Grid of Run Conditions in SE and SV using Different Optical Systems

	absorbance optics (ABS)	interference optics (IF)
selectivity:	selective detection (e.g., in the presence of non-absorbing components)	not selective: sensitive to all solution components (including buffer salts)
linearity and concentration range:	linear to ~ 1.5 OD, a large concentration range may be achieved by the use of multiple wavelengths	unlimited linearity, 10^4-fold concentration range
buffer considerations	buffer cannot contain large amounts of DTT, TRIS, HEPES, other absorbing components for use in far UV (e.g. 230 nm)	advantageous in the presence of strongly absorbing components (e.g., nucleotides, nucleic acids), **but** requires an exact chemical match of reference buffer volume and composition (through dialysis or gel filtration)
baselines	small time-invariant (TI) radial baseline profile[9]	generates significant time-invariant radial-dependent (TI) and radial-invariant time-dependent (RI) baselines, unproblematic in SV, but not trivial in SE
maximum signal/noise ratio	~ 300	> 3000
data acquisition	~ minutes/scan, may be limiting rotor speed in SV, depends on scanning mode	few seconds/scan
windows	quartz windows	sapphire windows
conditions for velocity sedimentation (SV) high speed, single speed typical sample requirements: stability for 3 hours [3] several cells with a range of loading concentrations; for example, stock solution with serial dilutions [4]	volume 400 microliters (as low as 150 microliters) rotor speed high: 40 – 60,000 rpm[1] optimal loading absorbance: 0.5 – 1.3 OD typical minimal desirable loading absorbance ~ 0.05 OD[5] requires thorough temperature equilibration controlled start from 0 rpm[6] constant baseline usually with small radial-dependent features[7] scan settings for fast scans (continuous mode, 0.003 cm radial increment)	volume 400 microliters (as low as 150 microliters), sample/reference precisely matched rotor speed usually 50 – 60,000 rpm[1] optimal loading concentration: > 0.1 mg/ml (> 0.3 fringes) typical minimal desirable loading concentration: ~ 0.05 mg/ml[5] requires thorough temperature equilibration controlled start from 0 rpm, may need pre-adjustment of optics[6] generates radial baseline profile and radial-invariant offsets in each scan, which can be computationally eliminated after modeling[8]
conditions for equilibrium sedimentation (SE) low speed, multiple speeds typical sample requirements: stability for 2 – 5 days [3] use gel-filtration to remove small Mw contaminants several cells with a range of loading concentrations; for example, stock solution with serial dilutions [4]	volume 180 microliters sample and 190 microliters reference (150 microliters sample for Mw > 100 kDa) two or three rotor speeds, lowest at c(b)/c(m) ~ 3, highest generating meniscus depletion c(m) ~ 0 [2] optimal loading absorbance: 0.2 to 0.5 OD[4] typically scan at multiple wavelengths: 280 nm, 230 nm, 250 nm usually no prior temperature equilibration required constant baseline usually with small radial-dependent features[9] scan settings for slow, precise scans (step mode, 0.001 cm radial increment)	volume 180 microliters, sample/reference precisely matched (150 microliters sample for Mw > 100 kDa) two or three rotor speeds, lowest at c(b)/c(m) ~ 3, highest meniscus depletion c(m) ~ 0; can tolerate steeper gradients leading to higher sample concentration[2] optimal loading concentration: > 0.1 mg/ml (> 0.3 fringes) require 'aging' of cell assemblies, water blanks usually no prior temperature equilibration required radial baseline profile and radial-invariant offsets, requires water blanks or TI noise elimination from global analysis of different rotor speeds[10]

(1) Choice of rotor speed: generally as fast as possible but dependent on protein size and optical system; the acquisition of at least 5 – 10 scans during the complete sedimentation process is desirable in SV; for molar mass determination slightly lower rotor speeds may be desirable (2) The ratio of concentration at the bottom relative to the meniscus, c(b)/c(m), can be theoretically predicted by simulating the approach to equilibrium with SEDFIT. This also provides a lower limit for the time to attain equilibrium and allows assessing the concentration profiles and gradients in equilibrium; (3) Stability may depend on temperature – SV and SE can be run at 4 °C; sedimentation equilibrium can be shortened by reducing column volume. [4] Concentration choice will depend on the purpose of the experiment. [5] Lower values are possible, but with deteriorating level of detail due to limiting signal/noise ratio. [6] Controlled start from 0 rpm excludes the use of a low-speed (typically 3,000 rpm) phase for adjustment of optical and scan settings or temperature equilibration prior to high-speed acceleration. [7] Ideally exhibits a constant flat baseline, but ordinarily shows some time-invariant features from imperfections in the windows, which can be computationally eliminated after data analysis [8] Computational elimination is

usually unproblematic in conjunction with modeling the time-course of sedimentation. [9] Baseline may shift at different wavelengths or when using buffer components with unstable absorbance, such as DTT (may by substituted by TCEP). Radial-dependent features may be eliminated computationally in the global analysis of equilibrium at a sufficient range of rotor speeds. [10] Computational treatment of TI noise in sedimentation equilibrium depends on the use of a sufficiently large range of rotor speeds, but may be improved by global multi-signal analysis in conjunction with absorbance data.