

Sample specifications for B23

CD spectra are measured in two regions:

1) far UV region: 170-260nm: This gives information about the protein backbone mainly secondary structure. In this region the protein concentration varies from 0.2-0.4 mg/mL and cell volume is about 0.2mL.

For the backbone region, to obtain the lowest cut off measurement, it is necessary to keep the concentration of chloride ion (NaCl) as lowest as possible (max 25mM), normally, 10-20mM. The same limitation applies to the type of buffer: phosphate, Tris, Hepes, MES and others.

Bear in mind that the protein conformation might be sensitive to the buffer composition, in other words, the folding might be different or slightly different on varying the type of buffer used.

At last do not use DTT or DTE. If you need reducing agents, use small amount of Mercaptoethanol.

2) near UV region: 250-330nm: This is sensitive to the tertiary structure of Aromatic residues (Phe, Tyr and Trp) and Disulphide bonds.

For a pathlength of 1cm, the total volume is 450-520 micro litre. The Absorption should be between 0.8-1.4 for which the protein concentration depends in terms of their extinction coefficient. Usually we need 1-2 mg/ml concentration with a volume of 0.5ml per experiment.

This is most often done by measuring the absorbance, **A**, near 280 nm and using the Beer-Lambert law:

$$A = \epsilon C l$$

where ϵ is the molar absorption coefficient ($M^{-1} \text{ cm}^{-1}$), l is the pathlength (cm), and C is the protein concentration (M).

If the extinction coefficient is unknown, you can calculate them, by how many Trp, Tyr, and disulphide bonds are present in proteins using the following values for each of the residues (*Protein Science (1995)*, 4:2411-2423, Pace et al):

$$\epsilon (280 \text{ nm } (M^{-1} \text{ cm}^{-1})) = (\#Trp)(5,500) + (\#Tyr)(1,490) + (\#cystine)(125).$$

For the aromatic disulphide region, buffers you can use 150mM and even higher because the Cl ion are transparent in this region.

For binding studies experiments, bring enough ligand to be used in several molar excesses (up to 10) than those of the proteins.

Do not forget to bring buffers for your baseline measurement.