Sample Preparation for CD Measurements

When preparing a sample for CD measurements the absorption of light must be considered. For normal light the optical density (OD) of the sample is given by the Beer-Lambert law: $OD = \varepsilon * l * c$, where ε is the extinction coefficient (OD/cm*Molar), l the path length (cm) and c the sample concentration (Molar). CD is based on the difference on the absorption of left and right circularly polarized light, so the Beer-Lambert law still applies. The difference though is that instead of the extinction coefficient (ε) the difference in the extinction coefficients for left and right circularly polarized light is used ($\Delta\varepsilon = \varepsilon_L - \varepsilon_R$). Therefore the CD signal linearly tracks the sample. From this relationship one would naturally assume that higher sample concentrations will give better CD signals. Unfortunately this case is not true. The $\Delta\varepsilon$ is small compared to the absolute levels of light. Increasing the concentration results in less light for the measurement, and the small difference in absorbance can become lost in the noise. Remember that absorbance is the log scale of transmission, which is the true measure of the light energy. An absorbance of 1 means that 90% of the light is being absorbed, 2 means 99%, 3 equals 99.9%, and so on. The noise level is not constant but is also a function of the light level, being higher at low light levels. Therefore the signal-to-noise profile peaks at approximately 0.8 OD before deteriorating. The profile is relatively broad so deviations are acceptable. However, it is always desirable to have an absorption level in the range of 0.8 OD at the wavelength of interest.

The researcher must consider what is actually absorbing the light. The ideal situation is for the sample molecules and nothing else to be the active chromophores. Unfortunately, in the UV and particularly the deep UV (below 250 nm), many things absorb light. This only reduces the amount of light available for the measurement and adds nothing to the CD signal. When taken to the extreme there is so little light passing through the sample that CD measurements are impossible. This is frequently the case when taking wavelength scans and it is desirable to penetrate the UV as far as possible. Table 1 shows the wavelengths at which a given solvent has an OD of 1. Values are given for optical path lengths of 1.0 and 0.05 mm. For a given solvent and path length wavelengths longer than the ones in the table will have lower absorbances while shorter wavelengths result in rapidly increasing absorbances. The problem of solvent absorbance can be reduced by using short path length cuvettes and scaling up the sample concentration. For example, a 0.5 mm cuvette with 2X the sample concentration will have the same CD signal as a 1X sample concentration in a 1 mm cuvette, but with 1/2 the solvent absorbance.

The same absorbance problems occur with buffers and salts added to the sample. Table 2 shows the absorbance values for different compounds over a range of wavelengths. The conditions are for a 1.0 mm cuvette containing 10 mM solutions. Changing path length or concentration will scale the absorbance linearly. The researcher can adjust concentrations and path length to optimize CD measurements.

Table 1: Solvent Transparency

Compound	Wavelength (nm) for OD = 1.0	
	1.0-mm Path Length	0.05-mm Path Length
H_2O	182	176
MeOH	195.5	184
F_6iPrOH	174.5	163
F_3EtOH	179.5	170
EtOH	195	186
MeCN	185	175
Dioxane	231	202.5
Cyclohexane	180	175
α-Pentane	172	168

Table 2: Absorbance of Various Salt and Buffer Substances in the Far-UV Region

Compound	pH	No Absorbance Above	Absorbance of a 10 mM solution in a 1.0 mm Cuvette at:			
			210 nm	200 nm	190 nm	180 nm
$NaClO_4$		170 nm	0	0	0	0
NaF, KF		170 nm	0	0	0	0
Boric Acid		180 nm	0	0	0	0
NaCl		205 nm	0	0.02	>0.5	>0.5
Na_2HPO_4		210 nm	0	0.05	0.3	>0.5
NaH_2PO_4		195 nm	0	0	0.01	0.15
Na Acetate		220 nm	0.03	0.17	>0.5	>0.5
Glycine		220 nm	0.03	0.1	>0.5	>0.5
Diethylamine		240 nm	0.4	>0.5	>0.5	>0.5
NaOH	pH 12	230 nm	>0.5	>2	>2	>2
Boric Acid, NaOH	pH 9.1	200 nm	0	0	0.09	0.3

Table 2: Absorbance of Various Salt and Buffer Substances in the Far-UV Region

Compound	pH	No Absorbance Above	Absorbance of a 10 mM solution in a 1.0 mm Cuvette at:			
			210 nm	200 nm	190 nm	180 nm
Tricine	pH 8.5	230 nm	0.22	0.44	>0.5	>0.5
TRIS	pH 8.0	220 nm	0.02	0.13	0.24	>0.5
HEPES	pH 7.5	230 nm	0.37	0.5	>0.5	>0.5
PIPES	pH 7.0	230 nm	0.2	0.49	0.29	>0.5
MOPS	pH 7.0	230 nm	0.1	0.34	0.28	>0.5
MES	pH 6.0	230 nm	0.07	0.29	0.29	>0.5
Cacodylate	pH 6.0	210 nm	0.01	0.20	0.22	

Selection of the appropriate cuvette is also important. Cuvettes are available from a wide range of materials, not all of which are suitable for CD measurements. First, the cell should be capable of transmitting light in the range of interest. Table 3 shows the approximate wavelength range for which the light transmission is better than 80%. For CD measurements in the deep UV the best material is QS quartz. At wavelengths below 200 nm all commonly available cuvettes absorb light, so measurements become difficult regardless of the sample characteristics.

Table 3: Selection of Cuvette

Material	Transmission >80%
QS Quartz	200 - 2500 nm
QH Quartz	230 - 2500 nm
QX Quartz	200 - 3500 nm
OS “Special Optical Glass”	320 - 2500 nm
OG “Optical Glass”	360 - 2500 nm
PY “Pyrex”	340 - 2500 nm
Methyacrylate Plastic	300 - 750 nm
Polystyrene Plastic	350 - 750 nm

Second, the cuvette should not be mechanically strained. Strain in the cuvette will depolarize the light so that the CD baseline is not flat. When buying cuvettes it is possible to specify “Strain Free” cuvettes from the manufacturer, but this specification is not always sufficient. The

manufacturer screens the cuvettes in the visible and near UV then sells the best as strain free. Unfortunately strain becomes more apparent at shorter wavelengths, and even a “Strain Free” cuvette could have a poor CD baseline. Buying cells from Aviv eliminates the risk of receiving an unsuitable cuvette. Aviv purchases cuvettes made to it’s specifications, then screens the cuvettes using a CD spectrometer before reselling. The least expensive solution to obtain a “Strain Free” cuvettes is to screen all available cuvettes in the laboratory, and to set aside the suitable ones as being used for “CD Only”.