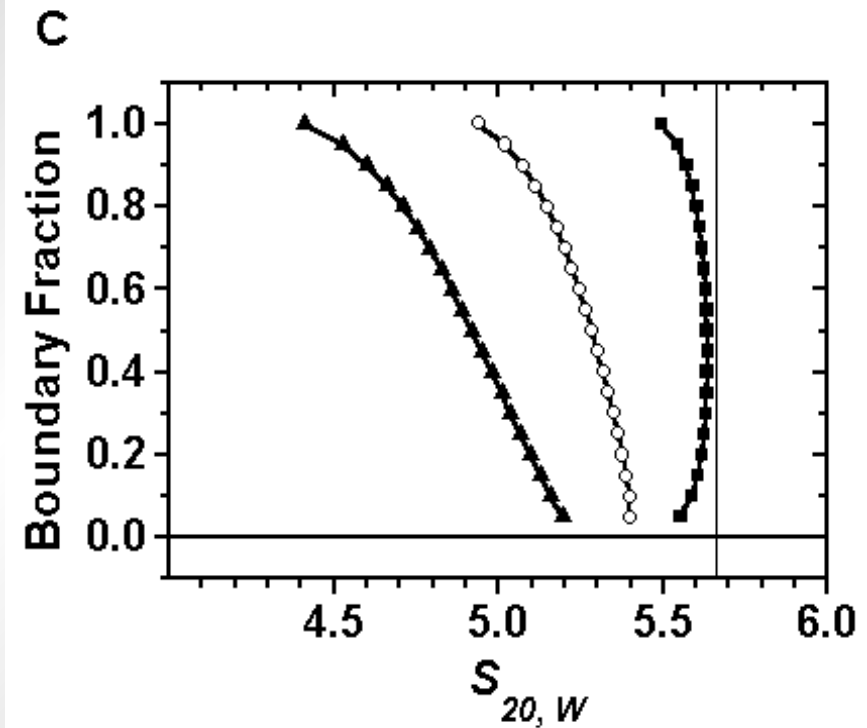
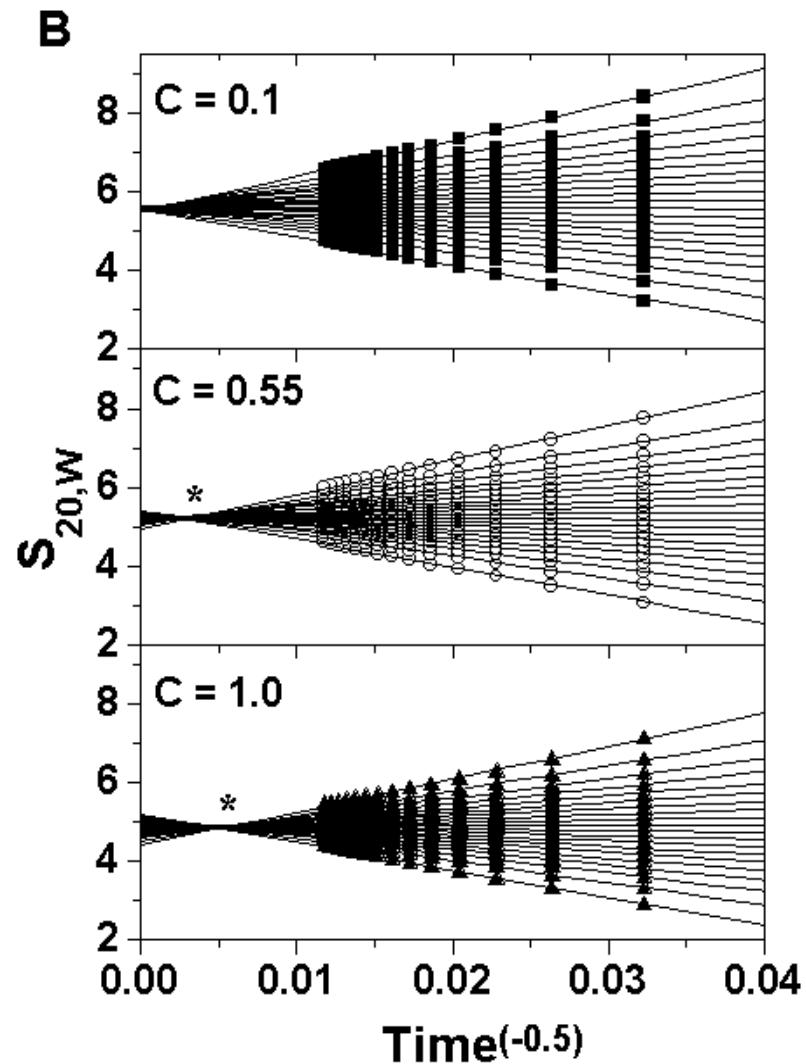
Concentration Dependency of the Sedimentation Coefficient



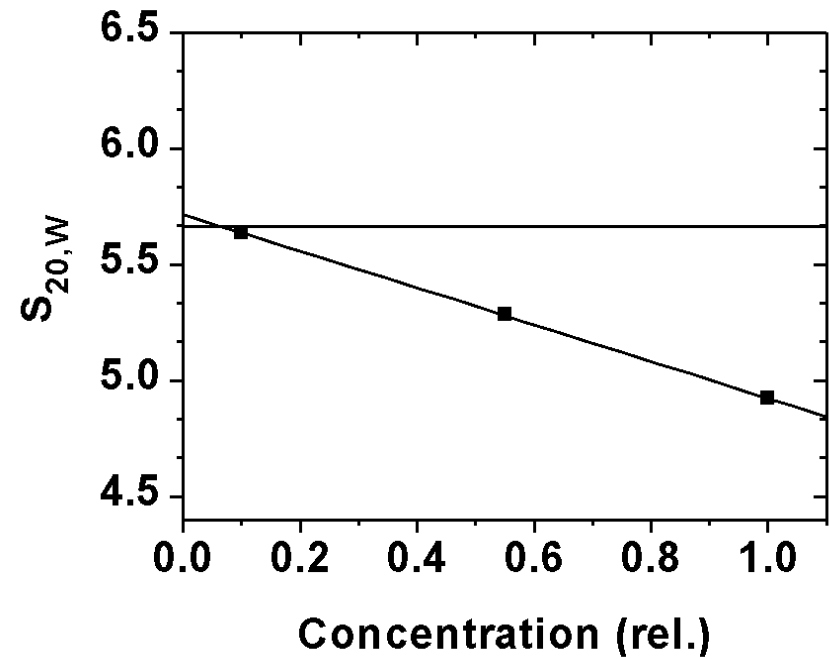
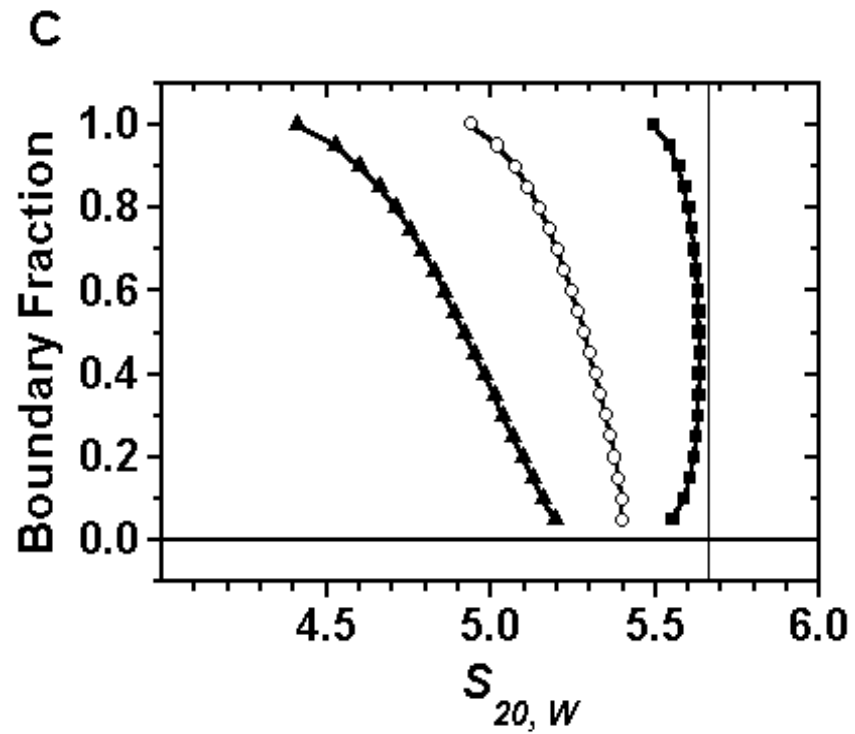
Concentration Dependency of the Sedimentation Coefficient



Concentration Dependency of the Sedimentation Coefficient



Concentration Dependency of the Sedimentation Coefficient



van Holde – Weischet Analysis: Limitations

The van Holde – Weischet method cannot be used for fitting diffusion coefficients, molecular weights, association constants or frictional coefficients, only sedimentation coefficients and partial concentrations are reported.

van Holde – Weischet Applications: Summary

Model independent analysis

Initial characterization of an unknown sample

Composition analysis:

Homogeneous or heterogeneous?

Aggregation?

Binding Stoichiometry

Molecular weight distribution transformations

Relative quantification of individual components

Conformational analysis

Qualitative information about diffusion

Identify concentration dependency:

Self-association or non-interacting?

Reversible or irreversible?

Concentration dependent solution nonideality?