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Short Notes on Circular Dichroism (CD)

CIRCULAR DICHROISM

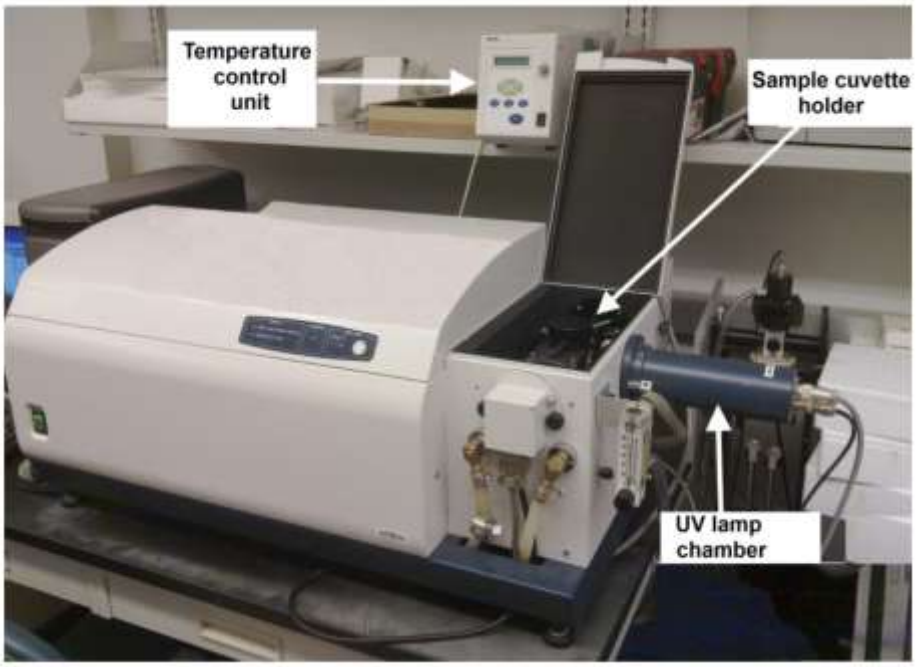

Fig: A CD instrument

Working Principle:

The physical basis of CD is utilization of circularly polarized light; a CD instrument records the unequal absorption of left-handed and right-handed circularly polarized light.

Polarisation of light

A. Unpolarized light-
The directions of oscillations randomly change in a same plane with time.

B. Plane polarized light-
The magnetic and electric fields components oscillate in a definite plane being perpendicular to each other.

C. Circularly polarized light-
The magnetic field keeps oscillating but the electric field vector changes direction in a rotary motion.

Origin of circularly polarized light:

- It is obtained by superimposing two plane polarized light of same wavelength and amplitude which are polarized in two perpendicular planes; but there is a phase difference of 90° between them.
- The wavelength range of 190nm-250nm (far- UV region) is used.

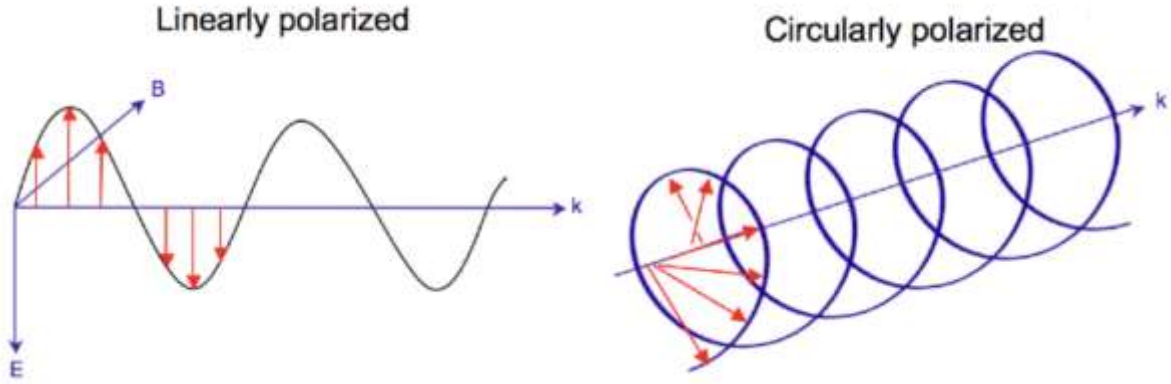

Fig: Linearly and circularly polarized light

Mode of working:

- The right circularly (R) and left circularly polarized (L) light is incident on a molecule/sample.
- Circularly polarized light when passed through a dichroic sample will be elliptically polarized.
- This becomes possible since the circular polarized components of the original linear polarized light will now not be of equal magnitudes due to differential absorbance (i.e., *circular dichroism*).
- Different deviation (in degrees) occur in each as the molecule is chiral; giving 2 different color illuminations; hence the name circular dichroism.
- It is measured ellipticity (millidegrees or degrees) or the difference in absorption of left and right circularly polarized light.
  [Left circularly polarized light- Anti-clockwise rotation of electric vector
  Right circularly polarized light- Clockwise rotation of electric vector]
- Thus, an elliptically polarized light is obtained by superimposing two plane polarized light vibrating at right angle to each other (phase difference of 90° between them) having same wavelength but unequal amplitude.

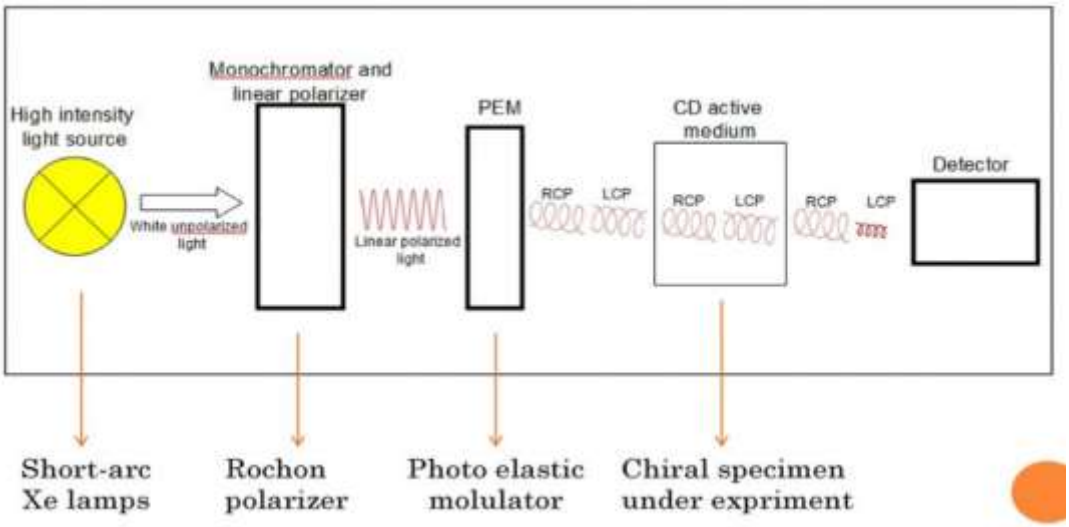

Fig: Mode of working of Circular Dichroism

So, CD = AL-AR
= ΔA = (εL-εR) cl
= Δεcl
[Δε - Molar circular dichroism]

Δε is typically <10 $M^{-1}cm^{-1}$
So, the CD signal is a very small difference between 2 large originals
The ellipticity is proportional to the difference in absorbance of 2 components (left and right circularly polarized light). So, CD is equivalent to ellipticity.

$\theta = 2.303 (A_L – A_R) 180/4\pi$
$\theta = 33(A_L - A_R) = 32.98 \Delta A = 33\Delta A$

Unit of θ = Degree cm^2 $dmol^{-1}$

Hence, CD measures the ellipticity of the transmitted light (i.e the light that is not absorbed)
Ellipticity value can be both positive and negative [$A_L > A_R$: Positive and $A_L < A_R$: Negative]

Typical initial concentrations:

- Protein concentration: Around 0.5mg/ml (adjustments made so as to produce the best data)
- Cell path length: For any problem arising in the absorption, at such times cells with a shorter path (0.1mm) with a correspondingly increased protein concentration and a longer scanning time can be utilized.
- Buffer concentration: It should be as low as possible around 5mM or even lower, while maintaining protein stability. Generally, 10mM phosphate buffer is used in CD spectra, although low concentrations of Tris, perchlorate or borate are also acceptable.

Sample preparation and measurement:

- Additives, buffers and stabilizing compounds: Those compounds which absorbs in the region of interest should be avoided.
- Solvent selectivity: A large number of organic solvents like THF, $CHCl_3$, CH_2Cl_2 cannot be used
- Protein solution: The protein solution should only contain those chemicals necessary to maintain protein stability/solubility and at the lowest concentration possible. The protein should be pure devoid of any sort of contamination.
- Lamp selectivity: In place of traditional Xe-arc lamps, high pressure short Xe lamps are used for performing low UV-CD spectroscopy.
- Contaminants: Any particulate matter (scattering particles) that adds a significant noise to the CD spectra should be avoided. Solutions must be filtered to improve the signal to noise ration.

Some standard CD patterns for secondary structure

A. For α-helical proteins:
Negative peak at 222nm and 208nm
Positive peak at 193nm

B. For β-sheet proteins:
Positive peak at 195nm
Negative peak at 218nm

C. For random-coil:
Positive peak at 215nm
Negative peak at 195nm

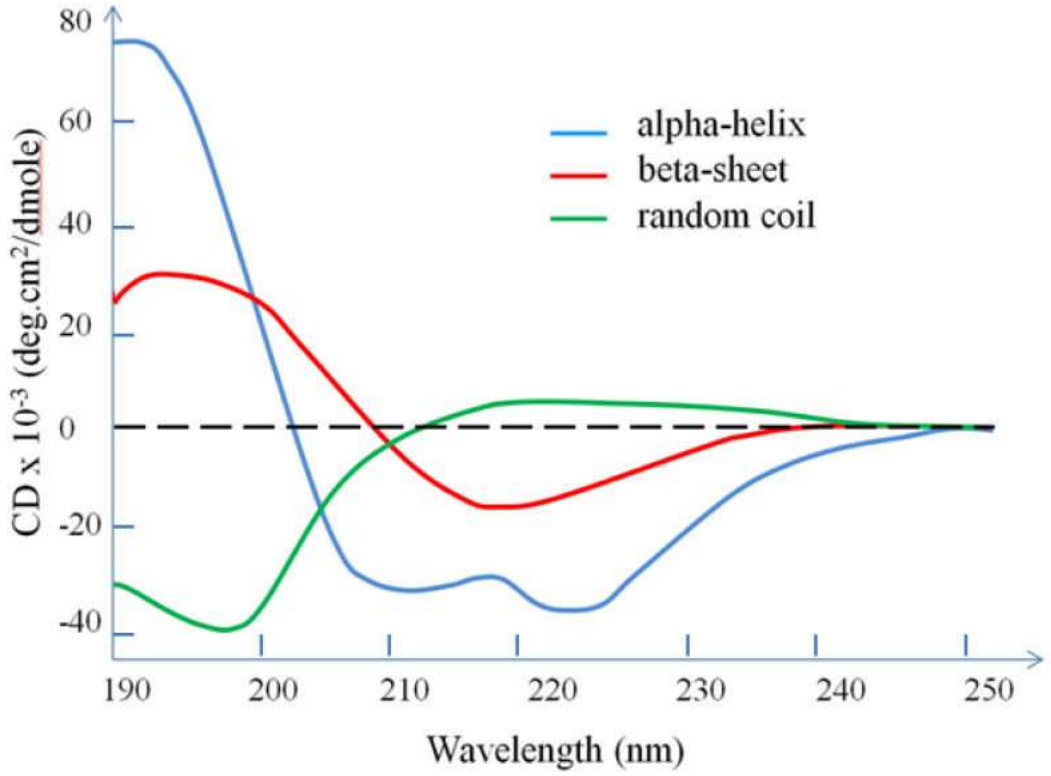

Fig : Standard CD graphs of secondary structures

Enantiomers and Cd-spectra:
Two enantiomers (mirror image of each other) are in equal amount in a sample; then the resultant CD-signal will be zero. Both the signals will cancel out each other in this racemic mixture.

Units of CD-data:
CD data is represented either in ellipticity (θ) or differential absorbance (ΔA)

Mean Residue Weight (MRW) = $\frac{M}{N-1}$

M- Molecular mass of polypeptide
N- No. of amino acid residues

Mean Residue Ellipticity (MRE):

$$[\theta]_{MRE} = \frac{MRW * \theta\lambda}{10 * l * c}$$

θ_λ = Observed ellipticity (in degrees)
l = Path length (in cm)
c = concentration (in g/mol)

Advantages of Circular Dichroism

- Relatively low concentrations/amount of sample is required for analysing
- Microsecond time resolution
- Timescale is much shorter thus allows to study dynamic system and kinetics

Limitations

- Certain buffer compounds in the sample strongly absorb in the Far-UV region and cause interference.
- Carbohydrates cannot be easily studied through CD.
- Oxygen must be completely absent from the system to perform the experiment below 200nm
- It does not provide atomic level structural analysis.
- It can be used only for qualitative analysis of data.
- Not able to provide a detailed residue-specific information as in NMR and X-Ray Crystallography

Applications of CD-spectra

- Determine the protein's secondary structure (at far-UV region: 180-240nm) and the protein's tertiary structure (at near-UV region: 280-380nm)
- It is the best method for monitoring structural alterations due to pH, temperature and ionic strength
- Structural, kinetic and thermodynamic information about macromolecules can be derived from CD spectra,
- It can be used to estimate α-helix, β- sheet and random coil configuration.
- It is used to determine the conformational changes due to protein-protein interactions, protein-DNA and protein-ligand interactions.
- It is used to measure the folding and unfolding state of proteins due to temperature changes.

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