Calculation of the Sedimentation Coefficient:

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

$$v = \frac{dr}{dt}$$

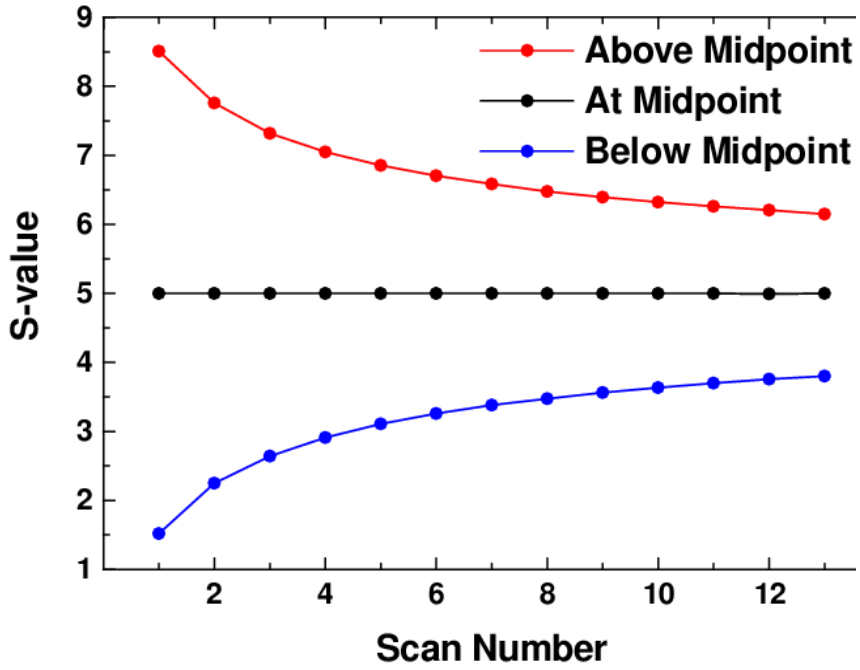
$$\frac{dr}{r} = \omega^2\ s\ dt$$

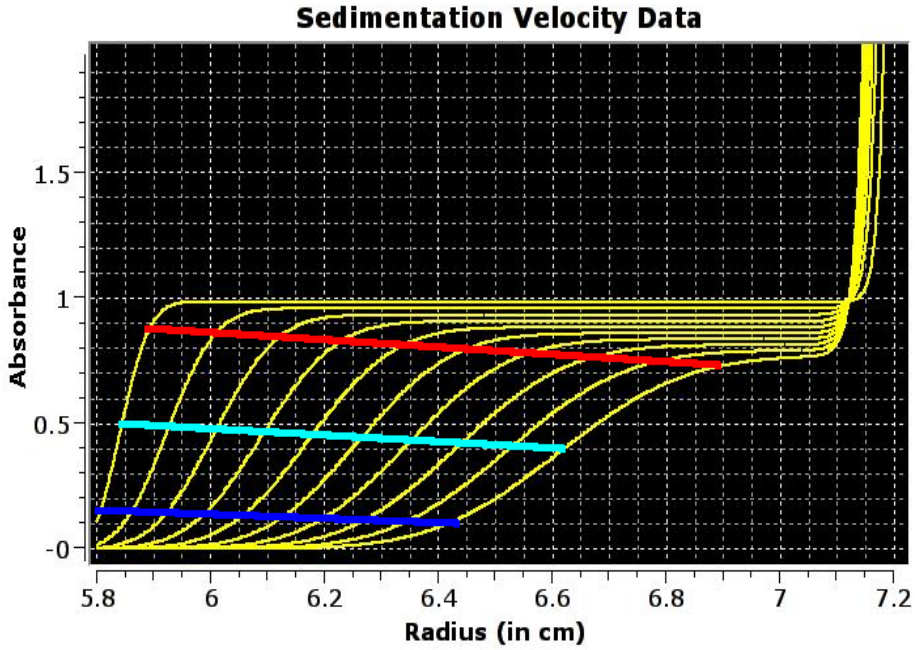
$$\int_{r=m}^{r=b} \frac{1}{r}\,dr = \int_{t=0}^{t=scan} s\,\omega^2\,dt$$

$$\ln r_b\left(t\right) - \ln\ r_m\left(t_0\right) = s\ \omega^2\left(t - t_0\right)$$

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

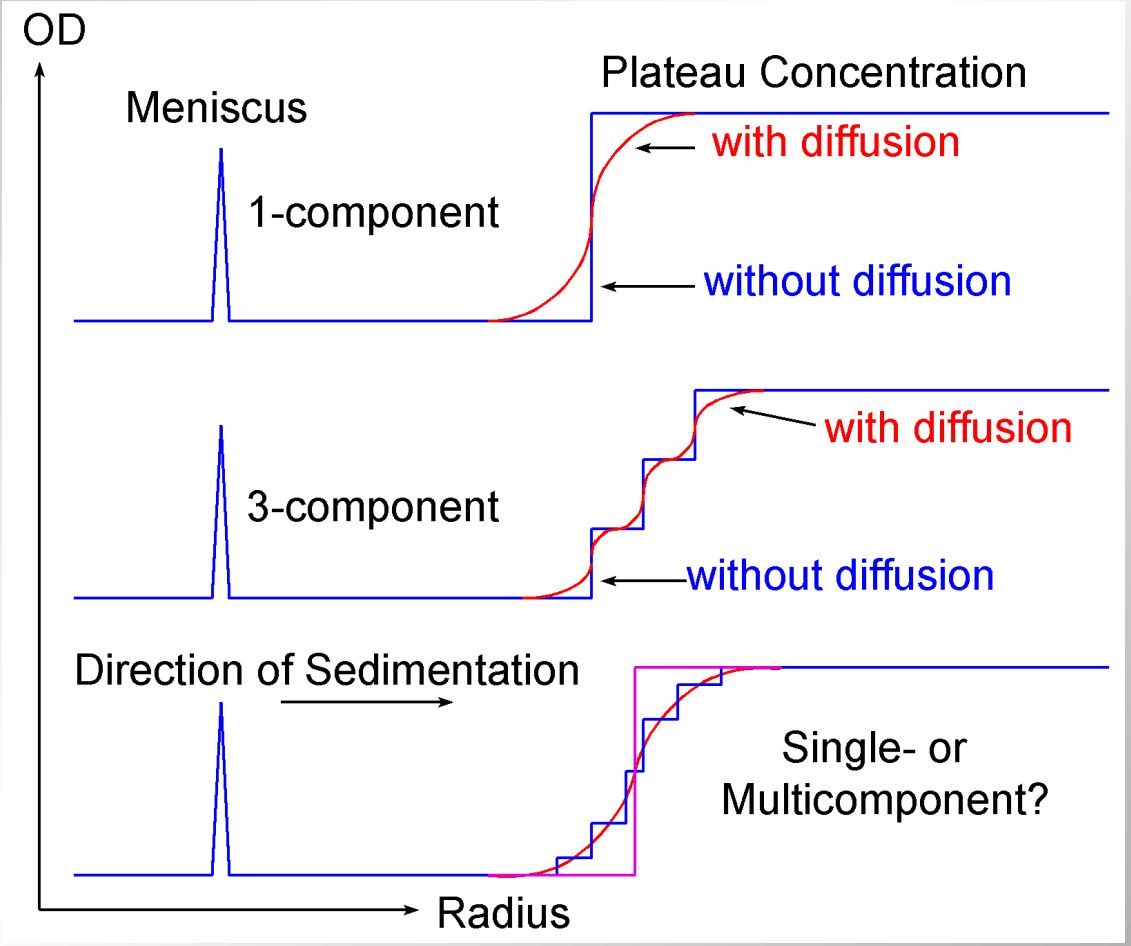
Calculation of the Sedimentation Coefficient:





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

What about Diffusion and Heterogeneity?



Enhanced 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?

Enhanced van Holde – Weischet Method:

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

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

transport due to sedimentation ~ t

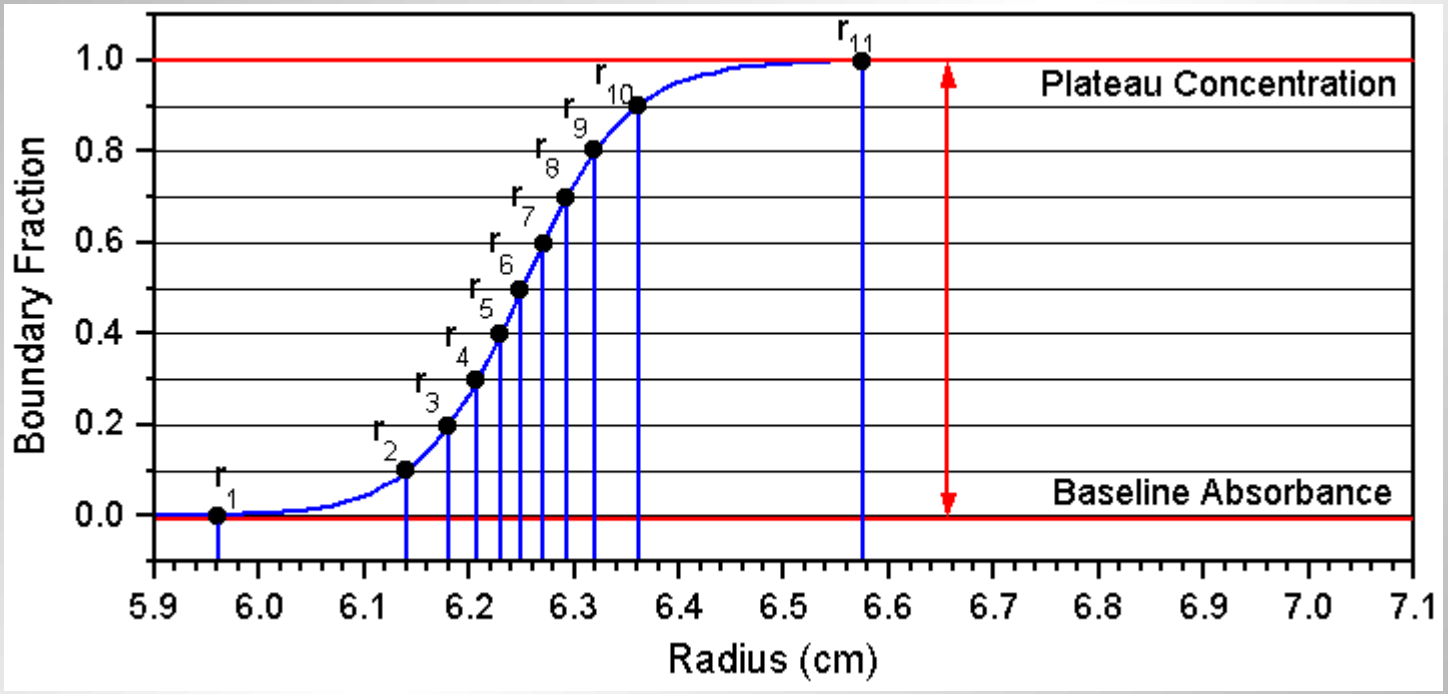
At infinity transport due to diffusion will be negligible compared to transport due to sedimentation - i.e., all components will separate out if the rotorspeed is fast enough.

...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

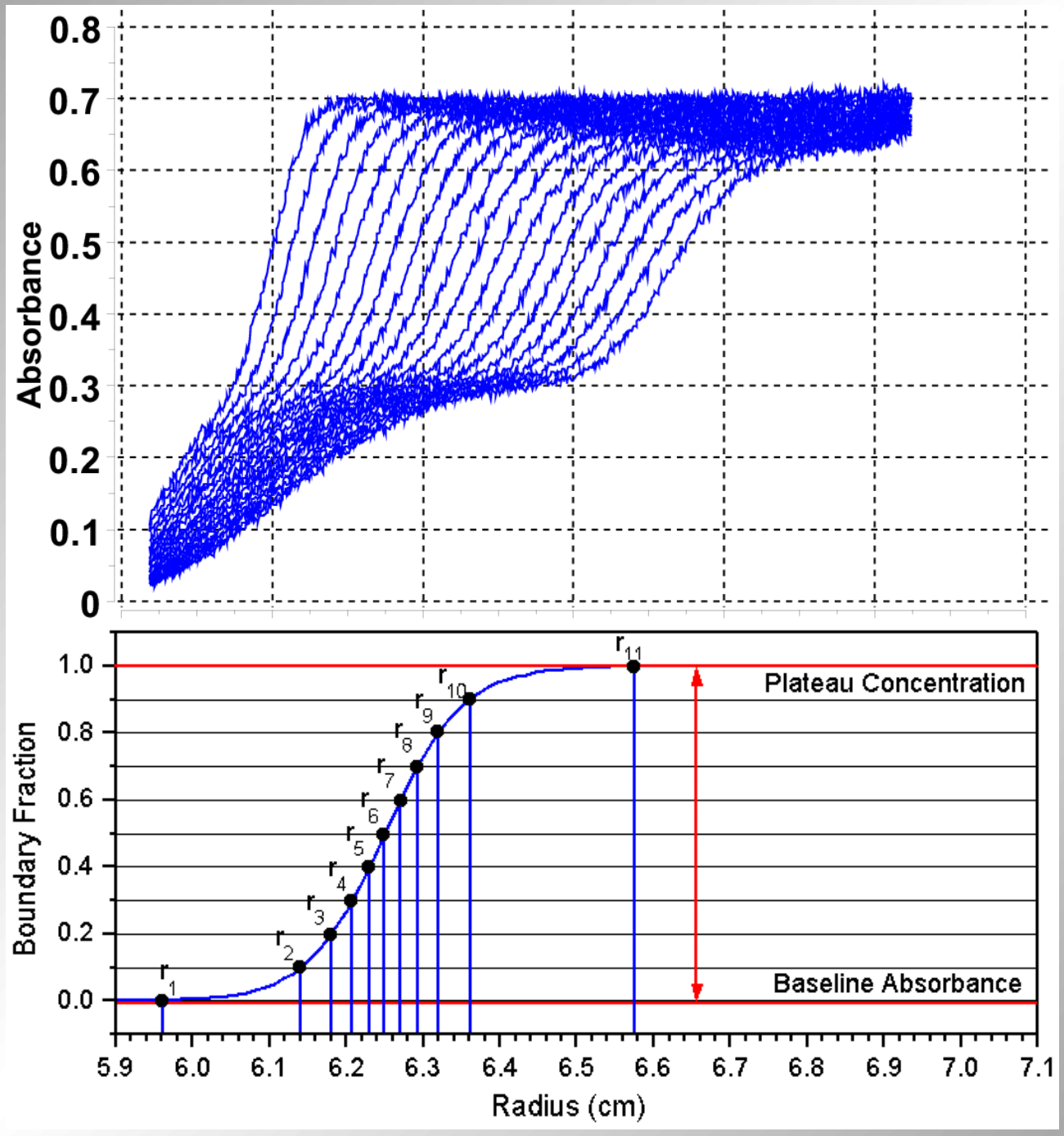
Calculation of apparent Sedimentation Coefficients:



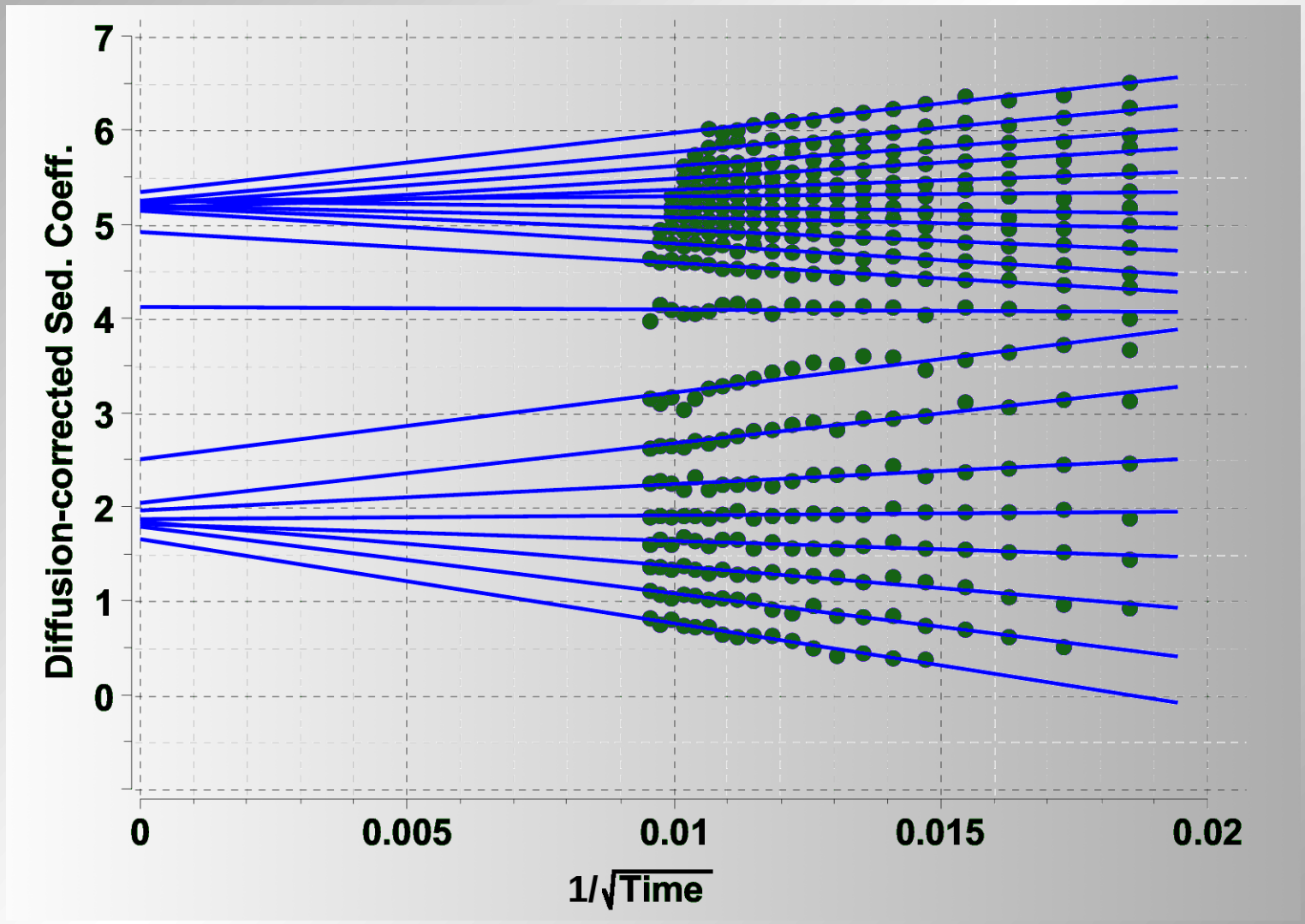
$$\frac{v}{\omega^2 r} = s$$

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

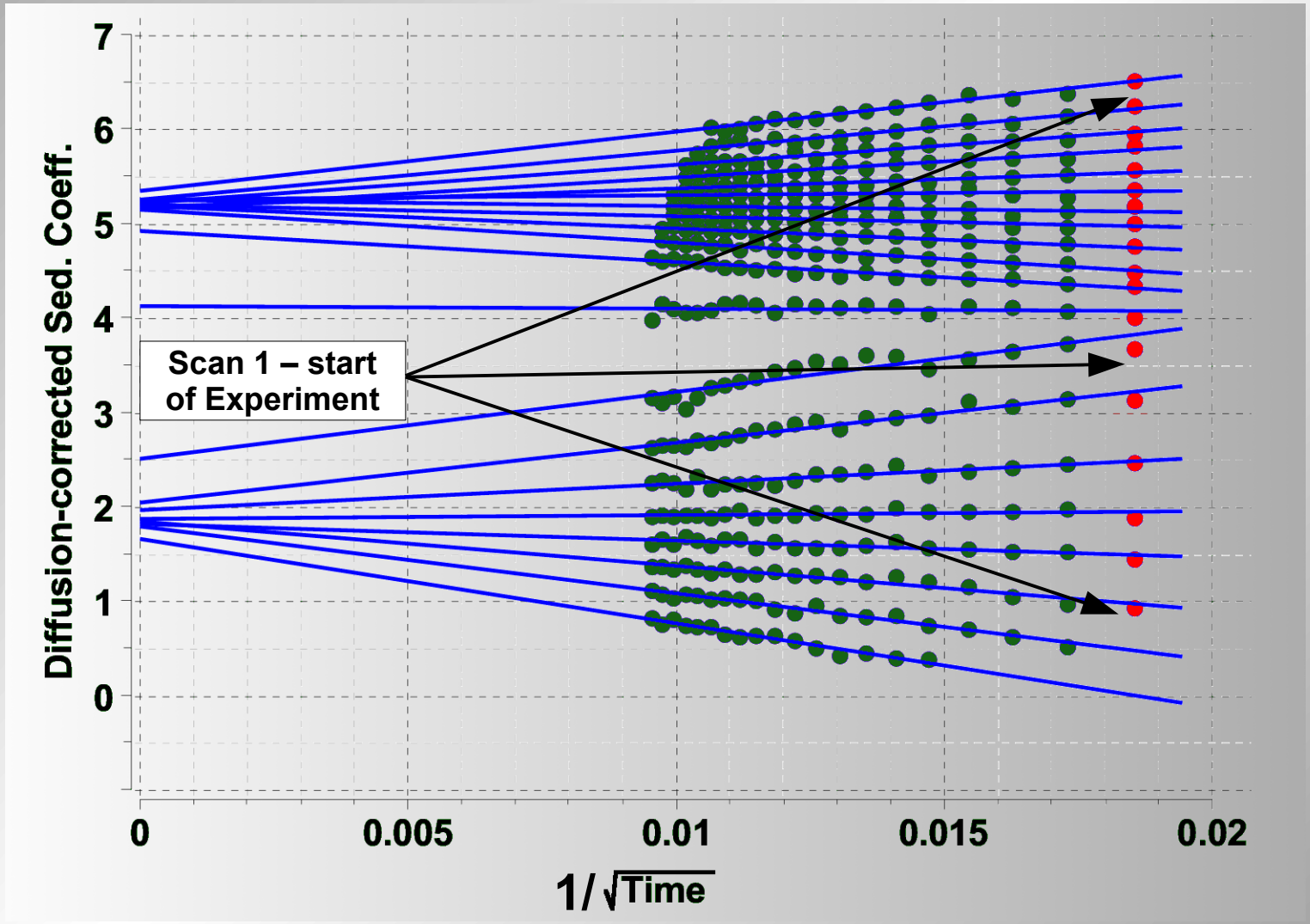
$$\hat{s}_b = \ln\left(\frac{r_b(t)}{r_a(t_0)}\right) \left[\omega^2 (t - t_0)\right]^{-1}$$


Absorbance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Boundary Fraction
1.0
0.8
0.6
0.4
0.2
0.0
r1
r2
r3
r4
r5
r6
r7
r8
r9
r10
r11
Plateau Concentration
Baseline Absorbance
5.9
6.0
6.1
6.2
6.3
6.4
6.5
6.6
6.7
6.8
6.9
7.0
7.1
Radius (cm)

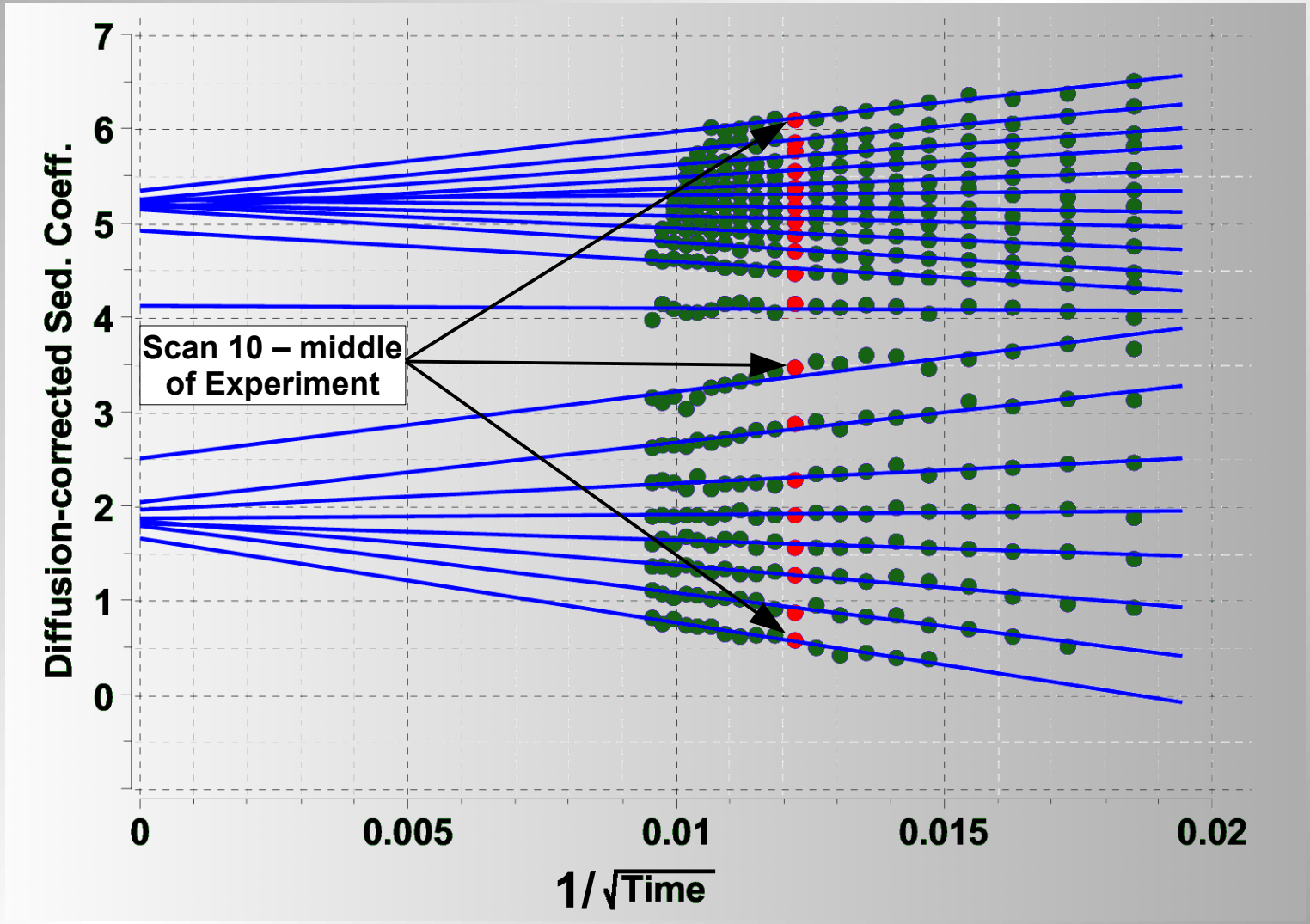
van Holde – Weischet Extrapolation Plot:



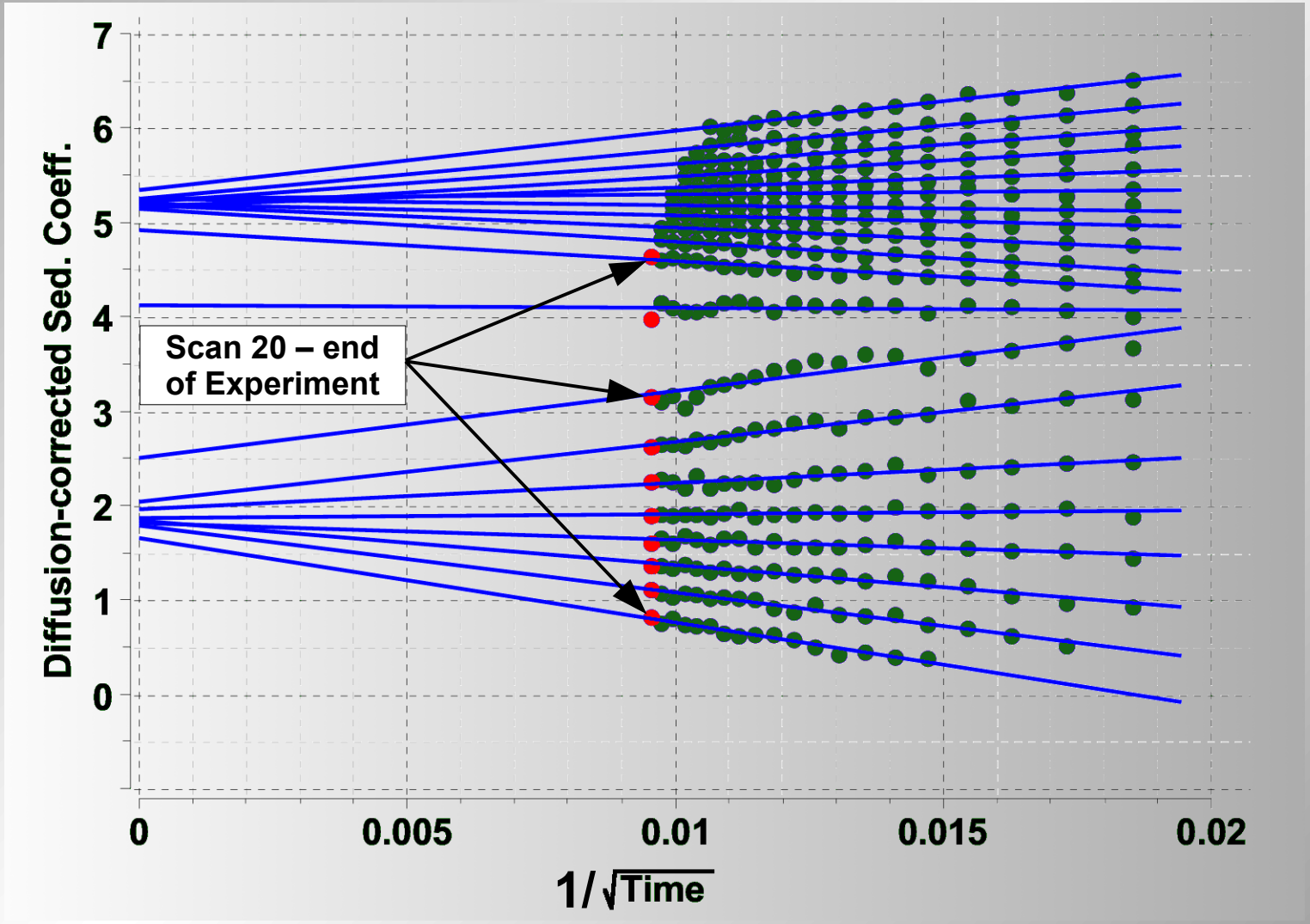
van Holde – Weischet Extrapolation Plot:



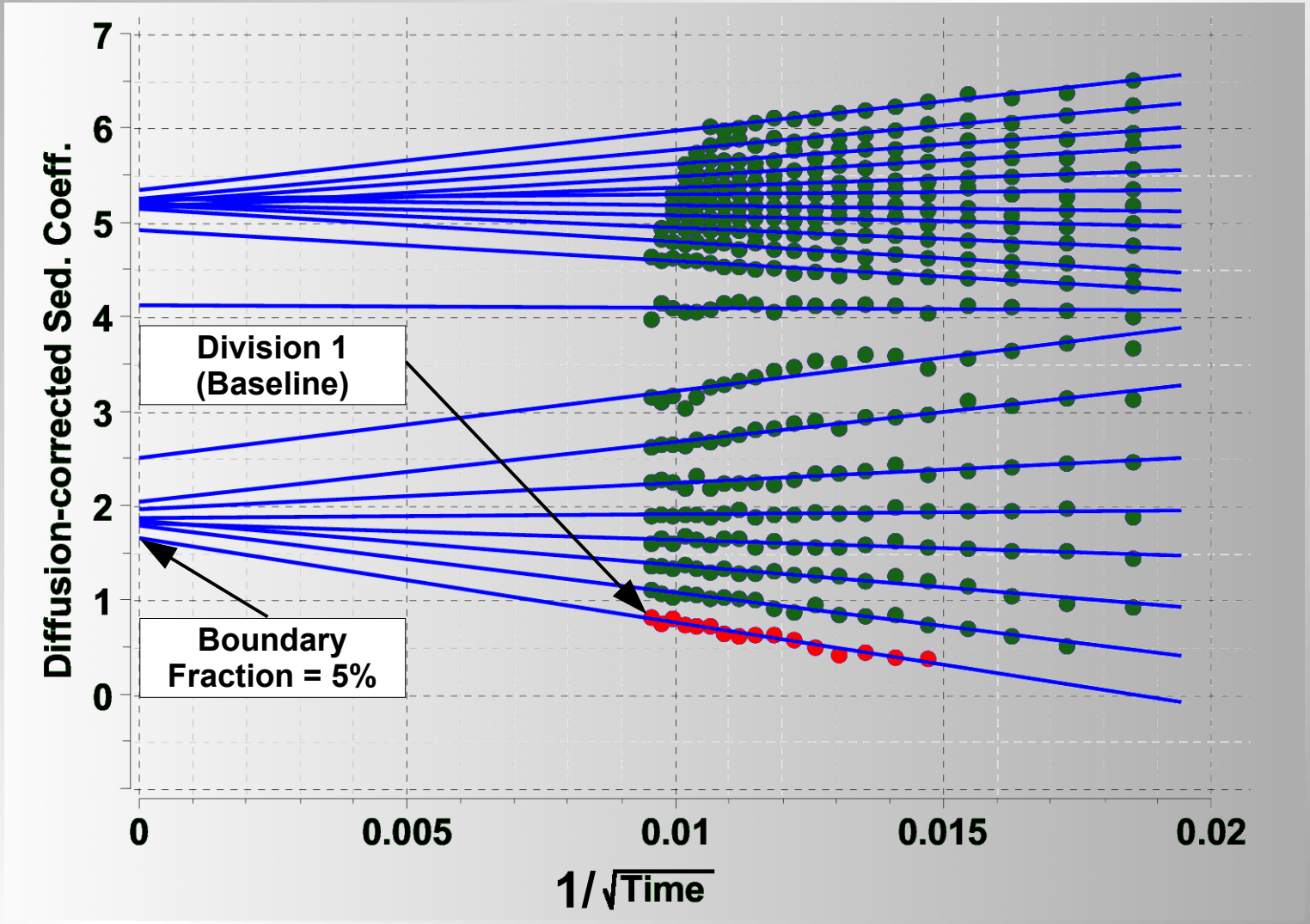
van Holde – Weischet Extrapolation Plot:



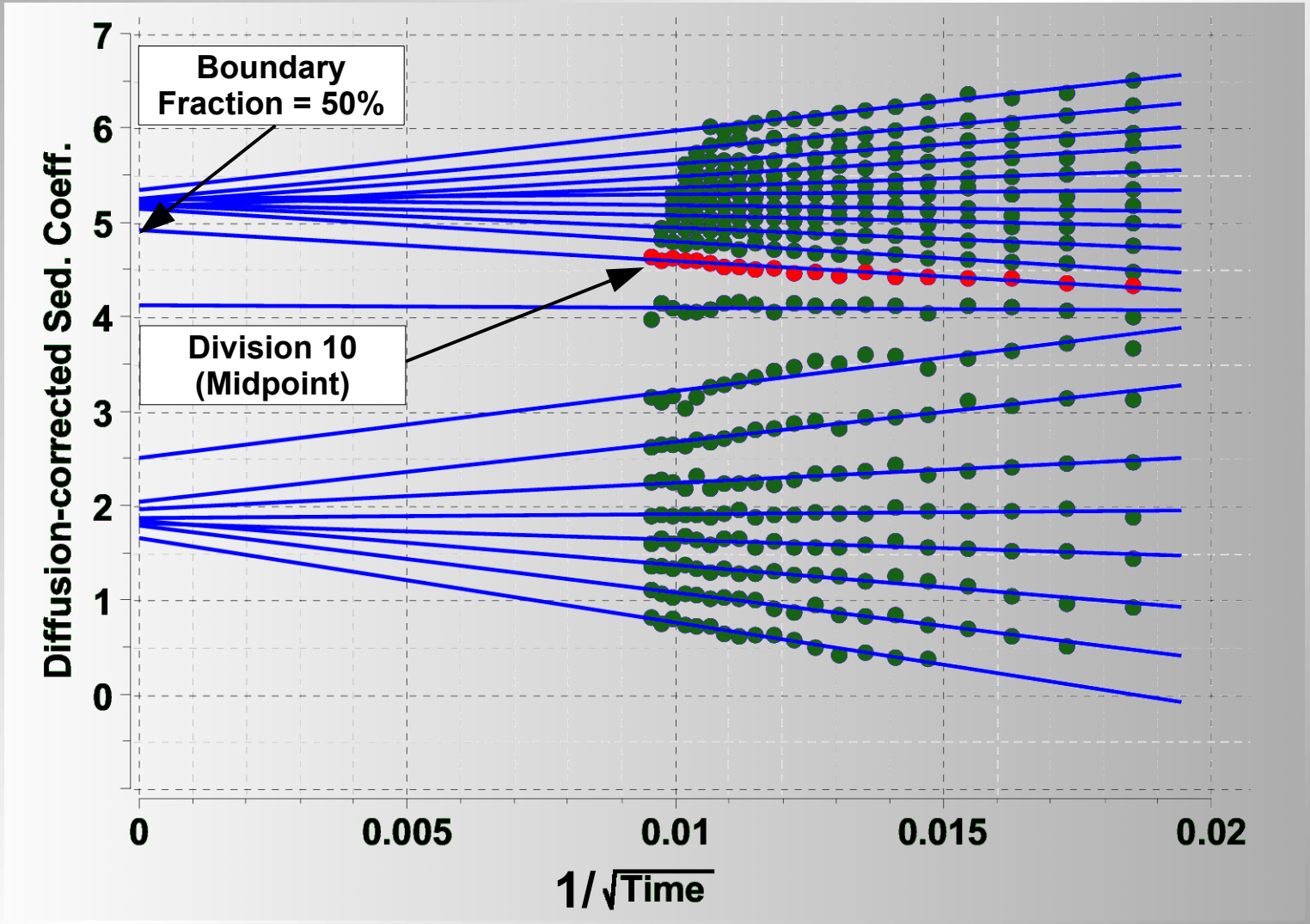
van Holde – Weischet Extrapolation Plot:



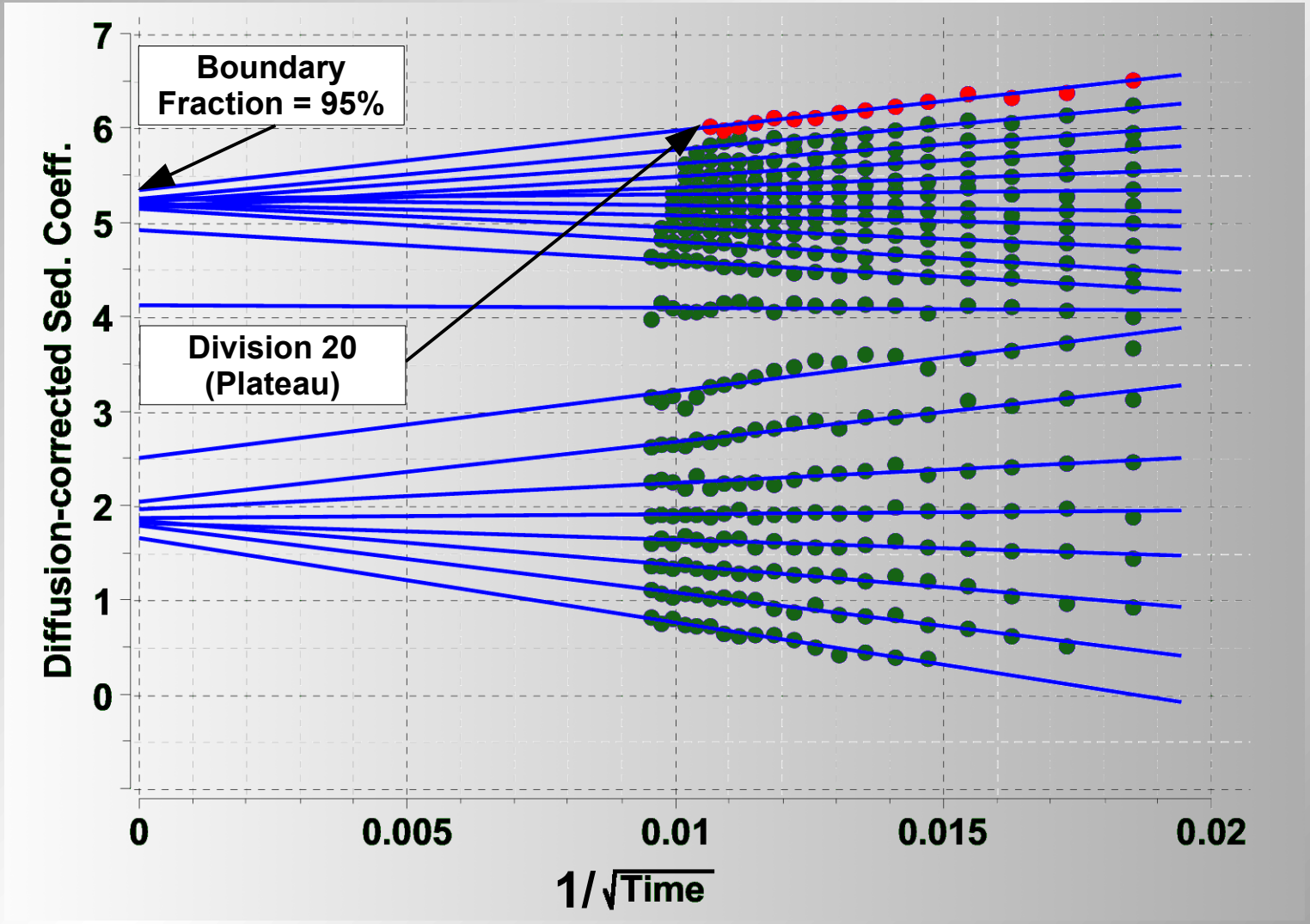
van Holde – Weischet Extrapolation Plot:



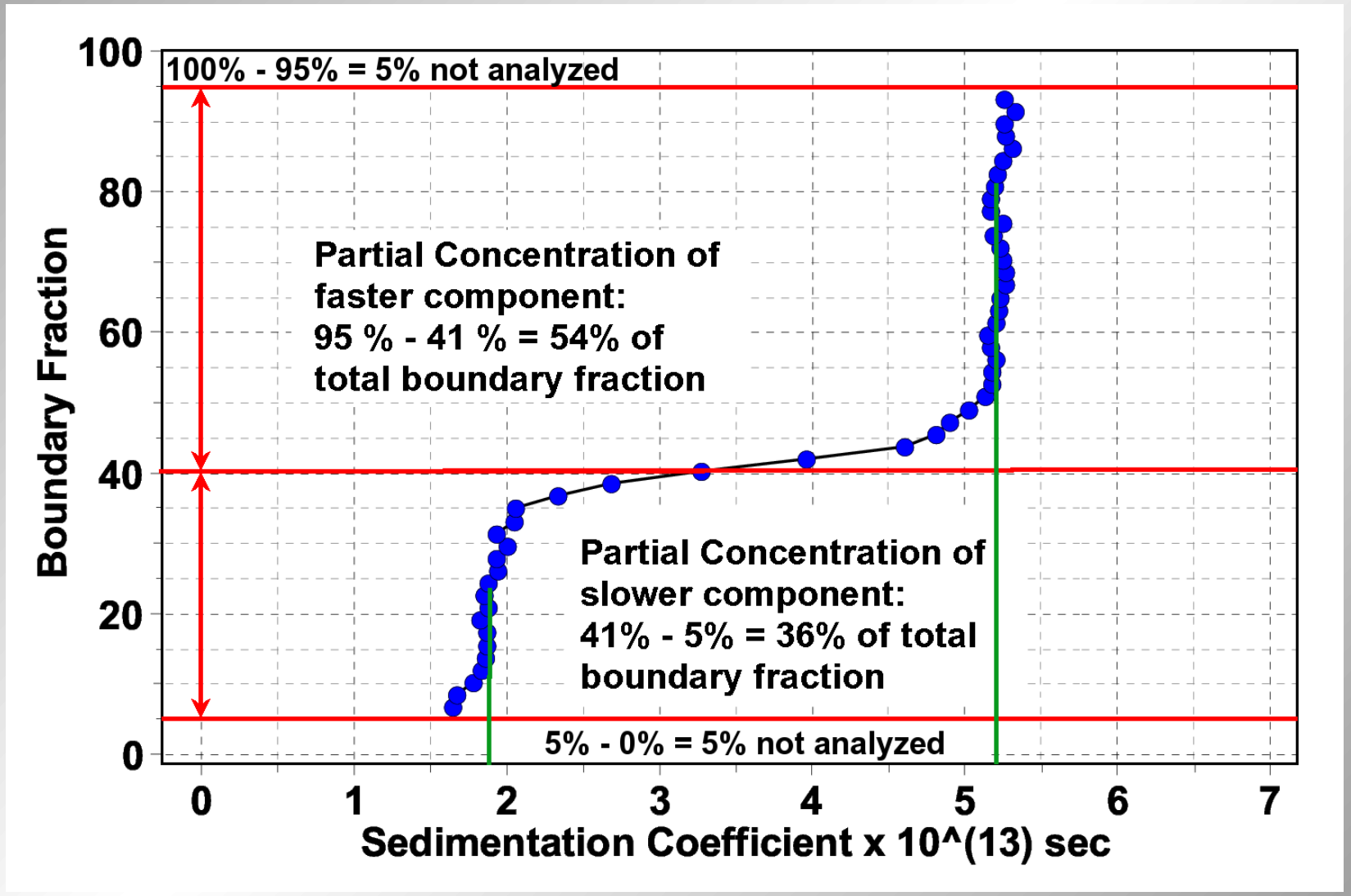
van Holde – Weischet Extrapolation Plot:



van Holde – Weischet Extrapolation Plot:



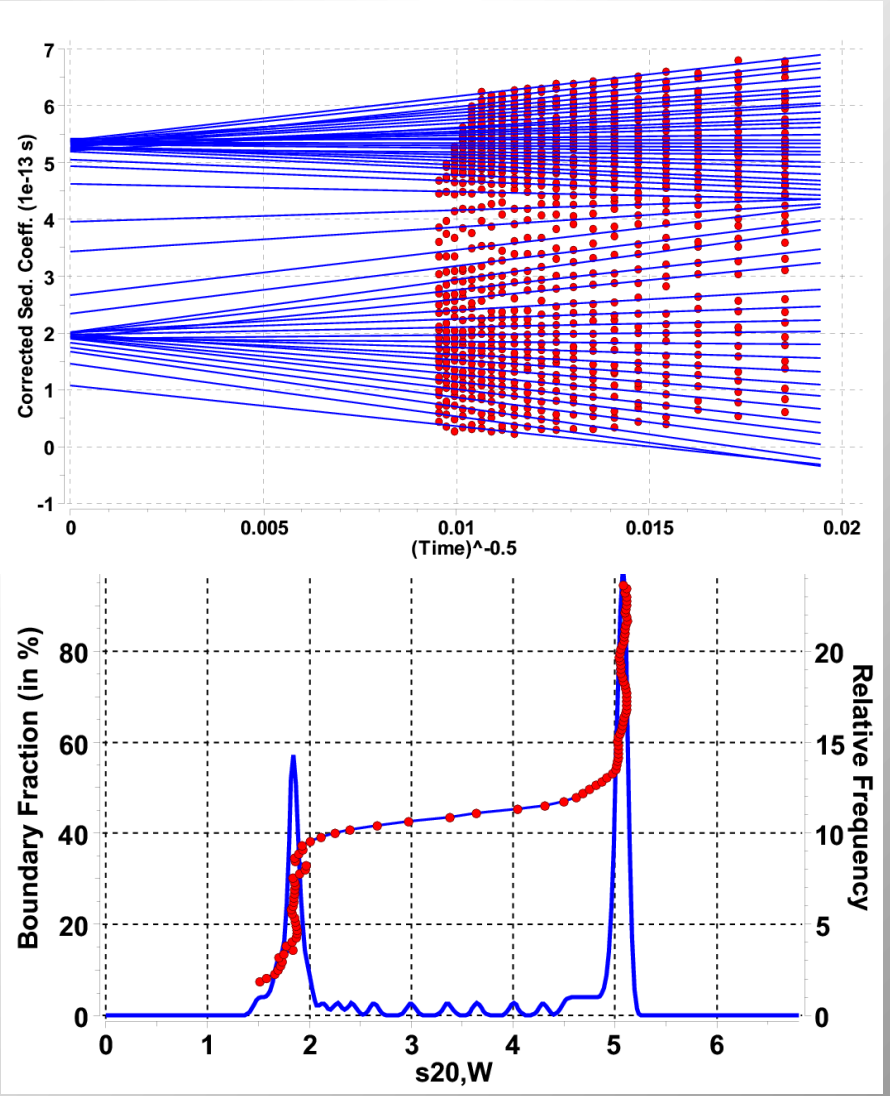
van Holde – Weischet Integral Distribution Plot (G(s)):

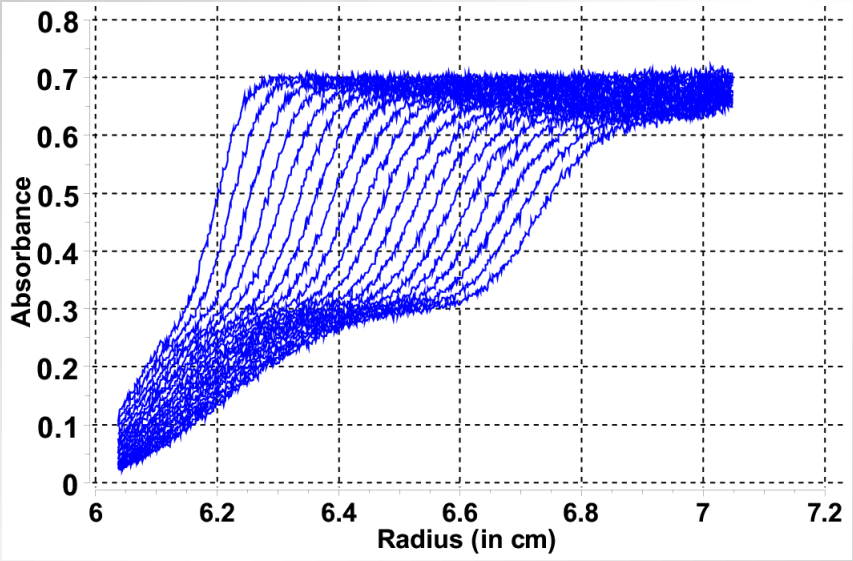


Enhanced van Holde – Weischet Method:

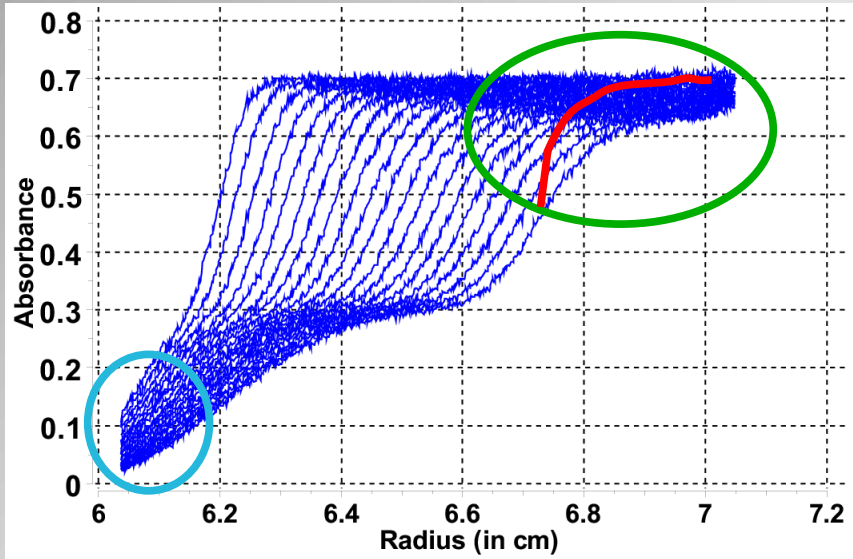
van Holde – Weischet method:

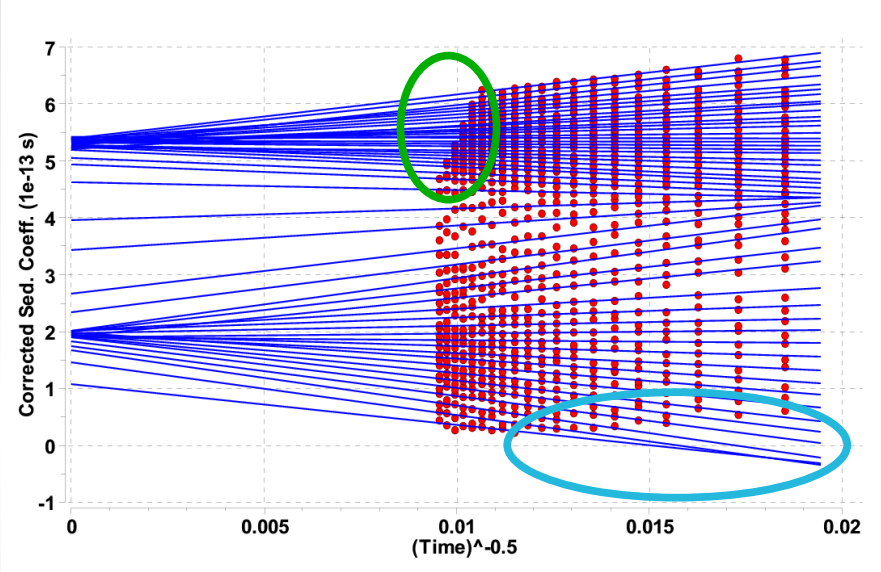
s_1: 5.35 x 10^{-13} (52 %)
s_2: 1.87 x 10^{-13} (39 %)





Enhanced van Holde – Weischet Method:





Green: Back diffusion distorts boundary – data points are excluded

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

DEMO