Polarimetry

Polarimetry

Introduction of Polarimetry

- Polarimetry is an optical analytical technique used to measure the rotation of plane-polarized light as it passes through optically active substances.
- It is particularly important in pharmaceutical analysis for characterizing compounds that exhibit chirality, such as enantiomers and diastereomers, which can have vastly different biological activities.
- Chirality in pharmaceutical compounds is of major significance because different enantiomers of a drug may have different pharmacodynamic and pharmacokinetic profiles.
- Therefore, polarimetry is a valuable tool for assessing the identity, purity, and concentration of chiral substances.

Optically Active Compounds

- A compound is optically active if it can rotate the plane of polarized light.
- This typically occurs in chiral molecules, which lack an internal plane of symmetry and exist as non-superimposable mirror images (called enantiomers).

Plane-Polarized Light

- Ordinary light waves vibrate in multiple directions perpendicular to the direction of propagation.
- In plane-polarized light, vibrations are restricted to a single plane.
- Polarized light is used in polarimetry to observe how the orientation of light changes when it interacts with optically active substances.

Optical Rotation

- When plane-polarized light passes through an optically active substance:
    - If the light rotates clockwise, the compound is **dextrorotatory (+)**.
    - If it rotates counterclockwise, the compound is **levorotatory (–)**.
- The angle of rotation (α) is proportional to the path length, concentration, and the specific rotation of the substance.
- The amount of rotation depends on several factors:
    - Nature of the substance
    - Concentration of the solution
    - Length of the path through which light travels
    - Wavelength of light used

- Temperature

- This phenomenon is referred to as **optical rotation**, and the instrument used to measure this rotation is called a **polarimeter**.

Optical Rotatory Dispersion (ORD)

Definition and Concept

- **Optical Rotatory Dispersion (ORD)** refers to the variation of optical rotation (or specific rotation) of a chiral substance as a function of the wavelength of incident plane-polarized light.
- In simpler terms, when polarized light of different wavelengths passes through a chiral (optically active) medium, the angle of rotation of the light changes depending on the wavelength.
- The resulting ORD spectrum is a plot of optical rotation (α or [α]) vs. wavelength (λ).

Underlying Physics / Physical Basis

- When plane-polarized light passes through an optically active medium, the following occurs:
  - The medium exhibits different refractive indices for left- and right-circularly polarized components of light — a phenomenon called circular birefringence.
  - This results in a phase difference between these two components.
  - Upon recombination, the resultant light has its plane of polarization rotated by an angle α.
- This interaction is wavelength-dependent due to the molecular electronic transitions within the chiral molecule.
  - **Near absorption bands** (wavelengths where the molecule absorbs light strongly), there is **anomalous dispersion** — a rapid change in optical rotation.
  - Away from absorption bands, the ORD follows a smooth curve.

Types of ORD Curves

1. Normal Dispersion Curve:

- ➢ Smooth change in optical rotation with increasing wavelength.
- ➢ Observed away from absorption bands.

2. Anomalous Dispersion Curve:

- ➢ Rapid and irregular changes in rotation near an absorption band.
- ➢ May show a **sign change** (crossing the x-axis), indicating peaks and troughs.

Applications of ORD

- ORD is a powerful tool in **stereochemical analysis** and **structural studies**, especially when other spectroscopic methods (e.g., UV-Vis, fluorescence) are not applicable.

1. Determining Absolute Configuration:

- Helps assign R/S configurations to chiral centers.
- Enantiomers show mirror-image ORD curves.

2. Studying Conformational and Structural Changes:

- Used to investigate secondary and tertiary structures in proteins, peptides, and polymers.

3. Analyzing Stereochemical Purity:

- Detects enantiomeric excess in pharmaceuticals.

4. Pharmaceutical Analysis:

- Differentiates chiral isomers.
- Monitors chiral stability and conformational changes during formulation or storage.

Example

- Two enantiomers of a drug exhibit mirror-image ORD spectra, allowing clear differentiation and assessment of enantiomeric purity.

Circular Dichroism (CD)

Definition and Fundamental Concept

- Circular Dichroism (CD) refers to the differential absorption of left-handed (L-CPL) and right-handed (R-CPL) circularly polarized light by a chiral molecule.
  - Unlike Optical Rotatory Dispersion (ORD), which measures the rotation of polarized light, CD measures the difference in absorbance between the two circular polarizations.
  - This difference gives rise to a CD spectrum, which provides insights into the electronic transitions and chiral environment of the molecule.

$$\Delta A = A_L - A_R$$

- **Where:**
  - A_L= Absorbance of left circularly polarized light
  - A_R= Absorbance of right circularly polarized light
  - ΔA= Differential absorbance
- The transmitted light becomes **elliptically polarized**, as one component is absorbed more than the other.

Physical Basis of CD

- Chiral molecules interact differently with left and right circularly polarized light during electronic transitions (e.g., $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$).
- This asymmetry in interaction arises due to the molecular geometry and electronic structure.
- CD is observed only in absorption regions—near wavelengths where electronic transitions occur.
- Therefore, unlike ORD, CD requires molecular absorption of light to occur.

CD Spectrum

- **The CD spectrum is typically plotted as:**
  - **Ellipticity (θ)** in millidegrees (mdeg), or
  - **ΔA** (difference in absorbance)
    versus **wavelength (λ)**.
- CD signals are strongest at absorption bands of chromophores and carry information about the structural and stereochemical features of the molecule.

Spectral Regions and Transitions

1. **Far-UV CD (180–250 nm):**
   - Sensitive to secondary structures of proteins.
   - Distinguishes α-helices, β-sheets, and random coils based on characteristic CD patterns.
2. **Near-UV CD (250–320 nm):**
   - Reflects the tertiary structure or chiral environment around aromatic residues (e.g., tryptophan, phenylalanine).
3. **Visible and Near-IR CD (>320 nm):**
   - Observed in metalloenzymes, pigments, and chiral transition metal complexes.

Applications of Circular Dichroism (CD)

1. **Protein and Biomolecule Analysis**
   - Determines secondary structures (α-helix, β-sheet, etc.).
   - Monitors conformational changes due to pH, temperature, or ligand binding.
   - Studies protein folding, stability, and dynamics.
2. **Nucleic Acids**
   - Differentiates DNA/RNA conformations (e.g., A-form vs. B-form).
   - Probes structural changes from drug or ion binding.
3. **Pharmaceuticals and Drug Development**
   - Assesses enantiomeric purity when chiral HPLC is insufficient.

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