Units of CD Measurement

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CD is reported in units of absorbance or ellipticity. Each of these can be normalized for molar concentration of the sample. The most direct data from the Olis CD instrument is absorbance (Abs(L)- Abs(R)). This value is often reported in milliabsorbance units (mA), which are a thousandth of an absorbance unit.

CD data are commonly reported as ellipticity (θ), which is related to absorbance by a factor of 32.98 (θ = 32.98 ΔAbs). Ellipticity is usually reported in millidegrees (mdeg or m°), which are a thousandth of a degree.

Molar ellipticity ([θ]) is CD corrected for concentration. The units of molar ellipticity are historical (deg×cm^2/dmol). Conversion from molar extinction (absorbance corrected for concentration) to molar ellipticity uses a factor of 3298 ([θ] = 3298Δε). To calculate molar ellipticity, the sample concentration (g/L), cell pathlength (cm), and the molecular weight (g/mol) must be known.

If the sample is a protein, the mean residual weight (average molecular weight of the amino acids it contains) is used in place of the molecular weight, essentially treating the protein as a solution of amino acids.

From ↓ To →	Absorbance[1]	Milliabsorbance[2]	Molar Extinction[3]	Degrees[4]	Millidegrees[5]	Molar Ellipticity[6]
(A)	A	A*1000	A*M/(C*L)	A*32.98	A*32980	A*M*3298/(L*C)
(mA)	mA/1000	mA	A*M/(C*L*1000)	mA*0.03298	mA*32.98	mA*M*3.298/(L*C)
(ε)	ε*C*L/M	ε*C*L*1000/M	ε	ε*C*L*32.98/M	ε*C*L*32980/M	ε*3298
(°)	°/32.98	°/0.03298	°*M/(C*L*32.98)	°	°*1000	°*M*100/(L*C)
(m°)	m°/32980	m°/32.98	m°*M/(C*L*32980)	m°/1000	m°	m°*M/(10*L*C)
[Θ]	[Θ]*C*L/(3298*M)	[Θ]*C*L/(3.298*M)	[Θ]/3298	[Θ]*C*L/(100*M)	[Θ]*C*L*10/M	[Θ]

[1]Units are Absorbance (Abs)
[2]Units are milliabsorbance (mAbs)
[3]Units are A*L/mol*cm
[4]Units are degrees (°)
[5]Units are millidegrees (m°)
[6]Units are deg*cm2/dmol

C is concentration in g/L
M is average molecular weight (g/mol)
L is path length of cell (cm)

Sample Concentration Effects

CD signals obey Beer's law – CD intensity is proportional to the concentration of the active species – so it is a tempting to increase the concentration of the sample to improve the signal to noise ratio. This strategy is not always useful, as the signal to noise is a function of the signal strength and the overall light intensity passing through the sample to the detectors. Since absorbance must occur at the CD active wavelengths, increasing the concentration also increases the overall absorbance, thus reducing the amount of light reaching the detectors. This necessitates the need for higher PMT high volts, which, in turn, increases the noise. The relationship between sample absorbance and signal to noise ratio is illustrated in Figure 3[1].

There is an optimum absorbance to use (Abs = 0.89). For a 1 mm pathlength cell, this absorbance is achieved with a protein concentration of about 0.1-0.3 mg/mL.

The optimal protein concentration is a function of the pathlength of the cuvette. Figure 4 shows a plot of the protein concentration required to produce an absorbance of 0.5 [2]. This is lower than the optimal 0.9 to account for absorbing buffer components. This plot indicates an optimal protein concentration of approximately 0.1 mg/mL for a protein solution, if the absorbance due to the buffer itself is minimized.

In the Olis dual-beam CD, no concentration calibration is required because the CD signal is directly digitally derived from the two photodetectors signals.

Figure 3

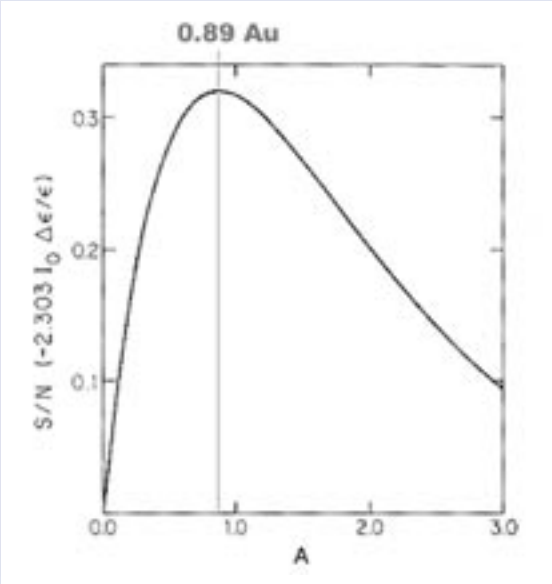
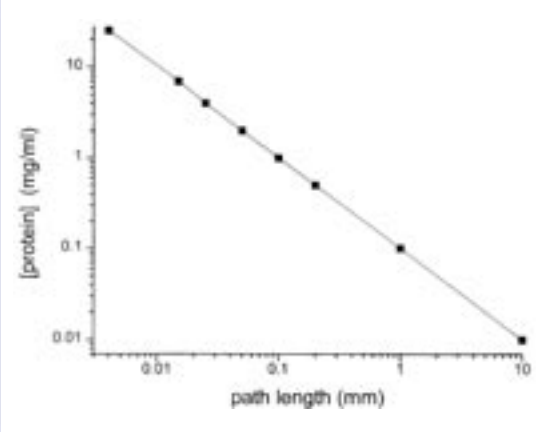


Figure 4



[1] 1 Johnson C. W. (1996) in Circular Dichroism and the Conformational Analysis of Biomolecules. Fasman G.D., Editor. Plenun Press, New York pp 635-352

[2] Sutherland, J.C. (1996) in Circular Dichroism and the Conformational Analysis of Biomolecules. Fasman G.D., Editor. Plenun Press, New York pp 599-633