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

<table>
<thead>
<tr>
<th>selectivity:</th>
<th>absorbance optics (ABS)</th>
<th>interference optics (IF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>selective detection (e.g., in the presence of non-absorbing components)</td>
<td>not selective: sensitive to all solution components (including buffer salts)</td>
</tr>
<tr>
<td>linearity and concentration range:</td>
<td>linear to ~1.5 OD, a large concentration range may be achieved by the use of multiple wavelengths</td>
<td>unlimited linearity, 10^4-fold concentration range</td>
</tr>
<tr>
<td>buffer considerations</td>
<td>buffer cannot contain large amounts of DTT, TRIS, HEPES, other absorbing components for use in far UV (e.g. 230 nm)</td>
<td>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)</td>
</tr>
<tr>
<td>baselines</td>
<td>small time-invariant (TI) radial baseline profile</td>
<td>generates significant time-invariant radial-dependent (TI) and radial-invariant time-dependent (RI) baselines, unproblematic in SV, but not trivial in SE</td>
</tr>
<tr>
<td>maximum signal/noise ratio</td>
<td>~ 300</td>
<td>&gt; 3000</td>
</tr>
<tr>
<td>data acquisition</td>
<td>~ minutes/scan, may be limiting rotor speed in SV, depends on scanning mode</td>
<td>few seconds/scan</td>
</tr>
<tr>
<td>windows</td>
<td>quartz windows</td>
<td>sapphire windows</td>
</tr>
<tr>
<td>volume 400 microliters (as low as 150 microliters)</td>
<td>volume 400 microliters (as low as 150 microliters), sample/reference precisely matched</td>
<td></td>
</tr>
<tr>
<td>rotor speed high: 40 – 60,000 rpm</td>
<td>rotor speed usually 50 – 60,000 rpm</td>
<td></td>
</tr>
<tr>
<td>optimal loading absorbance: 0.5 – 1.3 OD</td>
<td>optimal loading concentration: &gt; 0.1 mg/ml (&gt; 0.3 fringes)</td>
<td></td>
</tr>
<tr>
<td>typical minimal desirable loading absorbance ~ 0.05 OD</td>
<td>typical minimal desirable loading concentration: ~ 0.05 mg/ml</td>
<td></td>
</tr>
<tr>
<td>constant baseline usually with small radial-dependent features</td>
<td>requires thorough temperature equilibration</td>
<td></td>
</tr>
<tr>
<td>scan settings for fast scans (continuous mode, 0.003 cm radial increment)</td>
<td>controlled start from 0 rpm, may need pre-adjustment of optics</td>
<td></td>
</tr>
</tbody>
</table>

### Conditions for Velocity Sedimentation (SV)

#### High speed, single speed

- Typical sample requirements:
  - Stability for 3 hours
  - Several cells with a range of loading concentrations; for example, stock solution with serial dilutions

#### Low speed, multiple speeds

- Typical sample requirements:
  - Stability for 2 – 5 days
  - Use gel-filtration to remove small Mw contaminants
  - Several cells with a range of loading concentrations; for example, stock solution with serial dilutions

### Conditions for Equilibrium Sedimentation (SE)

#### Low speed, multiple speeds

- Typical sample requirements:
  - Stability for 2 – 5 days
  - Use gel-filtration to remove small Mw contaminants
  - Several cells with a range of loading concentrations; for example, stock solution with serial dilutions

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(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. 

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. 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.