CIRCULAR DICHROISM TIPS & TRICKS

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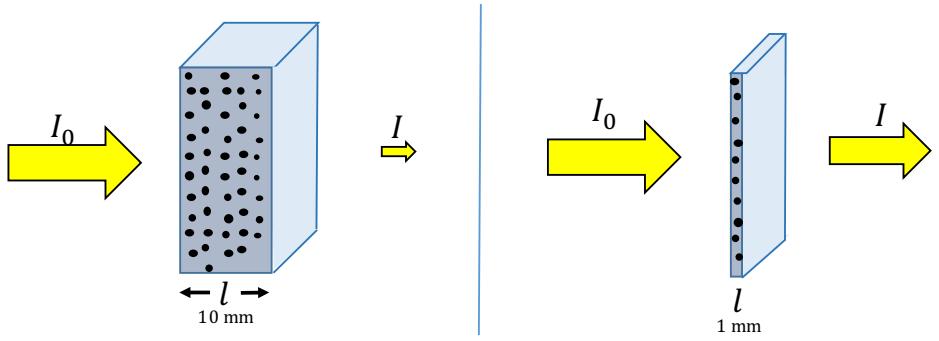

Sample Concentration and Pathlength

Since CD is an absorption technique based on Beer's Law, the sample concentration and pathlength are critical to obtaining good CD data. If a sample is too concentrated or the cell pathlength is too long, the sample will absorb too much light and not enough will pass to the detector for a reliable CD signal to be acquired. Conversely, if the sample is not concentrated enough or the cell pathlength is too short, the CD signal will be too weak or nonexistent. So what concentrations and pathlengths are necessary to obtain good CD data? For secondary structure studies (180-250 nm), 0.1 mg/mL of sample in a 1 mm pathlength cell is recommended. This ensures an optical density ~1 for most samples. For tertiary structure studies (250-350 nm), 1 mg/mL of sample in a 1 cm pathlength cell is required. If the sample concentration cannot be diluted to ensure enough transmittance, the cell pathlength can be decreased. For instance, if the working sample concentration must be 1 mg/mL, use a 0.1 mm pathlength cell instead. An ideal S/N is obtained when the optical density is ~0.7 so choosing a pathlength and sample concentration where the optical density is ~1 will provide good results. For far-UV studies below 200 nm, a 1 mm pathlength cell is necessary since water becomes opaque in a 1 cm pathlength cell below 200 nm and therefore, samples in aqueous buffer will have too much absorbance to obtain a CD signal.

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Buffer Conditions

The buffer or solvent the sample is made up in will also affect the CD spectrum. Especially for secondary structures studies in the far-UV, an ideal buffer is one that has a low absorbance in the wavelength region being probed. The addition of salt to any buffer will increase absorbance and potentially cause scattering, reducing the S/N. Phosphate buffers (potassium or sodium) are good to start with. Denaturing agents also have high absorbance below 200 nm so many protein denaturation studies must be measured at wavelengths above 200 nm. Buffers with sugars not only absorb highly in the far-UV but also have a CD signal and should be avoided for CD measurements.

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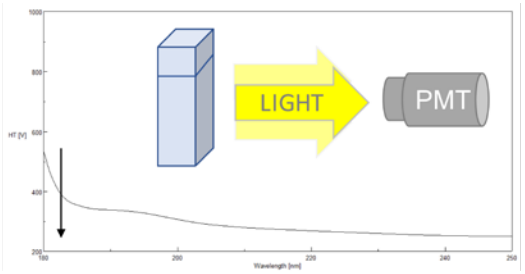

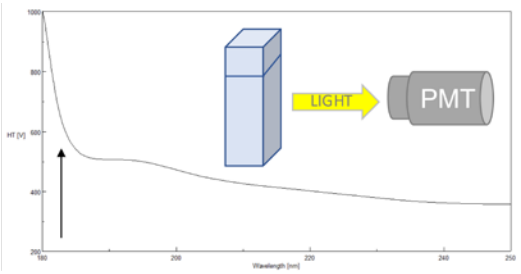

High Tension (HT) Voltage

The photomultiplier tube (PMT) detector has great sensitivity combined with a very wide linear dynamic range and is the best solution to measurement both low and high absorbing samples from the far UV to Visible regions of the spectrum. The HT voltage applied to the PMT is one of the most critical pieces of data to monitor in order to obtain good CD data. The applied HT voltage is used to amplify the detector's sensitivity. Since different wavelengths have different light energies, the HTV accommodates for fluctuations in the light levels by changing the gain. The gain either increases or decreases the amplitude of the signal depending on the DC voltage output. The standard DC is ~1 V. When a sample has an optimal concentration, enough light/photons is transmitted through the sample and reaches the detector, keeping the gain low. However, if a sample absorbs too much light, not enough photons will reach the detector and the gain must increase to amplify the signal and therefore the HT voltage will increase. If the HT voltage is equal to or above 700 V*, there are not enough photons being sampled by the PMT to measure a reliable or valid CD signal and the data points should not be used. The more photons reaching the detector, the better the S/N so at higher HT voltages, while the HT may be below 800 V, the CD signal may begin to look noisier. Additionally, farther into the far-UV (below 200 nm), less light is available so the HT voltage will also increase as the wavelength becomes shorter.

**for J-1000 Series CD Spectrometers*

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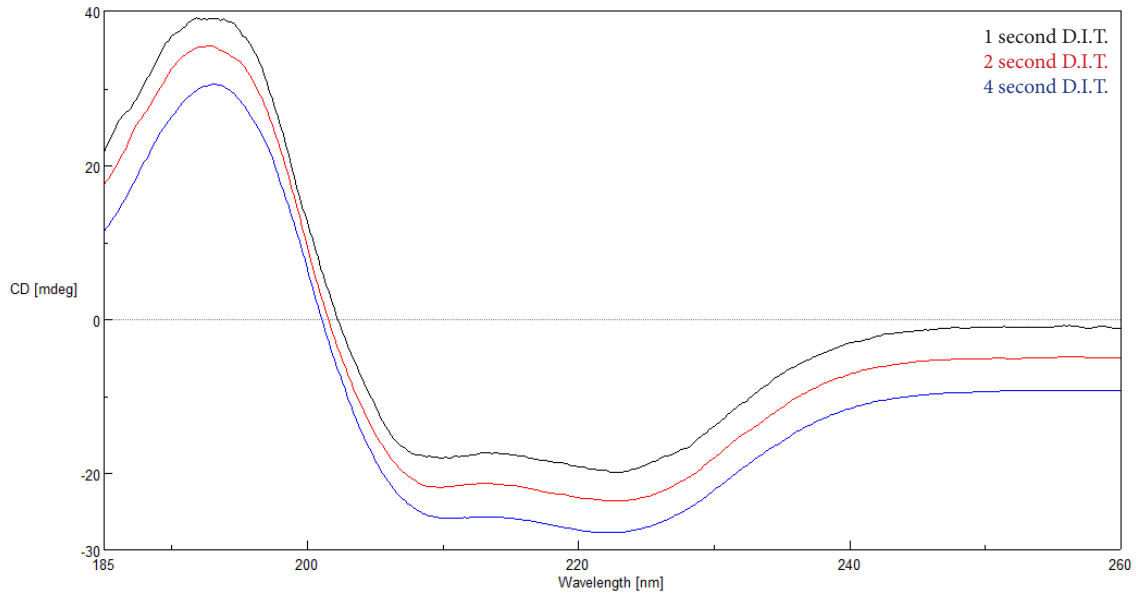
Digital Integration Time (D.I.T.)

The Digital Integration Time or D.I.T. is the amount of time that the data is integrated over or the length of time the detector collects photons before transferring the signal to the A/D converter for processing. The longer the D.I.T., the better the S/N. Therefore, increasing the D.I.T. has a more substantial effect when a sample's CD signal is small (<20 mdeg) since there is less signal. A 2 second D.I.T. with a 1 nm bandwidth is a good starting point.



**data offset*

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Scanning Speed

The scanning speed determines how quickly the monochromator scans to acquire data points at the specified data interval. Since JASCO circular dichroism spectrophotometers offer a continuous scan mode (and step-scan mode), the D.I.T. must be taken into account when setting the scanning speed. Together the two parameters determine the average interval of the CD signal. A good starting scanning speed is 50 nm/min.

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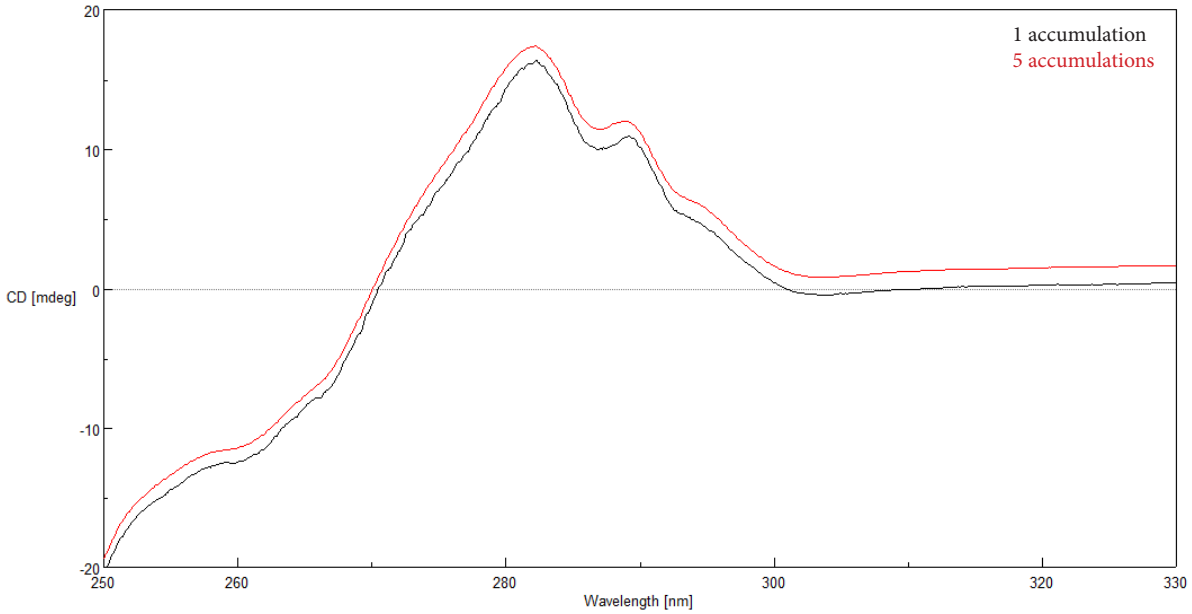
Accumulations

Spectral scans that are obtained and averaged together are referred to as ‘accumulations’. The following relationship exists between the signal-to-noise and the number of accumulations: S/N~√(Number of Accumulations). Once the previous scanning parameters have been optimized, increasing the number of accumulations can therefore further reduce the noise in a spectrum.



*data offset