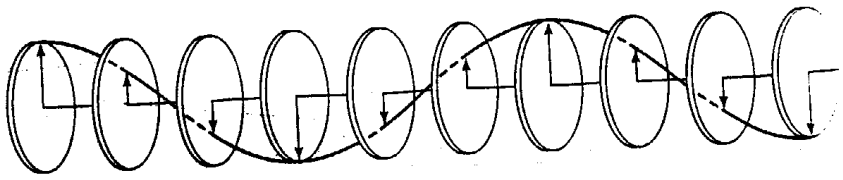
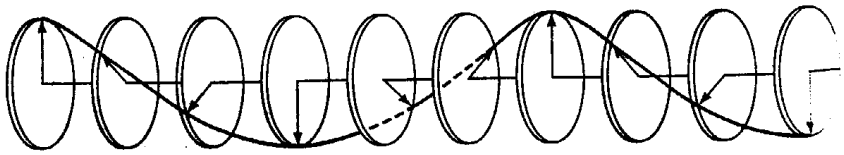
Circular Dichroism – A special kind of absorption spectroscopy, where the difference in absorption of left-handed & right-handed circularly polarized light is measured.

Definitions: In linearly polarized light, direction of electric vector is constant, magnitude of electric vector is varied:



linearly polarized light

In circularly polarized light, the magnitude of the electric vector is constant, & the direction of the electric vector is varied:



circular polarized light

Circularly polarized light is absorbed according to Beer's Law:

$$A = \epsilon l c$$

- A: absorption
- ϵ: extinction coefficient
- l: path length
- c: concentration

Some molecules (such as proteins) have a different extinction coefficient for left-handed and right-handed circularly polarized light.

Circular dichroism (CD) is defined as the difference in extinction coefficients for left & right handed circularly polarized light:

$$\Delta A = A_L - A_R = (\epsilon_L - \epsilon_R) lc$$

For historical reasons, CD is often reported as "ellipticity" Θ, where $\Theta = \Delta A \times 32.98$

How is circularly polarized light made?

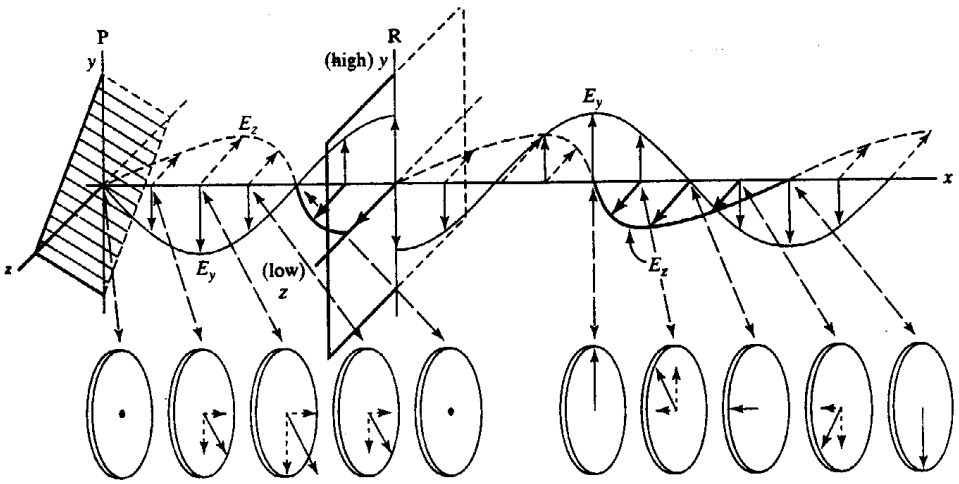


Figure 10.6 The optics for making circularly polarized light uses a linear polarizer P and a quarter-wave retarder R. Circularly polarized light can be decomposed into the sum of two mutually perpendicular linearly polarized waves that are one quarter of a wavelength out of phase. With E_y retarded one quarter of a wave relative to E_z, we have right circularly polarized light as diagrammed here. If E_z were retarded one quarter of a wave relative to E_y, then the circularly polarized light would be left-handed.

Different types of protein secondary structures give distinctive CD signals. Particularly noteable is the CD signal from α-helix, with minima at 207 and 222 nm.

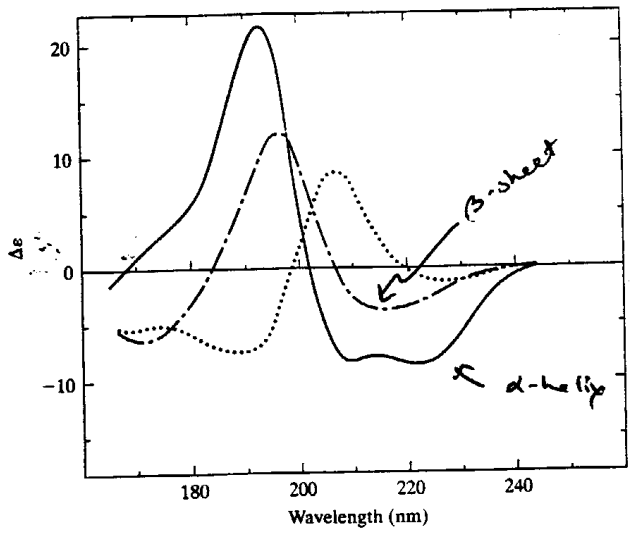


Figure 10.15 CD spectra for various secondary structures in aqueous solution: poly (L-glutamic acid) at pH 4.5 as an α-helix (———). [Adapted from W. C. Johnson and I. Tinoco (1972) *J. Amer. Chem. Soc.* 94, 4389–4390.] Poly (L-lysine-L-leucine) in aqueous solution at pH 7 as an antiparallel β-sheet (– – – – –). [Adapted from S. Brahms, G. Spach, and A. Brack (1977) *Proc. Natl. Acad. Sci. USA* 74, 3208–3212.] Poly (L-alanine$_2$-glycine$_3$) in aqueous solution at pH 7 (···) as a β-turn. [Adapted from S. Brahms, G. Spach, and A. Brack (1977) *Proc. Natl. Acad. Sci. USA* 74, 3208–3212.]

CD can be used to easily measure the α-helical content of proteins, due to the distinctive signal from α-helix.

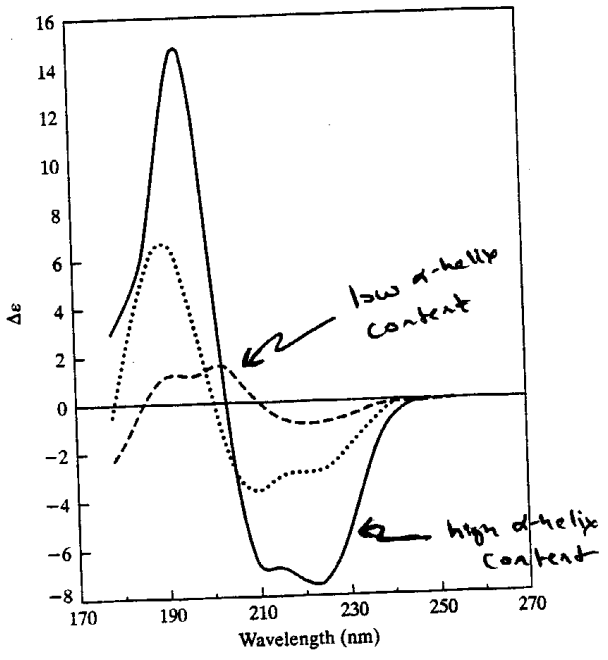


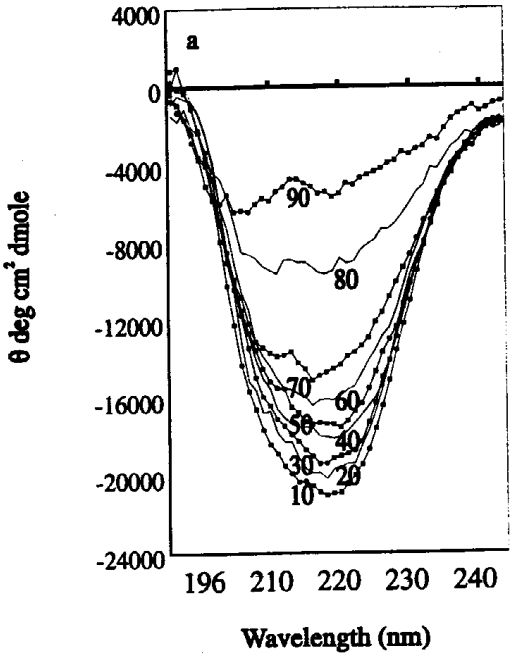
Figure 10.16 The CD of proteins hemoglobin (———), EcoRI endonuclease (···), and tumor necrosis factor-α (– – – – –).

At a wavelength of 207 nm, it has been found that the CD signal is (approximately) proportional to the % helix content of proteins.

If Θ (ellipticity) is expressed in units of deg·cm^2·dmole, then fractional helix content $\cong \Theta_{207}/-57{,}000$

Example: CD can be used to monitor protein denaturation in α-helical proteins, as shown below.

CD signal as a function of temperature for ribosomal protein L9



% helix content, estimated from $\Theta_{207}/-57{,}000$. Notice that α-helices unfold as temperature is increased.