Sedimentation velocity

- *Sedimentation velocity*
  - *High rotor velocity*
  - *Long solution column*
  - *Hydrodynamic information*

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Sedimentation velocity analysis

- *Simple theory*
- *Effects of hydrodynamic and thermodynamic nonideality*
- *Different methods of analysis*

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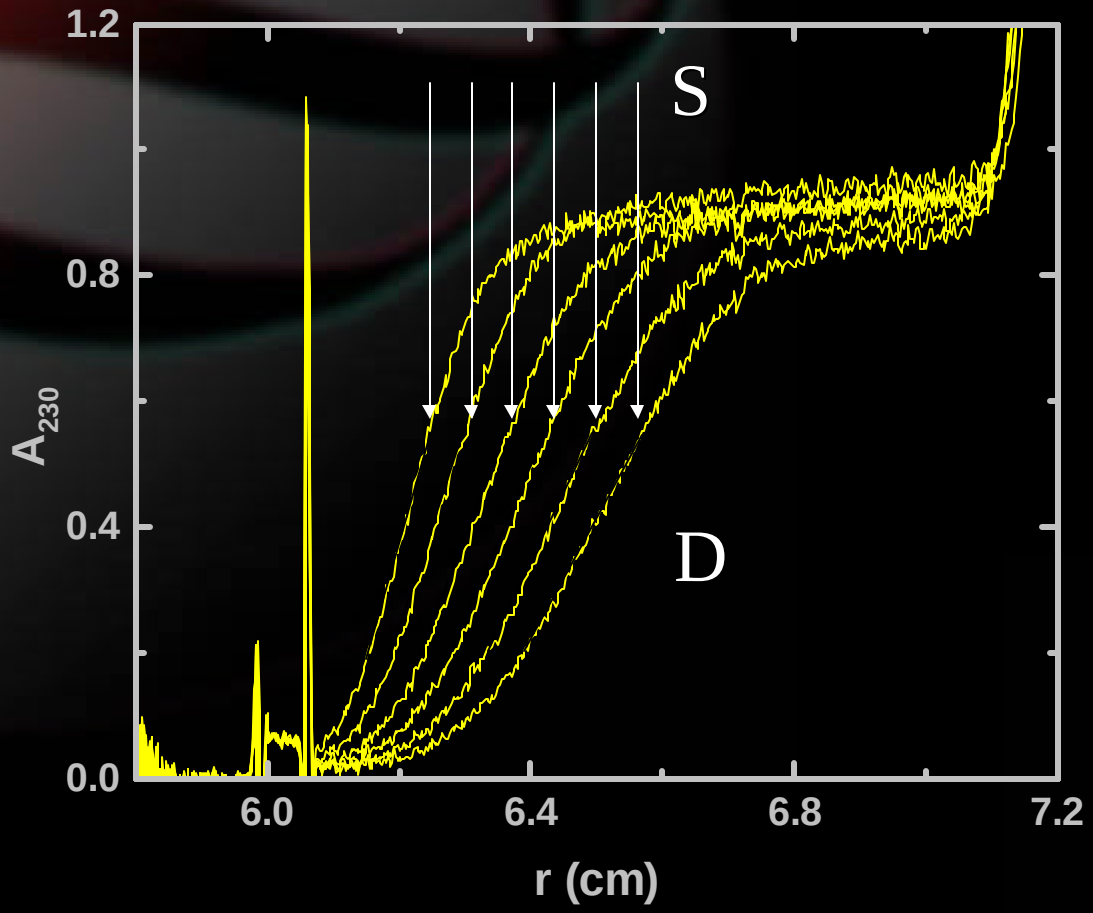
Sedimentation velocity



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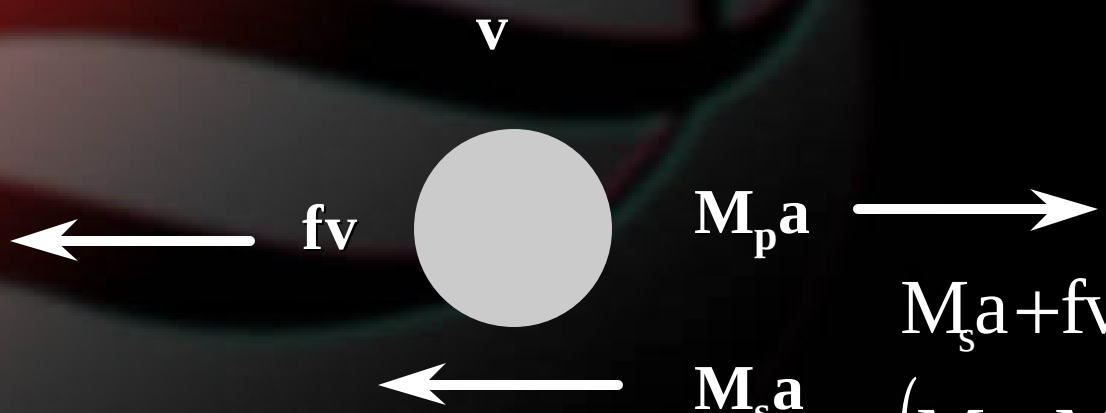
Simple theory: Analysis of forces



$$M_s a + fv = M_p a$$

$$(M_p - M_s)a = fv$$

$$\frac{(M_p - M_s)}{f} = \frac{v}{a}$$

$$\frac{M_p(1-\bar{v}\rho)}{f} = \frac{M_b}{f} = \frac{v}{a} \equiv s$$

$$\equiv s$$

$$a$$

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Velocity theory-continued

$$\frac{M_p\left(1-\bar{v}\rho\right)}{f} = \frac{M_b}{f} = \frac{\bar{v}}{a} \equiv s$$

Experimental definition

$$s = \frac{\bar{v}}{a} = \frac{\frac{dr}{dt}}{\omega^2 r} = \frac{d\ln r}{\omega^2 dt}$$

$$s = \frac{M_b}{f}$$

Molecular definition

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What do you want to know?

- *Number of species*
  - *Aggregate test*
- *Sedimentation coefficient*
  - *Shape/hydration*
- *Molecular weight*

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Preliminaries

- *What do you want to know?*
- *Sample handling*
- *Sample type*
- *Optical system*

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Sedimentation Velocity

Select Operating Conditions

Select rotor speed
Select temperature
Select optical system

General requirements

450 uL sample/cell
C depends on optical system

Select Method of Analysis

g(s) good for distributions
Transport equation (e.g.Svedberg) good for low M
Sw vs. [c] association constants
Van Holde-Weischet good for pauci-disperse systems

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Notes on Sedimentation velocity

- *Try to run 3 or more concentrations*
  - *From highest -> optical system limit*
- *For total unknown start run at low rotor speed (3000 rpm)*
  - *Run at multiple rotor speeds or use gravitational sweep*

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Preliminaries

- *What do you want to know?*
- *Sample handling*
- *Sample type*
- *Optical system*

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Sample type

Protein	**Polysaccharide**	**Nucleic Acid**
Choice of optics	Interference optics	Absorbance optics
$1\ A_{230\ or\ 280}$	C > 1 mg/ml	$1\ A_{260}$
1 mg/ml	Nonideality	Nonideality

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Preliminaries

- *What do you want to know?*
- *Sample handling*
- *Sample type*
- *Optical system*

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Choosing optical system

- ***Use absorbance if:***
  - *Need selectivity*
  - *Added sensitivity*
  - *Cannot dialyze sample*
- ***Use both:***
  - *Determine extinction coefficient*
  - *Test for sample purity*
  - *Extend concentration range*

- ***Use interference if:***
  - *Buffer absorbs*
  - *Sample does not absorb*
  - *Precision required*
  - *g(s)*
  - *Extinction coefficient varies*
  - *Short columns*

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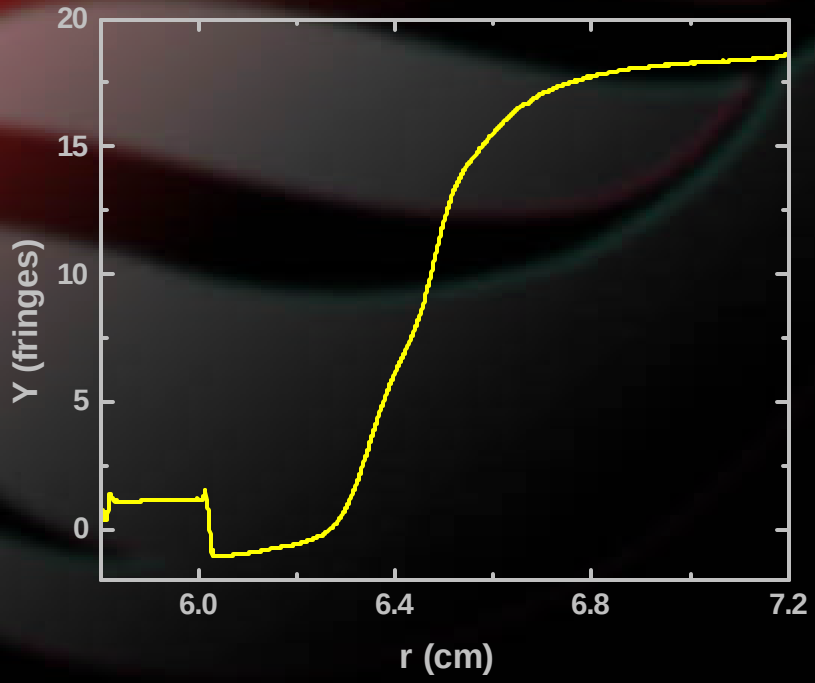
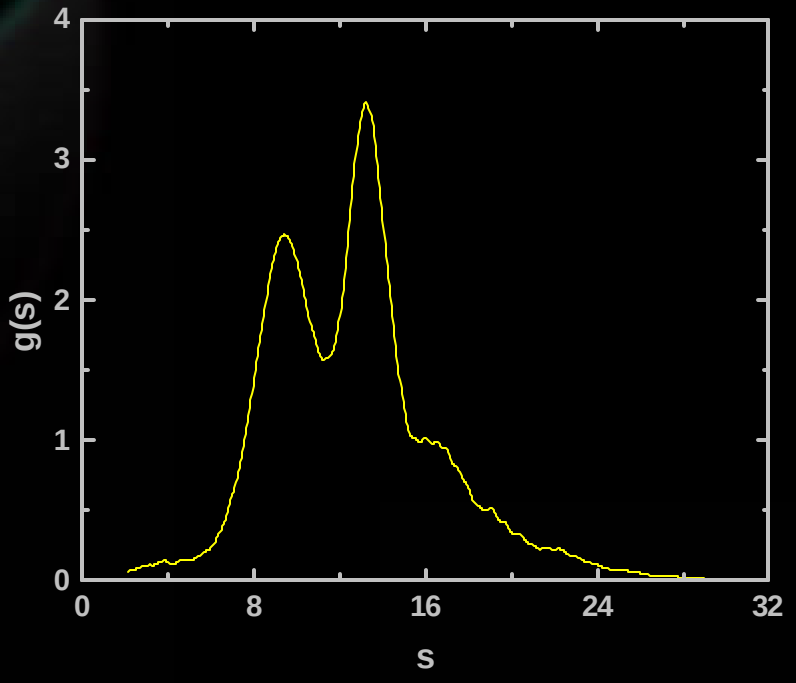
Number of species





Raw data → *Transformed*

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What do you want to know?

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  - *Shape/hydration*
- *Molecular weight*

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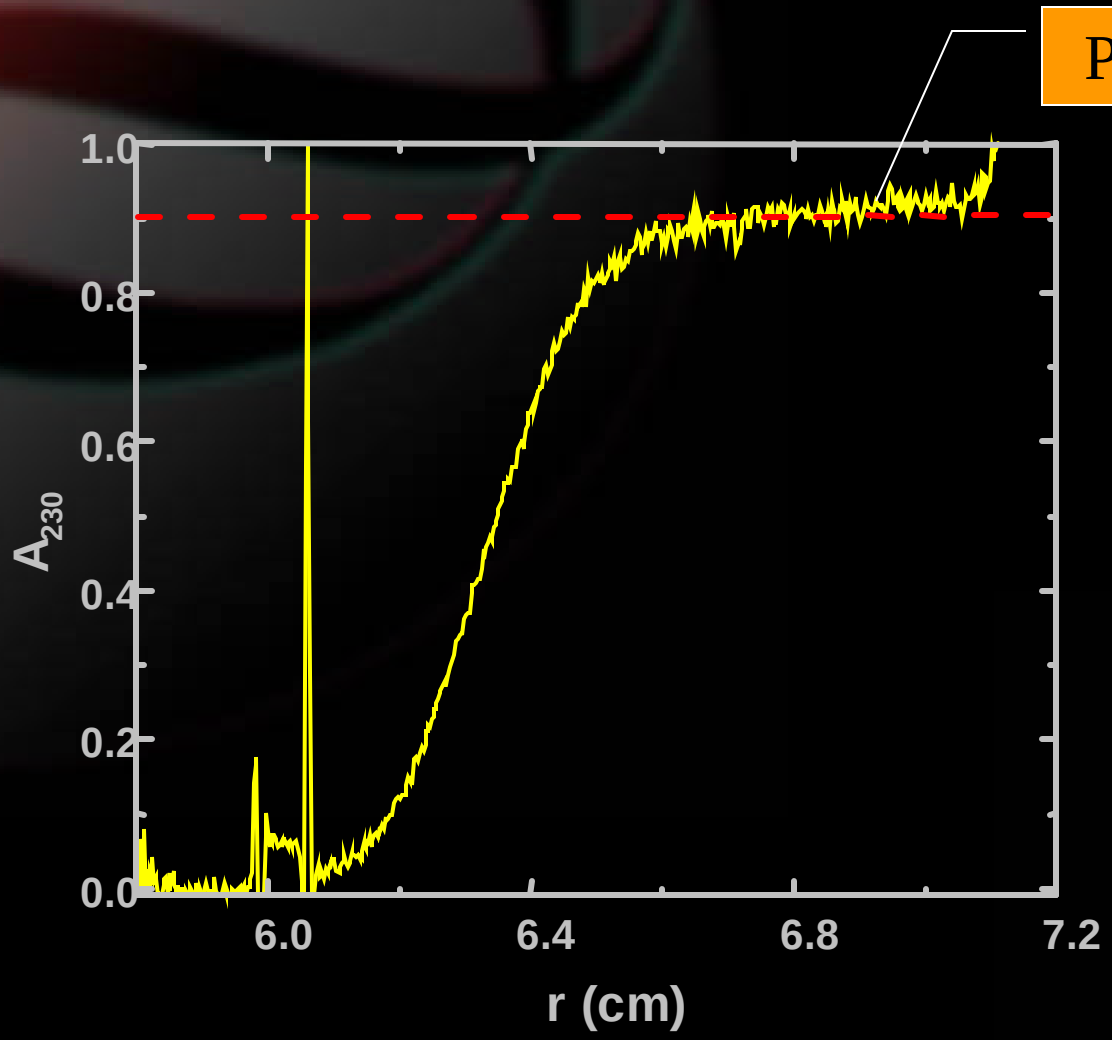
Aggregate test



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What do you want to know?

- *Number of species*
  - *Aggregate test*
- *Sedimentation coefficient*
  - *Shape/hydration*
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Determining s

- *Whole boundary methods*
  - *Transport equation*
  - *Fitting to simulation*
- *Time derivative*
- *van Holde Weischet*

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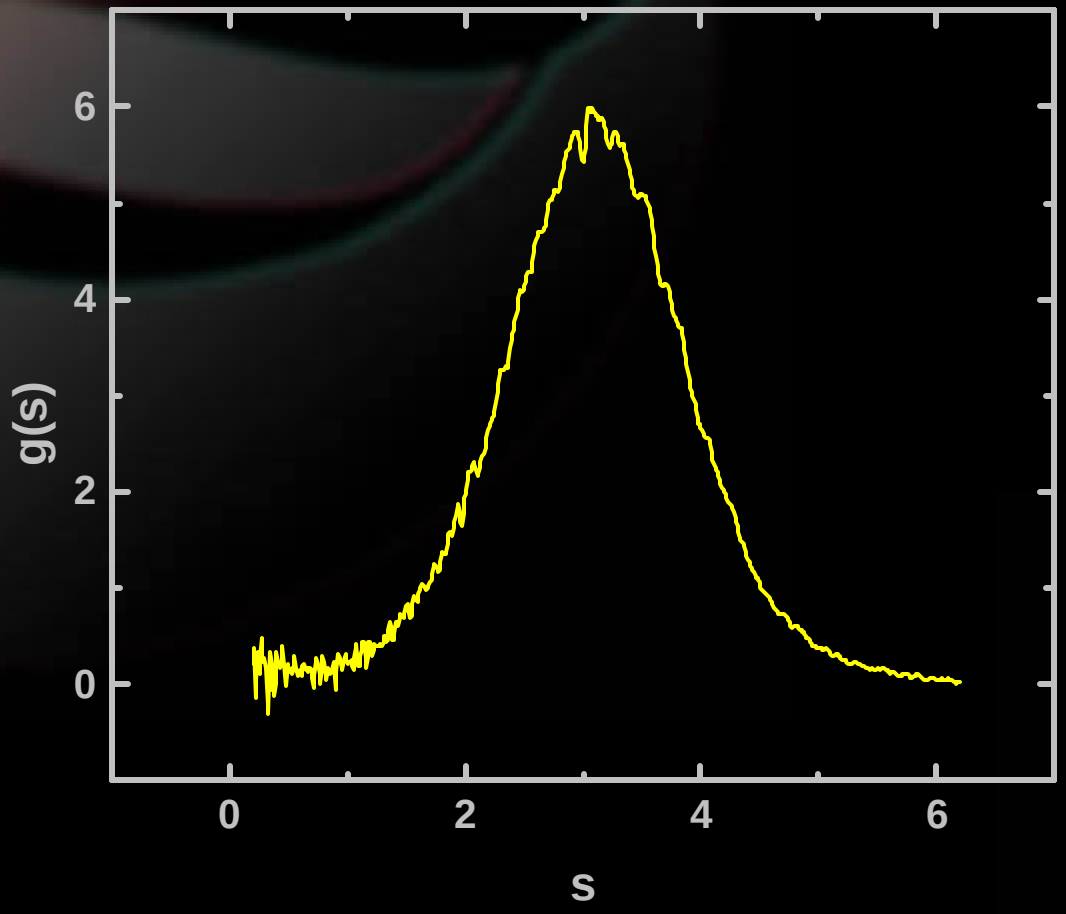
Determining s

Time derivative method



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What do you want to know?

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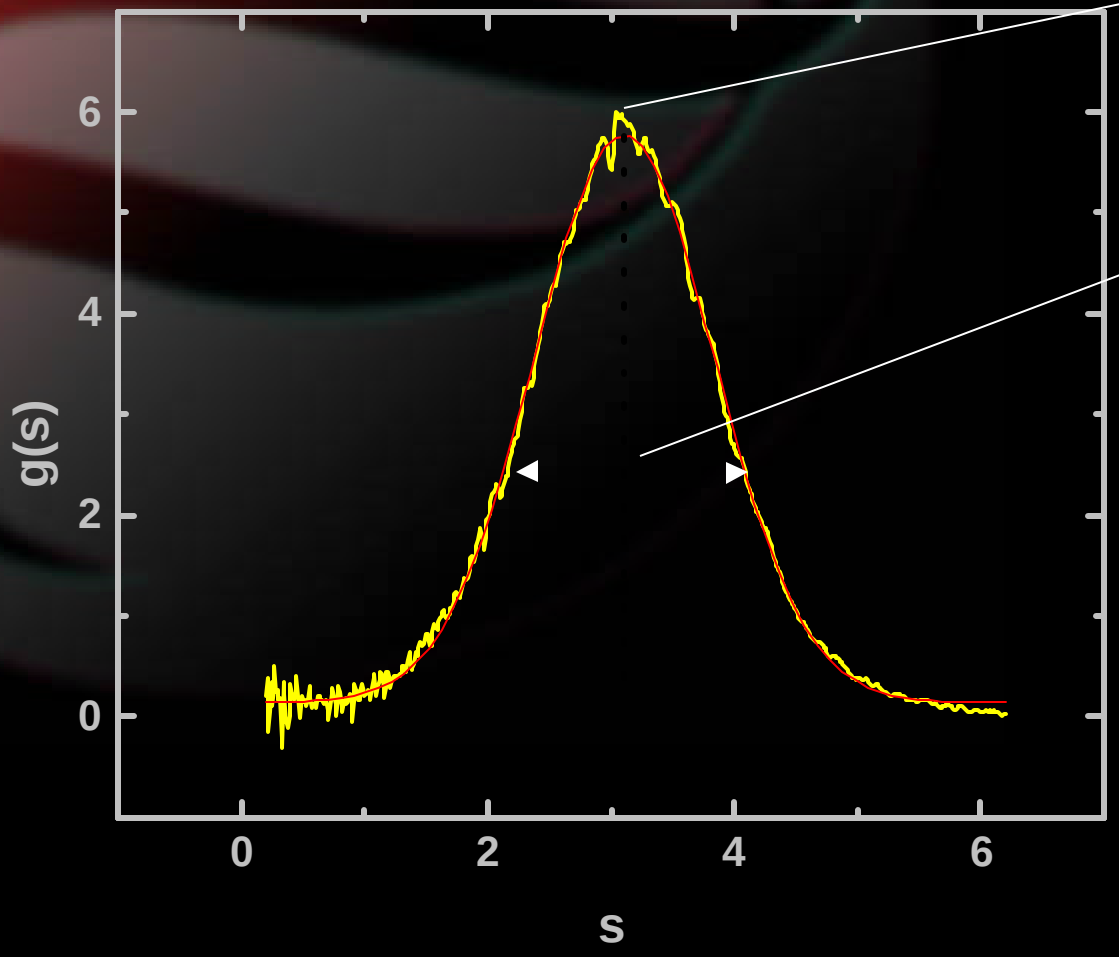
Molecular weight



s = 3.11 S

D = 6.23 F

$$M = \frac{s}{D} = \frac{M_b}{RT}$$

M = 44,000 g/mol

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General considerations

- *Correcting for buoyancy*
  - *Determining density*
  - *Partial specific volume*
- *Correcting for viscosity*

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Useful references

Books:

Analytical Ultracentrifugation in Biochemistry and Polymer Science. (1992) S.E. Harding, A.J. Rowe, and J.C. Horton, eds. Royal Society of Chemistry, Cambridge.

Modern Analytical Ultracentrifugation. (1995) T.M. Schuster and T.M. Laue, eds. Birkhauser, Boston.
Two fairly recent books devoted entirely to this field

K.E. van Holde, Physical Biochemistry. (1985) Prentice Hall, Englewood Cliffs, New Jersey.
Good introductory text for general theory of sedimentation, frictional coefficients, diffusion, and other hydrodynamic analysis

Freifelder, D. (1982). Physical Biochemistry: Applications to biochemistry and molecular biology. W.H. Freeman, New York.
Regarded as a good introductory text that is strong on centrifugation methods

van Holde, K.E., W.C. Johnson, Jr., and P.S. Ho. .(1998). Principles of physical biochemistry. Prentice-Hall, Upper Saddle River.

Cantor, C.R. and Schimmel, P.R. (1980). Biophysical chemistry. Part II: Techniques for the study of biological structure and function. W.H. Freeman, San Francisco.
These two are more advanced texts with good coverage of centrifugation methods

Special Journal Issue:

Chemtracts Biochemistry and Molecular Biology, vol. 11 no. 13 (pp. 933-1004), December 1998 (Jeffrey C. Hansen, Guest Editor)
Several review articles and condensation commentaries on current research

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Useful references

Review Articles:

Stafford, W.F. III. (1997). Sedimentation velocity spins a new weave for an old fabric. *Curr. Opin. Biotechnol*. 8, 14-24.

Laue, T.M. (1995). Sedimentation equilibrium as thermodynamic tool. Methods Enzymol. 259, 427-452.

Laue, T.M. Stafford, W.F., III (1999). Modern Applications of Analytical Ultracentrifugation. *Annu. Rev. Biophys. Biomol. Struct.* 28, *75-100.*

Articles:

Laue, T.M., Shah, B.D., Ridgeway, T.M., and Pelletier, S.L. (1992). Computer-aided interpretation of analytical sedimentation data for proteins. In: Analytical ultracentrifugation in biochemistry and polymer science. S.E. Harding, A.J. Rowe, and J.C. Horton, eds. Royal Society of Chemistry, Cambridge, pp. 90-125.
Procedures for calculating partial specific volume, density, sedimentation coefficient (corrected for water @ 20°C and extrapolated to zero concentration) hydration, frictional rations, ellipsoidal shapes, etc; basis for SEDNTERP software (but note that the formulae and tables contain a number of typographical errors that were corrected in SEDNTERP – see the SEDNTERP Help file for corrected formulas)

Stafford, W.F., III. (1992). Boundary analysis in sedimentaiton transport experiments: A procedure for obtaining sedimentation coefficient distributions using the time derivative of the concentration profile.
Anal. Biochem. 203, 295-301.
Initial publication describing the dc/dt method

Johnson, M.L. and Frasier, S.G. (1985). Nonlinear least-squares analysis. *Methods Enzymol*. 117:301-342.
Good overview of the fitting of experimental data

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