# Some Buffer Properties and Their Relation to AUC Detection

Note: Standard phosphate buffered saline works well with regard to all necessary considerations in both optical systems.

| | detection | details and recommendation |
|---|---|---|
| **ionic strength**<br>you need sufficient ions to screen protein charges and prevent long-range electrostatic interactions from affecting protein sedimentation | both<br>IF and ABS | *always use > 10 mM NaCl or other salt* |
| **absorption**<br>for ABS detection, the absorption of the buffer at the detection wavelength should not exceed 0.2 OD<br><br>*select appropriate detection system if in doubt, measure the absorption spectrum of the buffer* | ABS | *always use > 10 mM NaCl or other salt*<br>HEPES and TRIS buffers, as well as EDTA and EGTA, absorb in the far UV. At low concentrations they can be tolerable for 280 nm ABS detection (e.g., 10 mM TRIS), but this may not permit the 230 nm detection.<br>β-mercaptoethanol or DTT at low mM concentrations are compatible with IF and ABS detection at 280 nm, but they will generate an absorbance signal which may change with time. (For the ABS detection system, this requires a radial-invariant baseline ('RI-noise') to be considered in the data analysis)<br>the presence of nucleotides at > 50 μM usually prohibits the use of the ABS system |
| **refractive index**<br>the IF system detects the sedimentation of buffer salts (and any other buffer component) and will be sensitive to even very small mismatches in the concentration between the sample and reference buffer | IF | *use size-exclusion chromatography or equilibrium dialysis to change buffer, if necessary*<br>high concentrations > 1M of buffer components with large refractive index signal, e.g. guanidine hydrochloride, CsCl, glycerol, and others, are very difficult to match optically between sample and reference. In this case, either use the ABS system, or use pure $H_2O$ as a reference buffer and explicitly treat the buffer as a sedimenting component in the data analysis<br>if detergents are required, if possible, non-absorbing detergents in conjunction with the ABS system is usually advantageous over the IF system |
| **density**<br>buffer components that raise the density of the solution may create density gradients at high rotor speed | both | *glycerol or sucrose should be absent, if possible*<br>solutions of higher density decrease the sedimentation velocity and increase the time required to attain SE<br>self-forming density gradients may be a concern for mixed solvents and high concentrations of buffer components (such as CsCl or sucrose) |
| **viscosity**<br>buffer components that lead to increased viscosity extend the experimental time | both | if glycerol cannot be avoided, multiply the time-intervals for establishing SE of 6 h with the relative viscosity of the solution.<br>for SV experiments, use larger time intervals between scans and let the experiment continue until the protein is depleted from the solution column. |
| **preferential hydration**<br>when using buffer components that significantly increase the solvent density, preferential binding or exclusion of water from the protein solvation shell can lead to changes in the protein buoyancy and in its sedimentation behavior | both | this is usually not a concern for buffers with density close to water (< 1.02 g/ml), for which the density between the hydration shell and the buffer is nearly matched<br>the effect of buffer components strongly interacting with the protein, such as chaotropic agents or detergents, needs to be considered with regard to the altered partial-specific volume of the protein.<br>the characterization of heterogeneous interactions between proteins is usually insensitive of hydration effects, as the effective buoyant molar mass the solvated protein can be determined by sedimentation for each protein separately. |