CD SPECTROSCOPY

MFSN-2nd Sem-2019-20

Paper: MFSN 202 Food Science-II (Advance analytical techniques and food packaging)

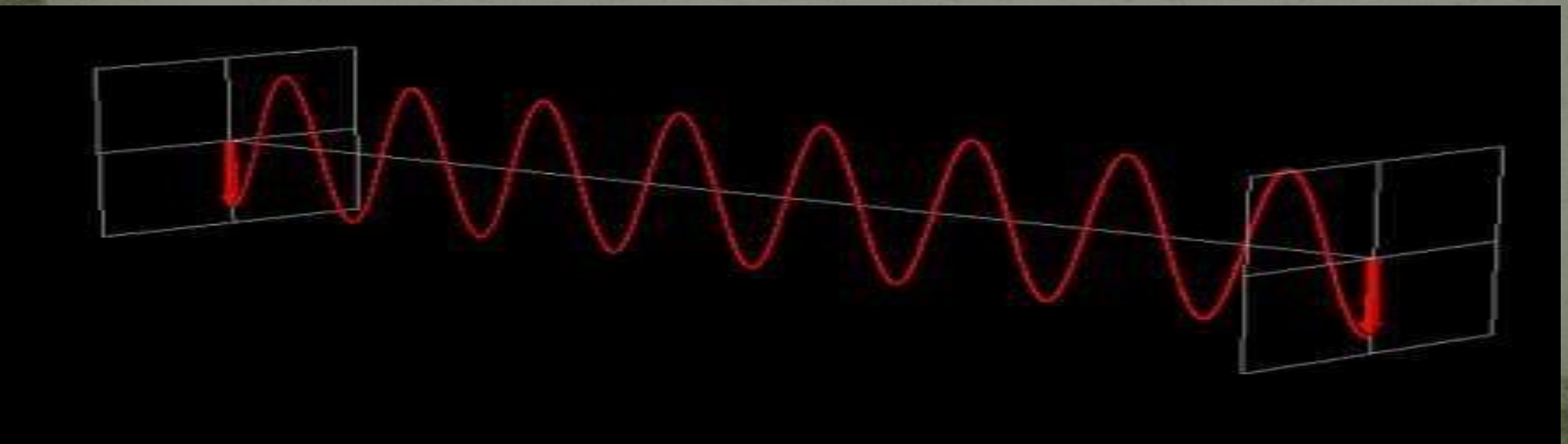
CIRCULAR DICHROISM SPECTROSCOPY

WHAT IS CIRCULAR DICHROISM?

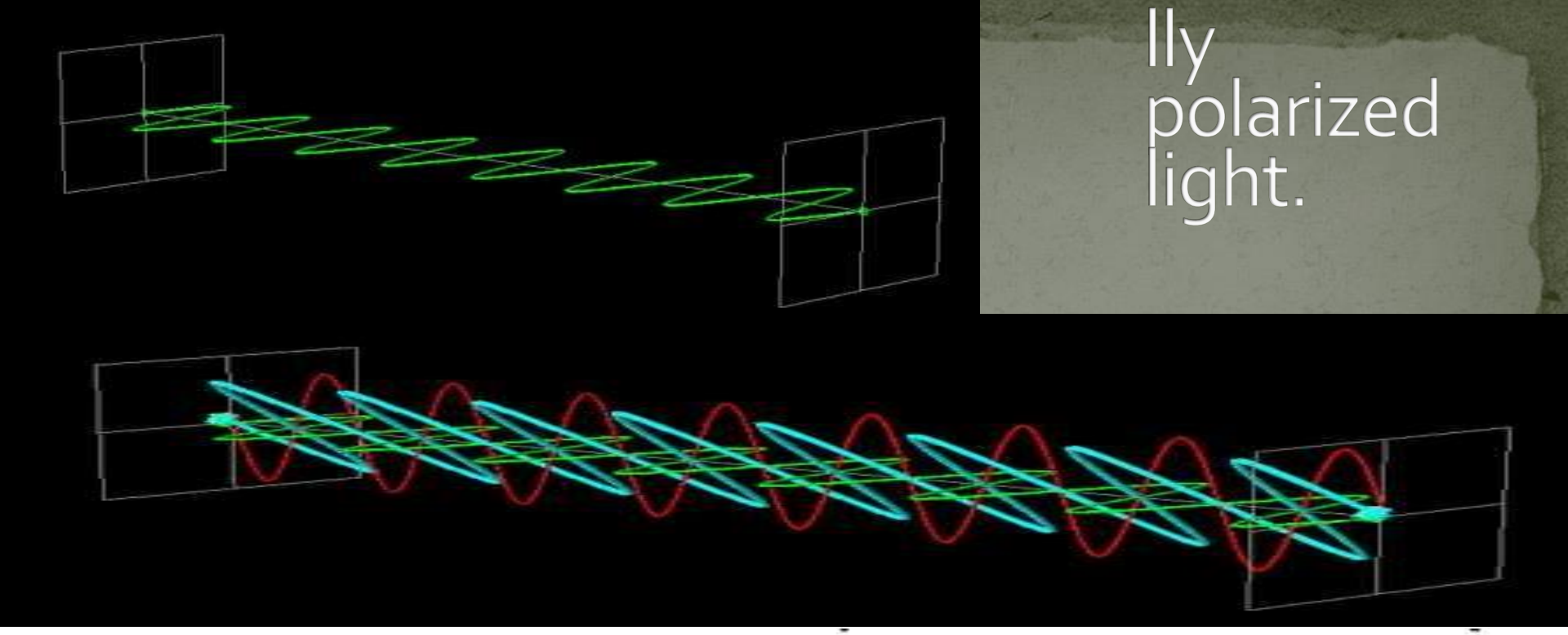
- Circular dichroism is observed when an optically active matter absorbs left and right hand circular polarized slightly differently.

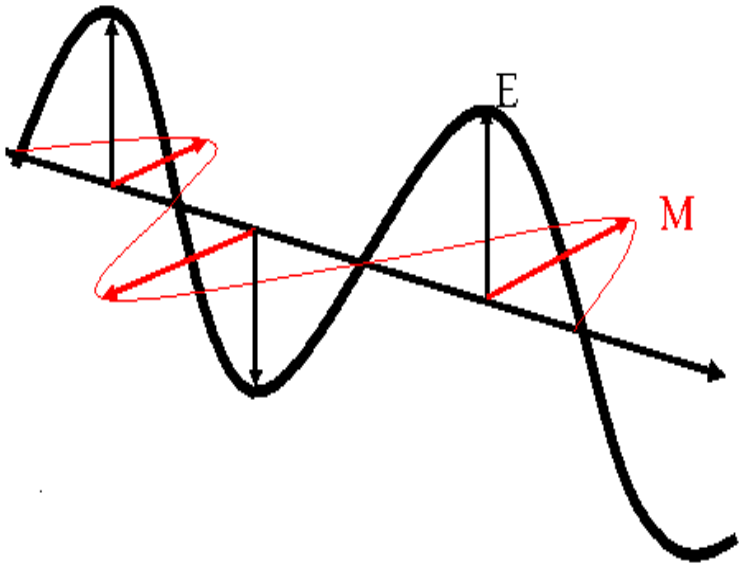
- Linearly polarised light is light whose oscillations are confined to a single plane.

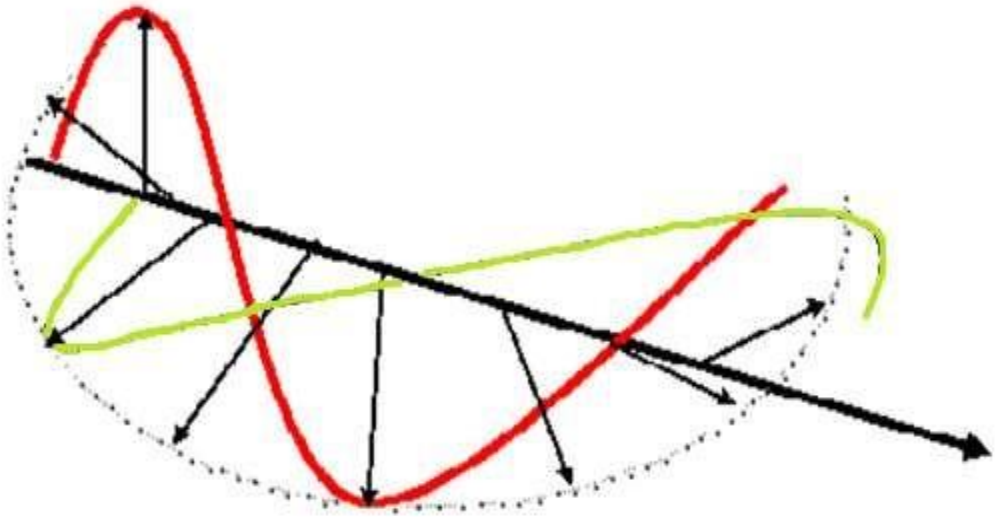
- All polarised light states can be described as a sum of two linearly polarised states at right angles to each other, usually referenced to the viewer as vertically and horizontally polarised light.



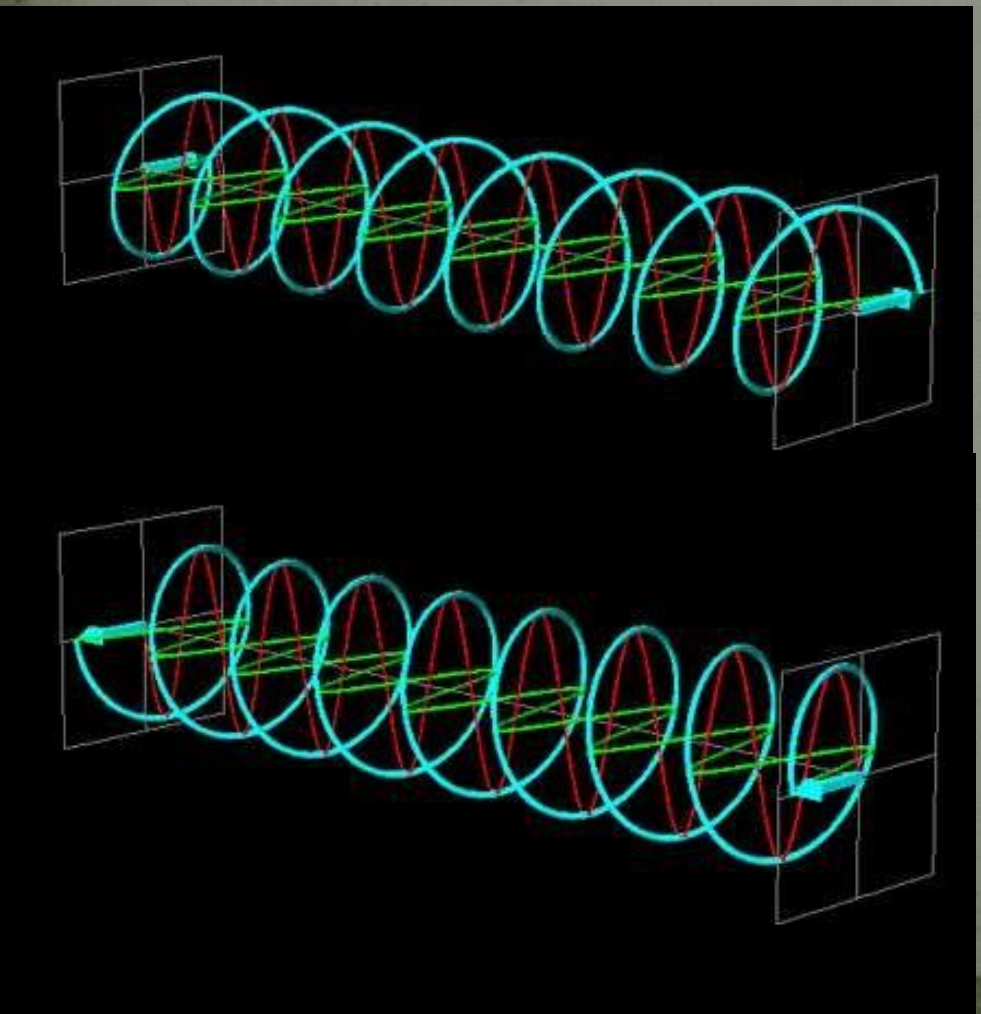
lly
polarized
light.







If one of the polarised states is out of phase with the other by a quarter-wave, the resultant will be a helix and is known as circularly polarised light (CPL). The helices can be either right-handed (R-CPL) or left-handed (L-CPL).



Left circularly polarised light

Right circularly polarised light

- Circular Dichroism (CD) is measured with a CD spectropolarimeter, which is relatively expensive (~$70k).

- The instrument needs to be able to measure accurately in the far UV at wavelengths down to 190-170 nm. In addition, the difference in left and right handed absorbance is very small (usually in the range of 0.0001) , therefore should be sensitive . The CD is a function of wavelength.

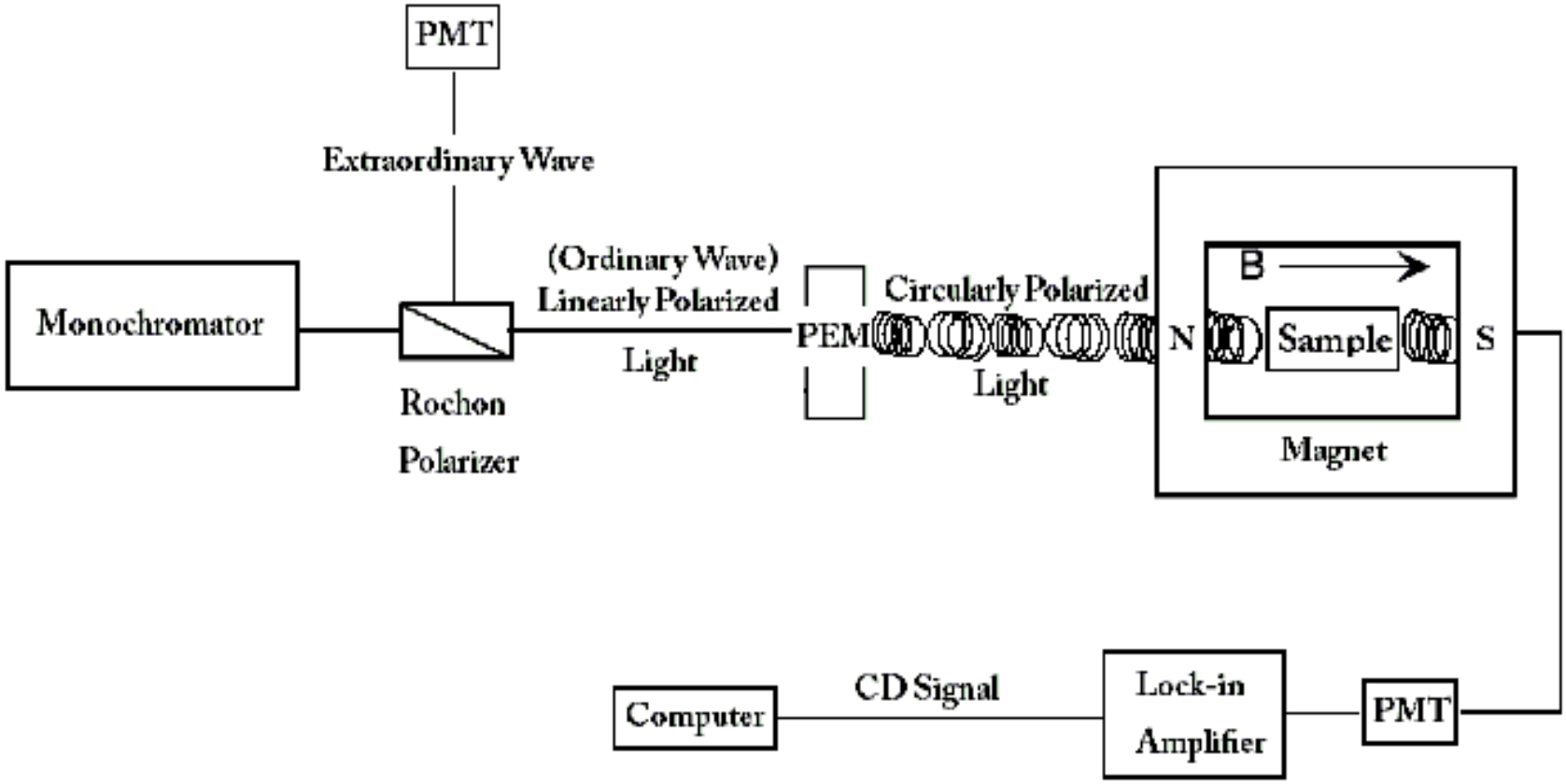
- When circularly polarized light passes through an absorbing optically active medium, the speeds between right and left polarizations differ as well as their wavelength and the extent to which they are absorb. Circular dichroism is the difference .

- CD spectra for distinct types of secondary structure present in peptides, proteins and nucleic acids are different. The analysis of CD spectra can therefore yield valuable information about secondary structure of biological macromolecules.

CD Instrumentation

- **So we need most of the same things as for a UV/visible:**
  - Lamp -Xe or Xe/Hg .
  - Monochromator.
  - Polarizer.
  - Photo-elastic modulator.
  - Sample compartment.
  - Detector (photomultiplier).

Block diagram of CD spectropolarimeter.



- **The Chirascan™ monochromator** uses two synthetic, single-crystal quartz prisms instead of the diffraction gratings that most people are familiar with from normal absorbance spectrophotometers.

- Quartz prisms are more efficient than diffraction gratings for a very wide range of wavelengths, particularly in the UV.

- Quartz is also birefringent and the prisms not only disperse light into the component wavelengths but also, because of their birefringence, disperse the linearly polarised components, one of which is selected for conversion to circularly polarised light.

- A further advantage of prisms is they do not pass second-order multiples of the desired wavelength, which is a major source of stray-light in grating-based monochromators.

Photo-elastic modulator:

- This is a piezoelectric element cemented to a block of fused silica.
- At rest, when the piezoelectric element is not oscillating, the silica block is not birefringent . when driven, the piezoelectric element oscillates at its resonance frequency (typically around 50 kHz), and induces stress in the silica in such a way that it becomes birefringent.
- The alternating stress turns the fused silica element into a dynamic quarter-wave plate, retarding first vertical with respect to horizontal components of the incident linearly polarised light by a quarter-wave and then vice versa, producing left- and then right- circularly polarised light at the drive frequency.
- The amplitude of the oscillation is tuned so that the retardation is appropriate for the wavelength of light passing through the silica block.

CD Spectropolarimeter

Operation of CD spectrometer.

The monochromator outputs linearly polarised light of a single wavelength(monochromatic).

The photo elastic modulator converts the linearly polarised beam into left and right circularly polarised light altering at 50 KHz.

With no CD active sample, the L-CPL and R-CPL have equal light intensities.

Consequently there is only a steady output from the light detector.

A CD active sample will absorb either L-CPL or R-CPL preferentially

The result of the unequal amount of L-CPL and R-CPL reaching the detector , is a signal that varies with the frequency of the PEM (50 KHz).

Difference in the intensity of L-CPL and R-CPL components is measured by amplifier tuned to the PEM frequency.

The average of the light intensity over time is measured and is termed as vDC. This can be used to scale the changes in CD measured by vAC signal.

The vAC signal is divided by vDC signal. The siganl is multiplied by a calibration factor (G) to give units of milidegrees or absorbance.

Plot of CD signal Vs. wavelength of light gives the CD spectrum.

Sample preparation

- Additives, buffers and stabilizing compounds: Any compound which absorbs in the region of interest (250 - 190 nm) should be avoided.

- A buffer or detergent or other chemical should not be used unless it can be shown that the compound in question will not mask the protein signal.

- Protein solution: From the above follows that the protein solution should contain only those chemicals necessary to maintain protein stability, and at the lowest concentrations possible. Avoid any chemical that is unnecessary for protein stability/solubility. The protein itself should be as pure as possible, any additional protein or peptide will contribute to the CD signal.

- Contaminants: Unfolded protein, peptides, particulate matter (scattering particles), anything that adds significant noise (or artificial signal contributions) to the CD spectrum must be avoided. Filtering of the solutions (0.02 um syringe filters) may improve signal to noise ratio.

- Nitrogen purging: the function of purging the CD instrument with nitrogen is to remove oxygen from the lamp housing, monochromator, and the sample chamber. The reason for removing oxygen is that oxygen absorbs deep UV light, thus reducing the light available for the measurement.

Typical Conditions for CD

- Protein Concentration: 0.25 mg/ml
- Cell Path Length: 1 mm
- Volume 400 µl
- Need very little sample 0.1 mg
- Concentration reasonable
- Stabilizers (Metal ions, etc.): minimum
- Buffer Concentration : 5 mM or as low as possible while maintaining protein stability
- A structural biology method that can give real answers in a day

CD Spectra of Protein 2ndary Structures

	-ve band (nm)	+ve band (nm)
α-helix	222 208	192
β-sheet	216	195
β-turn	220-230 (weak) 180-190 (strong)	205
L.H polypro II helix	190	210-230 weak
Random coil	200	

PROTEIN BACKBONE

CD spectrum sensitive to secondary structure change

AROMATIC AMINO ACIDS

CD spectrum sensitive to tertiary structure change

170 nm **Far-UV** 250 nm **Near-UV** 320 nm

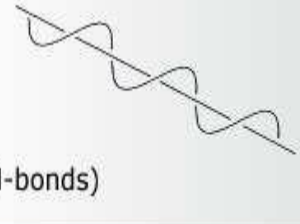
Structural types identified:

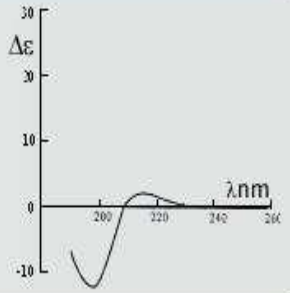
Alpha helix
Beta turn
Pollyproline
Irregular

Structural types identified:

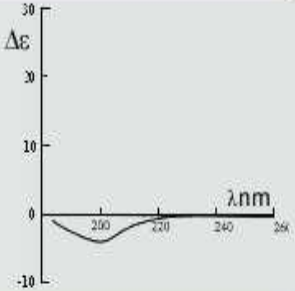
Phenylalanine
Tyrosine
Tryptophan
Disulphide bonds

Polyproline
PII helix
31-helix
(H-bonded)
(left hand
extended helix,
no intramolecular H-bonds)



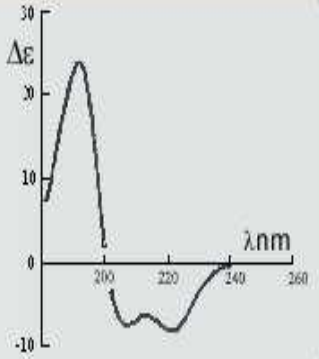


Irregular
(Disordered)

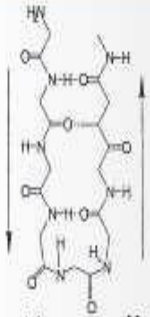


Conformation Molecular Shape CD spectrum

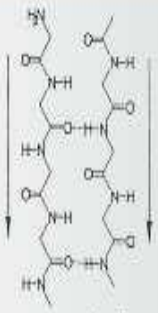
α-helix
(H-bonded)



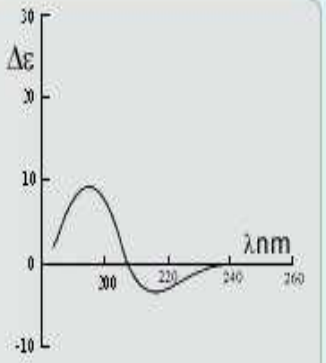
β–sheet
parallel and
anti-parallel
(H-bonded)



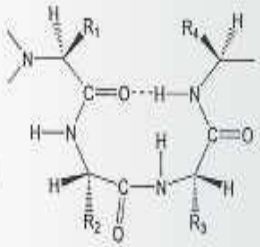
anti-parallel

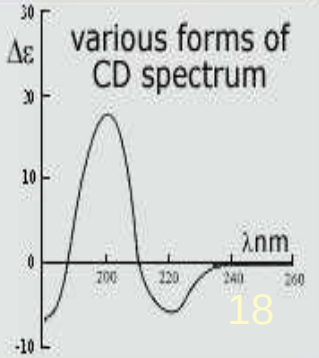


parallel



β–turn
(Type I,II,III.....)
γ–turns
(some turns not
H-bonded in proteins)





ADVANTAGES

- Simple and quick experiment.
- No extensive preparation..
- Relatively low concentrations/amounts of sample.
- Microsecond time resolution.
- Any size of macromolecule.

DISADVANTAGES

- Difficult to quantitate similarity or differences.
- Certain buffer components absorb strongly in Far-UV & can cause interference.

Applications

CD spectroscopy has wide range of applications in many different fields.

- UV CD – to investigate the secondary structure of proteins that cannot be crystallized.
- UV/ VIS CD – to investigate charge-transfer transitions.
- Near IR CD – to investigate geometric and electronic structure by probing metal transitions.
- Investigation of the effect of drug binding on protein secondary structure.
- Studies of the effects of environment on protein structure.
- Study of ligand -induced conformational changes.