The samples :

- The analyzed macromolecules must be soluble and stable (especially for sedimentation equilibrium experiments)

- The concentration depends on what you want to see and of what you have of available:

In Absorbance (sedimentation velocity), the comfort is DO=1 (we can modulate: optical path = 1.2, 0.3 or 0.15 cm, λ between 230 and 600 nm).

Using interference optics, there is no limit considering the large concentrations, but a number of reasonable fringes is needed to have informative data (the series 1.2/0.8/0.3 mg/ml for proteins is generally suitable using absorbance and interference). The minimum concentration depends on the homogeneity of the sample.

Usually, we make two dilutions - 2/3 and 1/3 - from a sample of DO=1.2. When we expect (or we observe) effects of the concentration, we are often brought to use various systems of detection and\or optical routes.

For polysaccharides: in our study on heparin fragments, we tested typically our samples at 3, 6 and 10 mg / ml (sometimes 1.5, 3 and 5 mg / ml).

- The usual volume in sedimentation velocity experiments is 450 µl for cells with an optical path of 12mm (it can be 150 or 80 µl for cells of optical path of 3 or 1.5 mm). If you have no constraints, prepare 1 mL (we cannot exclude a leak, thus a sample to be redone).

For sedimentation equilibrium experiments, the volumes are lower, typically 180 µL in 12 mm, as the concentrations (typically DO=0.6), but we often duplicate samples and we make series of dilution.

The solvent :

- It is necessary to know its exact composition, especially for sedimentation equilibrium experiments (dialysis is wished)
- It has to contain some salt (for example 100 mm), so that the transport is not perturbed by electrostatic interactions (the macromolecule migrating faster than ions).
- For acquisitions using absorbance: the solvent does not have to absorb (or little) at the considered wavelength.
- For acquisitions using interference: the composition of the solvent must be strictly controlled and identical to that of the reference solvent (except for membrane proteins: the reference solvent does not have to contain detergent)
- The volume of solvent: the comfort is 1ml reference solvent by sample. If the volume of solvent does not raise problem, plan 5-10 ml, for possible measurements of density and viscosity.
- In the case of membrane proteins, it is necessary to have 2 solvents: a solvent with the detergent (for possible dilutions and measurements of density and viscosity) and a second one without detergent (for the reference).