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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OFENGINEERING & TECHNOLOGY

Circular Dichroism

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Circular Dichroism (CD) is an absorption spectroscopy method based on the differential absorption of left and right circularly polarized light. Optically active chiral molecules will preferentially absorb one direction of the circularly polarized light. The difference in absorption of the left and right circularly polarized light can be measured and quantified. UV CD is used to determine aspects of protein secondary structure. Vibrational CD, IR CD, is used to study the structure of small organic molecules, proteins and DNA. UV/Vis CD investigates charge transfer transitions in metal-protein complexes.

Principle

Electromagnetic radiation consists of oscillating electric and magnetic fields perpendicular to each other and the direction of propagation. Most light sources emit waves where these fields oscillate in all directions perpendicular to the propagation vector. Linear polarized light occurs when the electric field vector oscillates in only one plane. In circularly polarized light, the electric field vector rotates around the propagation axis maintaining a constant magnitude. When looked at down the axis of propagation the vector appears to trace a circle over the period of one wave frequency (one full rotation occurs in the distance equal to the wavelength). In linear polarized light the direction of the vector stays constant and the magnitude oscillates. In circularly polarized light the magnitude stays constant while the direction oscillates.

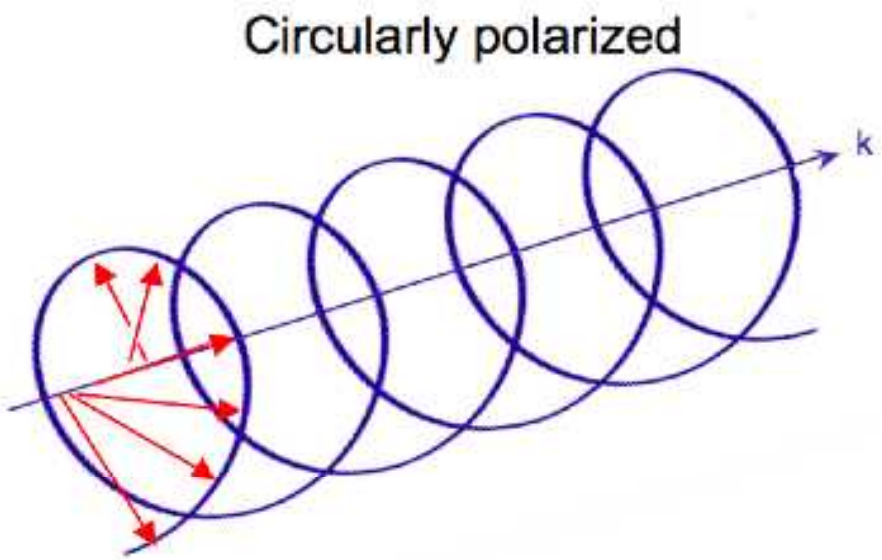


Diagram of linearly polarized and circularly polarized light. As the radiation propagates the electric field vector traces out a helix. The magnetic field vector is out of phase with the electric field vector by a quarter turn. When traced together the vectors form a double helix.

Light can be circularly polarized in two directions: left and right. If the vector rotates counterclockwise when the observer looks down the axis of propagation, the light is left circularly polarized (LCP). If it rotates clockwise, it is right circularly polarized (RCP). If LCP and RCP of the same amplitude, they are superimposed on one another and the resulting wave will be linearly polarized.

Interaction with Matter

As with linear polarized light, circularly polarized light can be absorbed by a medium. An optically active chiral compound will absorb the two directions of circularly polarized light by different amounts

$$\Delta A = Al - Ar \quad (1)$$

This can be extended to the Beer-Lambert Law. The molar absorpitivty of a medium will be different for LCP and RCP. The Beer-Lambert Law can be rewritten as

$$A = (\varepsilon l - \varepsilon r)cl \quad (2)$$

The difference in molar absorptivity is also known as the molar circular dichroism

$$\Delta\varepsilon = \varepsilon l - \varepsilon r \quad (3)$$

The molar circular dichroism is not only wavelength dependent but also depends on the absorbing molecules conformation, which can make it a function of concentration, temperature, and chemical environment.

Any absorption of light results in a change in amplitude of the incident wave; absorption changes the intensity of the light and intensity of the square of the amplitude. In a chiral medium the molar absorptivities of LCP and RCP light are different so they will be absorbed by the medium in different amounts. This differential absorption results in the LCP and RCP having different amplitudes which means the superimposed light is no longer linearly polarized. The resulting wave is elliptically polarized.

Molar Ellipticity

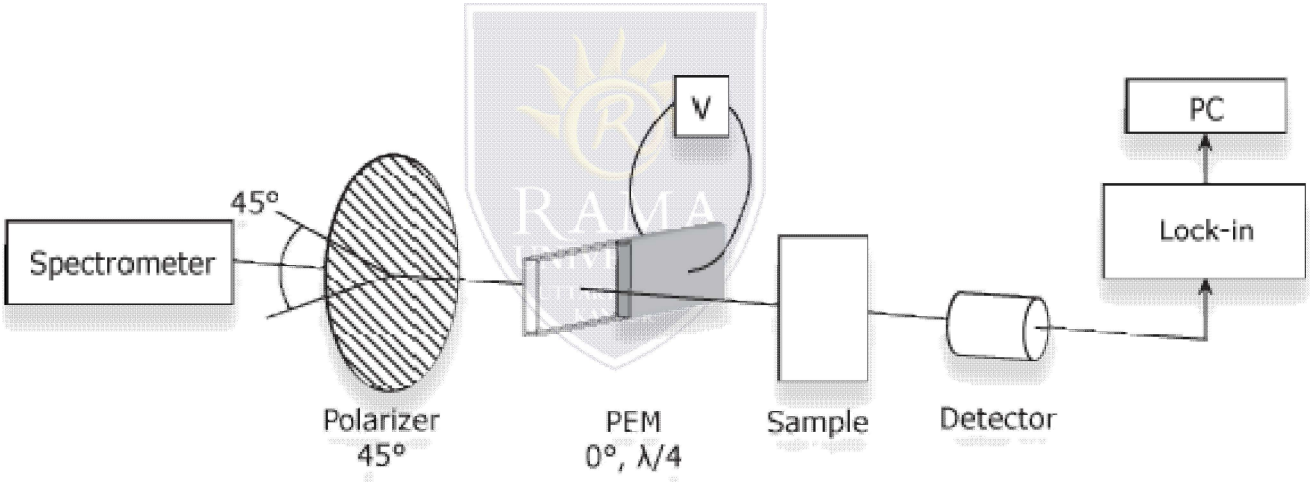
The CD spectrum is often reported in degrees of ellipticity, θ which is a measure of the ellipticity of the polarization given by:

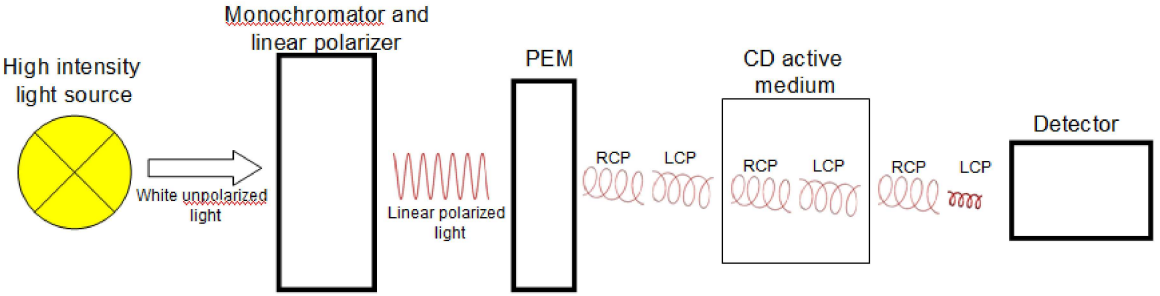
$$Tan\ \theta = El - Er / El + Er$$

where E is the magnitude of the electric field vector.

Working & Instrumentation

The basic layout of a CD spectrometer follows that of a single-beam UV absorption spectrometer. Owing to the nature of the measured effects, an electro-optic modulator, as well as a more sophisticated detector are needed.





•Generally, left and right circularly polarised light passes through the sample in an alternating fashion. This is achieved by an electro-optic modulator which is a crystal that transmits either the left- or right-handed polarised component of linearly polarised light, depending on the polarity of the electric field that is applied by alternating currents. The photomultiplier detector produces a voltage proportional to the ellipticity of the resultant beam emerging from the sample. The light source of the spectrometer is continuously flushed with nitrogen to avoid the formation of ozone and help to maintain the lamp.

•The most widely used application of CD spectroscopy is identifying structural aspects of proteins and DNA. The peptide bonds in proteins are optically active and the ellipticity they exhibit changes based on the local conformation of the molecule.

•The ordered *α*-helices, *β*-sheets, *β*-turn, and random coil conformations all have characteristic spectra. The peptide bond in proteins possesses UV absorption bands in the area of 220–190 nm. The carbon atom vicinal to the peptide bond (the C_α atom) is asymmetric and a chiral centre in all amino acids except glycine.

•The real value in CD comes from the ability to show conformational changes in molecules. It can be used to determine how similar a wild type protein is to mutant or show the extent of denaturation with a change in temperature or chemical environment.

•Some information about the tertiary structure of proteins can be determined using near-UV spectroscopy. Absorptions between 250-300 nm are due to the dipole orientation and surrounding environment of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, and cysteine residues which can form disulfide bonds. Near-UV techniques can also be used to provide structural information about the binding of prosthetic groups in proteins.

Metal containing proteins can be studied by visible CD spectroscopy. Visible CD light excites the d-d transitions of metals in chiral environments. Free ions in solution will not absorb CD light so the pH dependence of the metal binding and the stoichiometry can be determined.

Vibrational CD (VCD) spectroscopy uses IR light to determine 3D structures of short peptides, nucleic acids, and carbohydrates. VCD has been used to show the shape and number of helices in A-, B-, and Z-DNA. VCD is still a relatively new technique and has the potential to be a very powerful tool. Resolving the spectra requires extensive *ab initio* calculations, as well as, high concentrations and must be performed in water, which may force the molecule into a nonnative conformation.

Stability analysis of protein

CD spectroscopy can also be used to monitor changes of secondary structure within a sample over time. Frequently, CD instruments are equipped with temperature control units and the sample can be heated in a controlled fashion. As the protein undergoes its transition from the folded to the unfolded state, the CD at a certain wavelength (usually 222 nm) is monitored and plotted against the temperature, thus yielding a thermal denaturation curve which can be used for stability analysis.

Test your understanding

Circular Dichroism spectroscopy is used for determination of

a. Secondary structure of protein
b. Primary structure of protein
c. Tertiary structure of protein
d. To determine lipid concentration

Circular dischroism is a

a. absorption spectroscopy method based on absorption of infrared radiation
b. absorption spectroscopy method based on absorption of radiofrequency
c. absorption spectroscopy method based on the differential absorption of left and right circularly polarized light
d. emission spectroscopy method based on the differential absorption of UV rays.

Molar circular dichroism spectroscopy is affected by

a. Wavelength
b. absorbing molecules conformation
c. Temperature
d. All of the above

A biological sample should be……for analysis using circular dichroism spectroscopy

a. Optically inactive
b. Optically active
c. Should reflect incident radiation
d. None of the above

Vibrational circular dichroism uses……..for determination of 3D structure of protein

a. Infrared radiation
b. Visible light
c. Microwave radiation
d. None of the above

References & Further reading

1. Wilson, K, Walker, J., Principles and Techniques of Practical Biochemistry. 5th Ed. - Cambridge University Press,. Cambridge 1999.
2. Biotechniques, Theory & Practice: Second Edition by SVS Rana, Rustogi Publications.
3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.