Circluar Dichroism & Optical Rotation Dispersion Spectroscopy

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Principle

- Electromagnetic radiation oscillates in all possible directions.
- It is possible to preferentially select waves oscillating in a single plane.
- The phenomenon first known as mutarotation (described by Lowry in 1898) became manifest in due course as a special property of optically active isomers allowing the rotation of plane-polarised light.
- Optically active isomers are compounds of identical chemical composition and topology, but whose mirror images cannot be superimposed; such compounds are called chiral.

Linearly and circularly polarised light

- Light is electromagnetic radiation where the electric vector (E) and the magnetic vector (M) are perpendicular to each other.
- Each vector undergoes an oscillation as the light travels along the direction of propagation .
- A light source usually consists of a collection of randomly oriented emitters.
- Therefore, the emitted light is a collection of waves with all possible orientations of the E vectors.
- This light is non-polarised.
- Linearly or plane-polarised light is obtained by passing light through a polariser that transmits light with only a single plane of polarisation, i.e. it passes only those components of the E vector that are parallel to the axis of the polariser (Fig. 12.14).
- If the E vectors of two electromagnetic waves are ¼ wavelength out of phase and perpendicular to each other, the vector that is the sum of the E vectors of the two components rotates around the direction of propagation so that its tip follows a helical path. Such light is called circularly polarised (Fig. 12.16).

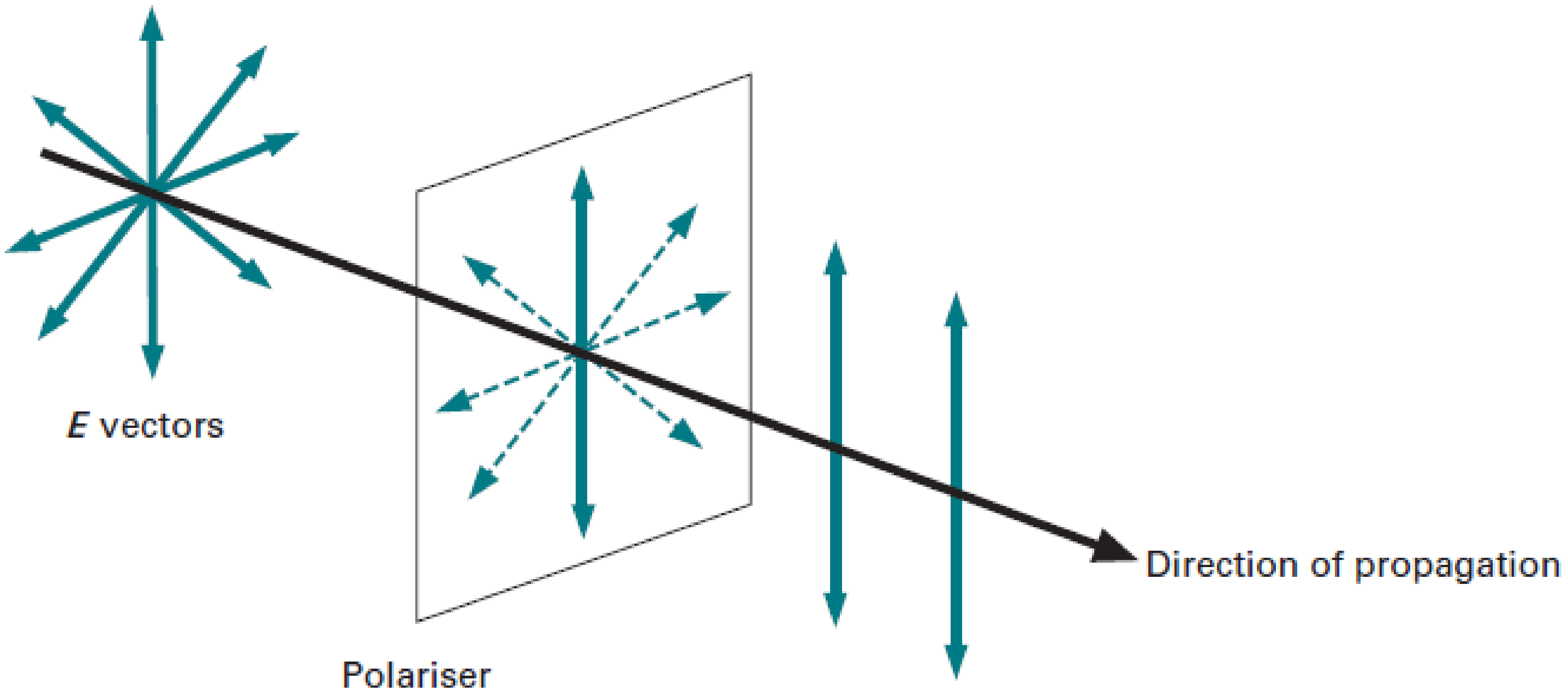


Fig. 12.14 Generation of linearly polarised light.

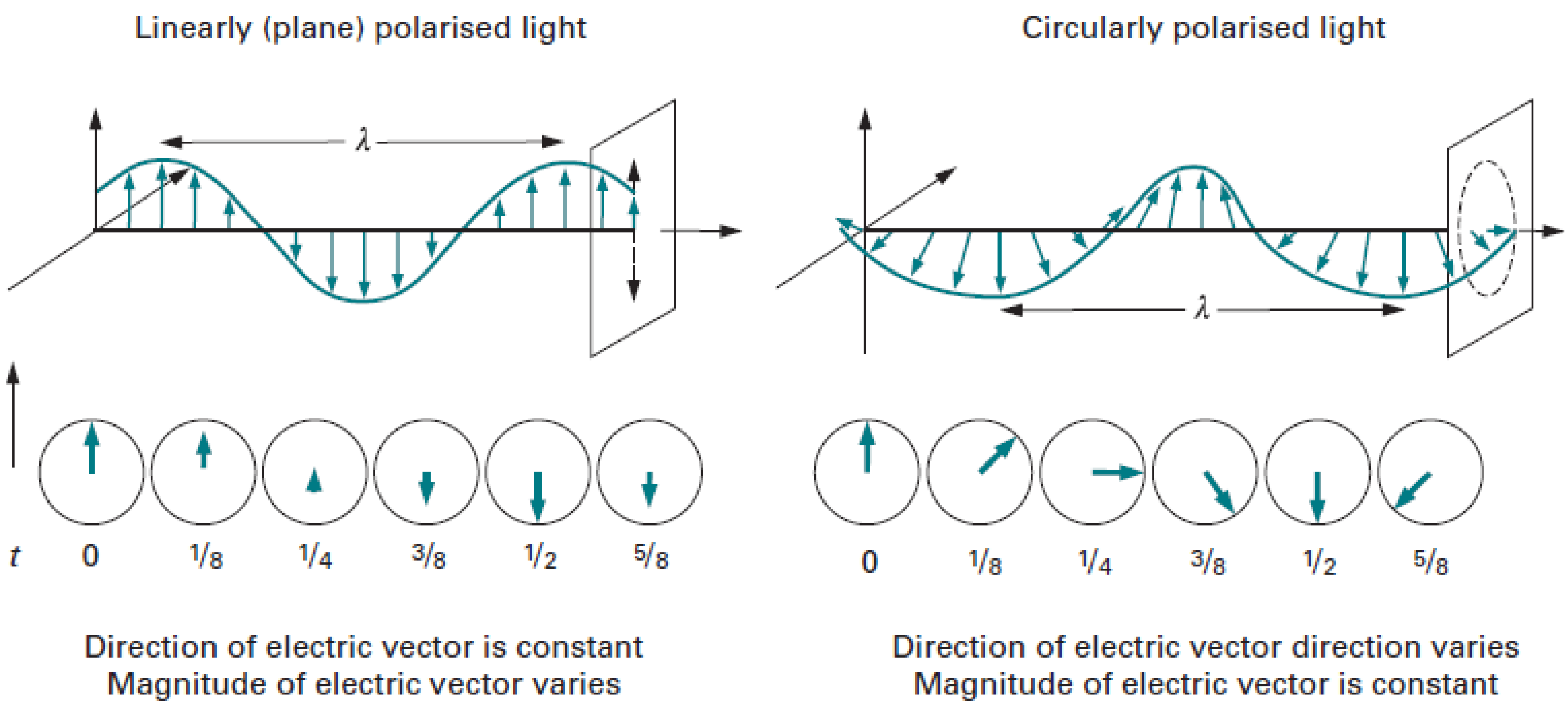
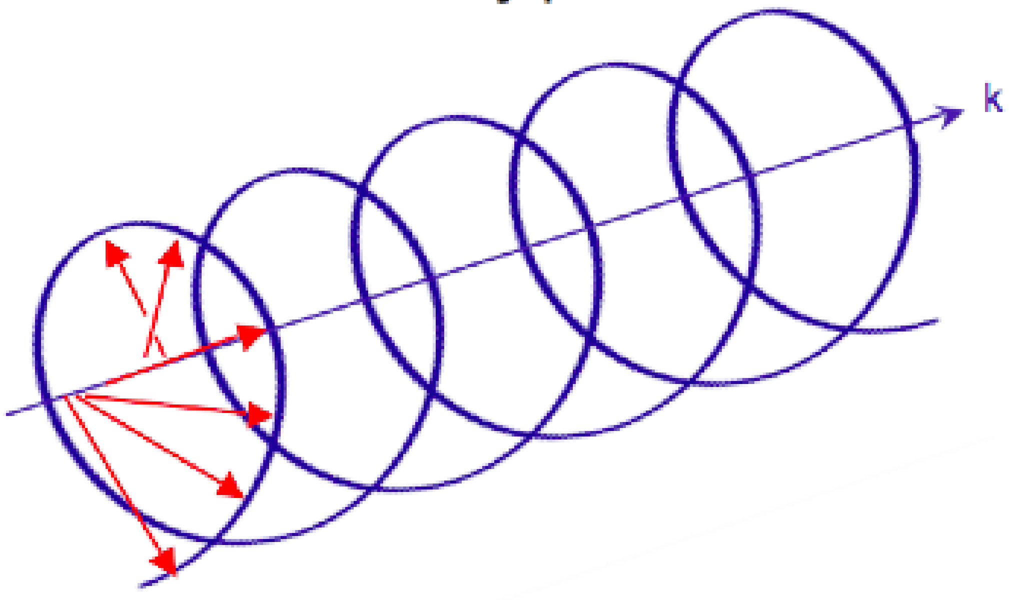


Fig. 12.16 Linearly (plane) and circularly polarised light.

- **While the E vector of circularly polarised light always has the same magnitude but a varying direction, the direction of the E vector of linearly polarised light is constant; it is its magnitude that varies.**

Circularly polarized



Polarimetry and optical rotation dispersion

- Polarimetry essentially measures the angle through which the plane of polarisation is changed after linearly polarised light is passed through a solution containing a chiral substance.
- Optical rotation dispersion (ORD) spectroscopy is a technique that measures this ability of a chiral substance to change the plane-polarisation as a function of the wavelength.
- Linearly polarised light can be thought of consisting of two circularly polarised components with opposite ‘handedness’.
- The vector sum of the left- and right-handed circularly polarised light yields linearly polarised light.

… **Polarimetry and optical rotation dispersion**

- The angle α_λ between the plane of the resulting linearly polarised light against that of the incident light is dependent on the refractive index for left (n_{left}) and right (n_{right}) circularly polarised light.
- The refractive index can be calculated as the ratio of the speed of light in vacuo and the speed of light in matter.
- After normalisation against the amount of substance present in the sample (thickness of sample/cuvette length d, and mass concentration ρ^*), a substance-specific constant $[\alpha]_\lambda$ is obtained that can be used to characterise chiral compounds.

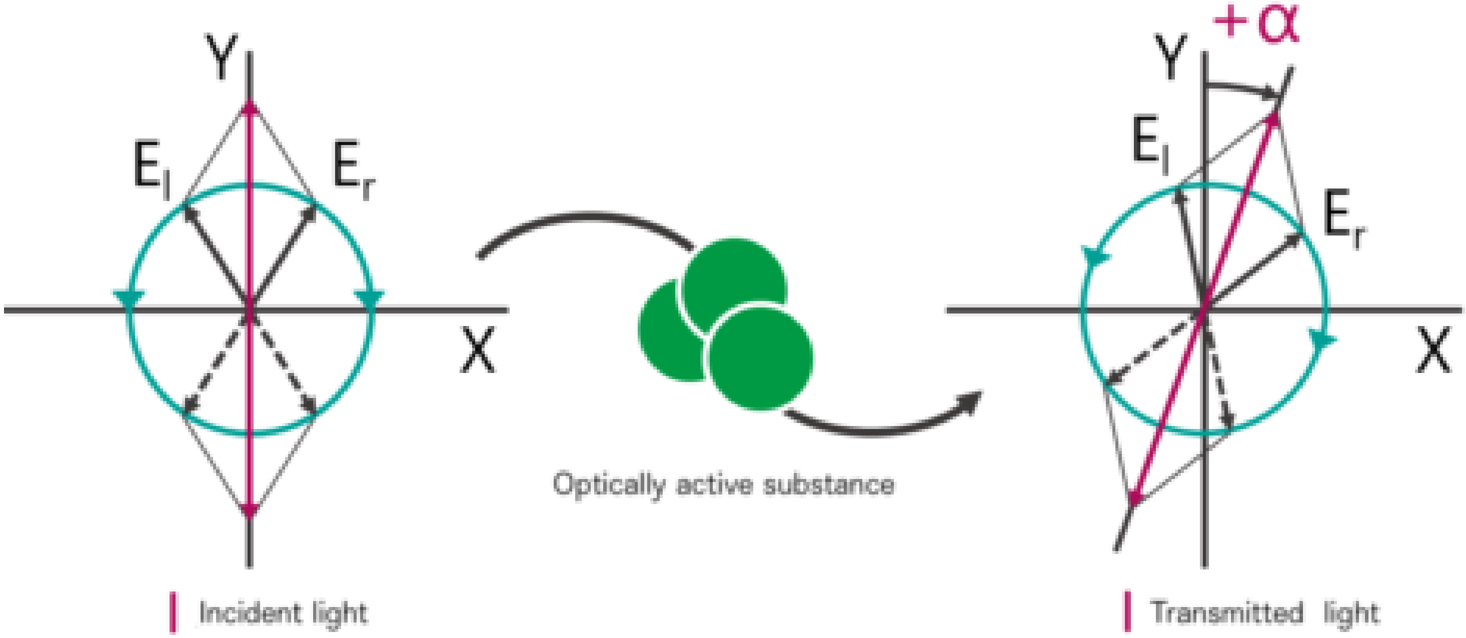


Fig. 3 Principle of optical rotation

Circular dichroism

- In addition to changing the plane of polarisation, an optically active sample also shows unusual absorption behaviour.
- Left- and right-handed polarised components of the incident light are absorbed differently by the sample, which yields a difference in the absorption coefficients $\Delta\varepsilon = \varepsilon_{left} - \varepsilon_{right}$.
- This latter difference is called circular dichroism (CD).
- The difference in absorption coefficients $\Delta\varepsilon$ (i.e. CD) is measured in units of $cm^2\ g^{-1}$, and is the observed quantity in CD experiments.
- If the amplitudes of left- and right-handed polarised components differ, the resulting light is elliptically polarised.
- The results from CD experiments are reported as ellipticity (The ellipse is characterised by a major and a minor axis.).

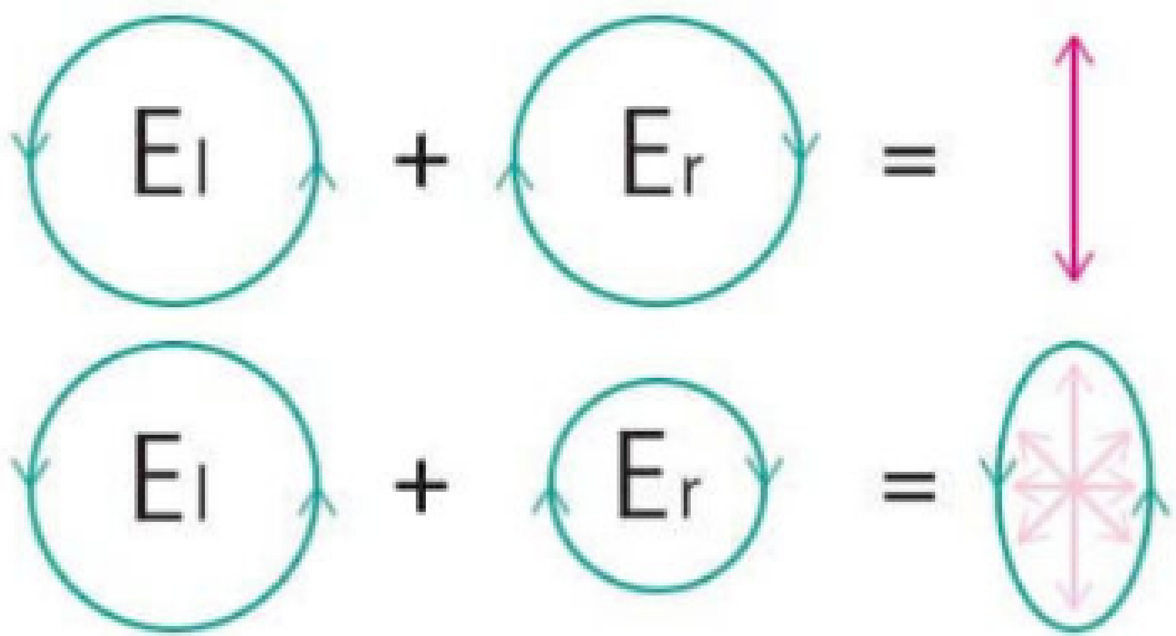


Fig. 2-1 Sum of circularly polarized light components

El = Er： linearly polarized light (upper)

El ≠ Er：elliptically polarized light (lower)

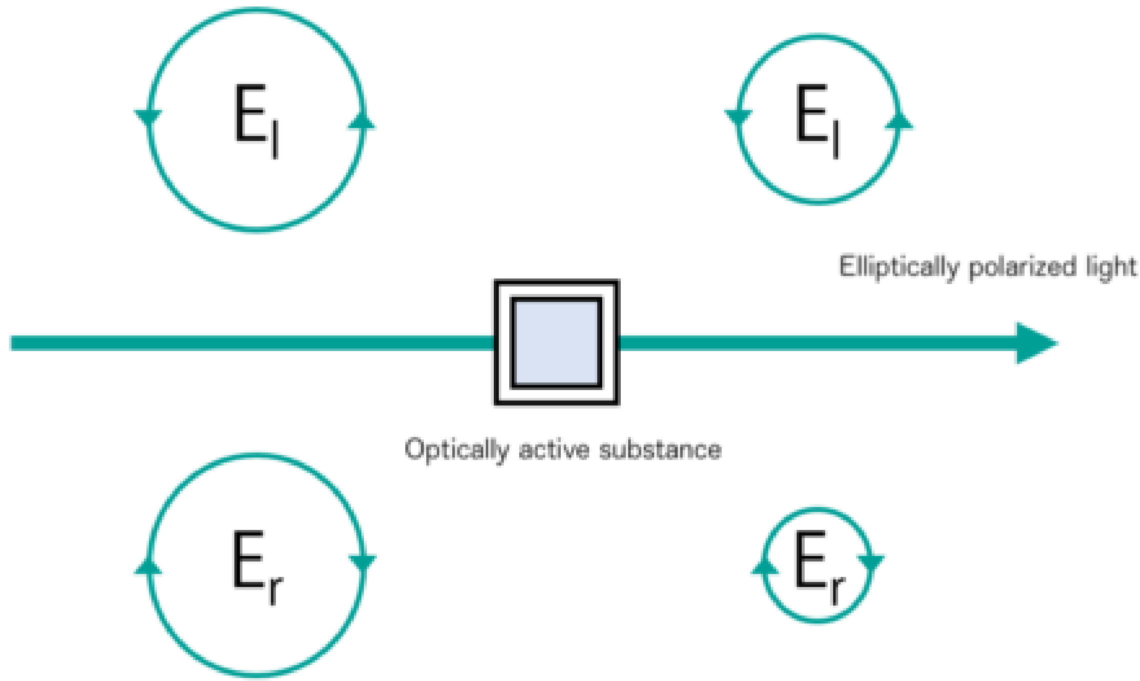


Fig. 4-1 Principles of CD：unequal absorption of circularly polarized light

... **Circular dichroism**

- Three important conclusions can be drawn:
  - ORD and CD are the manifestation of the same underlying phenomenon;
  - if an optically active molecule has a positive CD, then its enantiomer will have a negative CD of exactly the same magnitude; and
  - the phenomenon of CD can only be observed at wavelengths where the optically active molecule has an absorption band.

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, though.
- 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.
- CD spectrometry involves measuring a very small difference between two absorption values which are large signals.

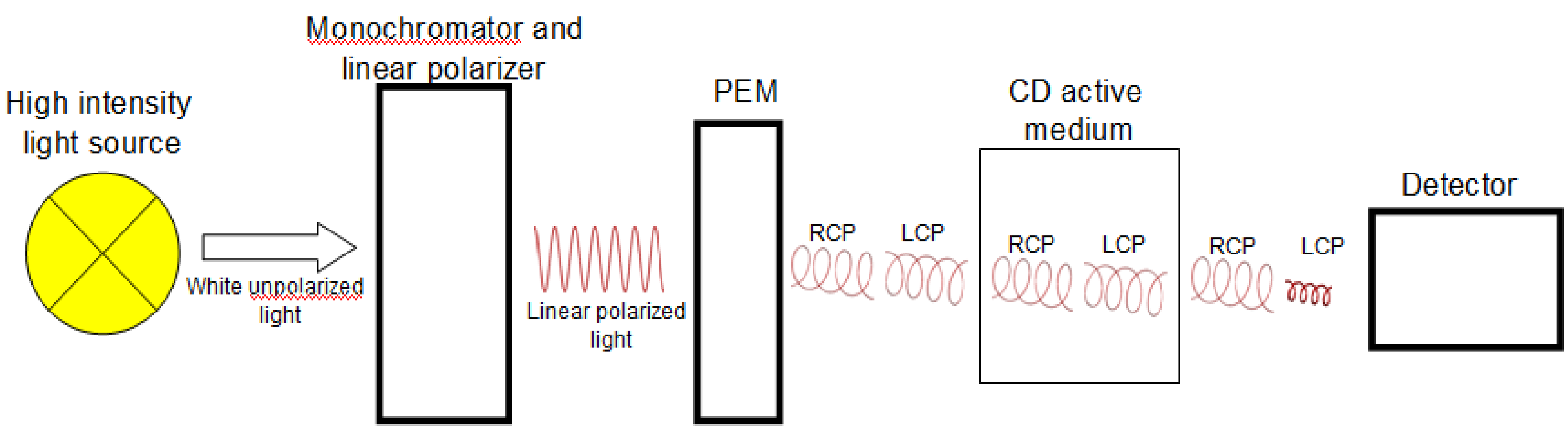


Figure 5: The instrumentation for a common CD spectrometer showing the polarization of light and the differential absorption of LCP and RCP light.

Applications

- The main application for CD spectroscopy is the verification of the protein adopted secondary structure.
- The application of CD to determine the tertiary structure is limited, owing to the inadequate theoretical understanding of the effects of different parts of the molecules at this level of structure.
- Rather than analysing the secondary structure of a 'static sample', different conditions can be tested.
- For instance, some peptides adopt different secondary structures when in solution or membrane-bound.
- 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.
- Further applications include the use of circular dichroism for an observable for kinetic measurements.