

CHAPTER 1

Introduction

Abstract

Asymmetry and symmetry are two significant aspects that are found in nature and are of great importance. This thesis work focuses on chiral materials, which are asymmetric molecules that exist in two stereoisomeric forms and are non-superimposable mirror images of each other. (Guijarro & Yus, 2008) For example, our hands show the chiral nature as our left hand is non-superimposable mirror image of our right hand as shown in Figure 1.1. With any set of rotations and translations, we can't superimpose them on each other due to the difference in alignment of fingers. Chirality also exists in the molecular world. And molecules are also non superimposable mirror images of each other as shown in Figure 1.2.

Chirality is significant and present all around us in nature, amino acids, sugars, DNA and drug molecules and have extensive applications. The specific properties of chiral molecules are to rotate plane-polarized light and show differential absorption for right and left circularly polarized light. This chapter of the thesis focuses on the literature, definition, and characteristic details of chiral molecules. The chapter gives an overview of why chiral materials exhibit optical activity and explains why enantiomers rotate plane polarized light in opposite directions. It defines chiral carbon centers and the different types of chirality found in stereoisomeric compounds. The different methods that are used to study the structural details of chiral molecules are discussed in detail. The chapter also discusses the applications of chiral compounds and the significance of particular chiral enantiomers.

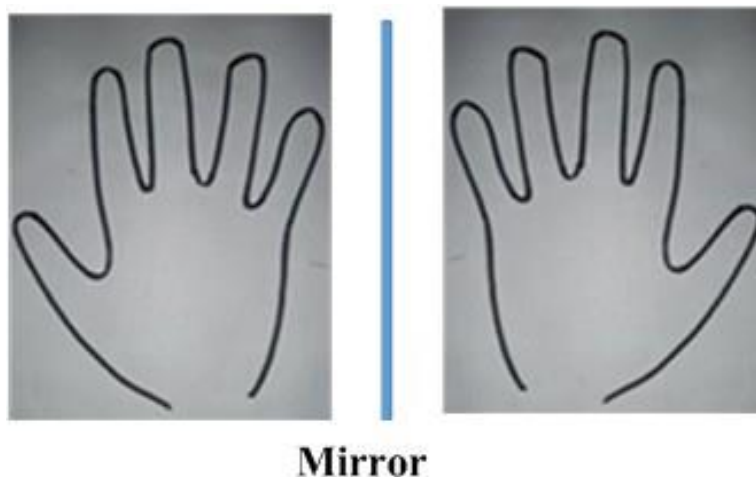


Figure 1.1: The left-hand palm is non-superimposable on the mirror image by any set of rotations and translations.

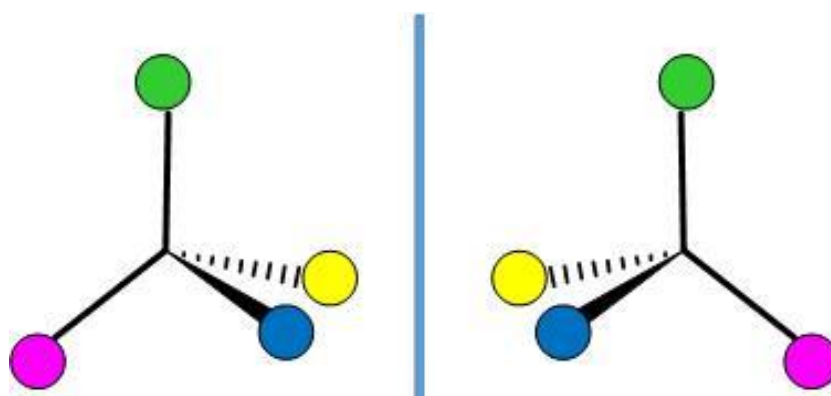


Figure 1.2: Schematic diagram of chiral molecule. Image formed on the mirror is non-superimposable mirror image.

1.1 Nature and Significance of Chirality

Chiral is derived from the Greek word "cheir," which means handedness. When the spatial arrangement of atoms in three dimensional space differs, and they exist in two stereoisomeric forms that are non-superimposable mirror images of each other, they are termed "chiral molecules".(Liu et al., 2017) Chirality is a significant property and depends on the geometrical arrangement of atoms in the molecule.(Solomons et al., 2017; Wagnière, 2007) The term "chiral" was originally used by Lord Kelvin in 1894, and according to his definition, a geometric shape is chiral if its image cannot be superimposed on itself. (Buda et al., 1992; Gal, 2011)

Chirality is important because it integrates with biology,(Gal, 2013; Inaki et al., 2016) chemistry,(Derewenda, 2008; Prelog, 1976; Ribó, 2020)physics, pharmaceuticals,(Blaser,

2013; Nguyen et al., 2006) nanotechnology, nanodots,(Đorđević et al., 2018) chiral carbon dots,(Döring et al., 2022) material science, quantum technology,(C. Zhang et al., 2021) electronics, optics, catalysis,(Noyori & Takaya, 1990) agriculture, and forestry.

The world we live in is completely chiral in nature. Our galaxies are chiral in nature and this chiral nature is linked with elementary particles(Laurence D. Barron, 2021); according to "The Carnegie Atlas of Galaxies," 540 of the 1,168 galaxies are chiral in nature. (Sandage & Bedke, 1994) Kondepudi et al. have described S- shaped and Z- shaped spiral chiral galaxies.(Kondepudi & Durand, 2001) Plants (Chinese wisteria twist in anticlockwise direction while Japanese wisteria twist in clockwise direction) (Nakamura & Hashimoto, 2020) animals (Chirality found in snails: In Lymnaea (snail species), the blastomere spindles slant clockwise, while in Pulmonata (snail species) the blastomere spindles slant anticlockwise) (Inaki et al., 2016) and biological molecules too exhibit chirality. As glucose, which is a building block of nucleic acid (example: deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA), and amino acids that are building blocks of proteins are chiral in nature, the majority of molecules in cells are chiral in nature. Proteins and nucleic acids found in the body are chiral in nature, and they are made up of small chiral molecules like amino acids and sugars.(Inaki et al., 2016) A range of pharmaceuticals (Blaser, 2013; Nguyen et al., 2006; Ötvös & Kappe, 2021) food components and beverages (The natural flavour of raspberries is of R-enantiomer of (E)- α -ionone but synthetic raspberry flavour is of racemic mixture) (Rivera et al., 2020) show chiral nature. Nonlinear optical processes are used to probe chiral molecules and chiral vibrations. According to the study, nonlinear optical processes are more sensitive to probe chemical and biological molecules. (Fischer & Hache, 2005) Recently, chirality is observed in molecular magnetic compounds with static and dynamic nature.(Inoue, 2021)

For a pharmaceutical industry, most drugs are made up of chiral molecules. A chiral molecule exists in two enantiomeric forms. A specific enantiomer of the chiral molecule is used to prepare drugs because one enantiomer of the drug can treat a specific disease while another is inactive or toxic in its property. It can be disastrous if the incorrect enantiomer is chosen, and horrific results can occur due to the toxicity of the enantiomer. Hence, it is crucial to study and differentiate chiral molecules (Sanganyado et al., 2017) for pharmaceutical industry.

1.2 Stereoisomers

Stereoisomers are molecules that have same molecular formula, same functional groups, and same constitution but different arrangements of atoms in three-dimensional space. According to the molecular arrangement of atoms, stereoisomers are of two types. 1. Enantiomers 2. Diastereomers.

Enantiomers are pairs of stereoisomeric molecules that are non-superimposable mirror images of each other. These compounds have the same molecular formula, same bond connectivity, and identical functional groups and constitutions, but differ in the three-dimensional arrangement of atoms. The physical and chemical properties of two pure enantiomers are similar, but their biological properties are different.

Diastereomers are compounds that have the same molecular formula, and same bond connectivity but with the difference, they are not mirror images of each other and their physical and chemical properties also differ. From Figure 1.3 it can be observed that D-glucose and L-glucose are enantiomers because they are non-superimposable mirror images of each other, whereas D-glucose and D-galactose are diastereomers.

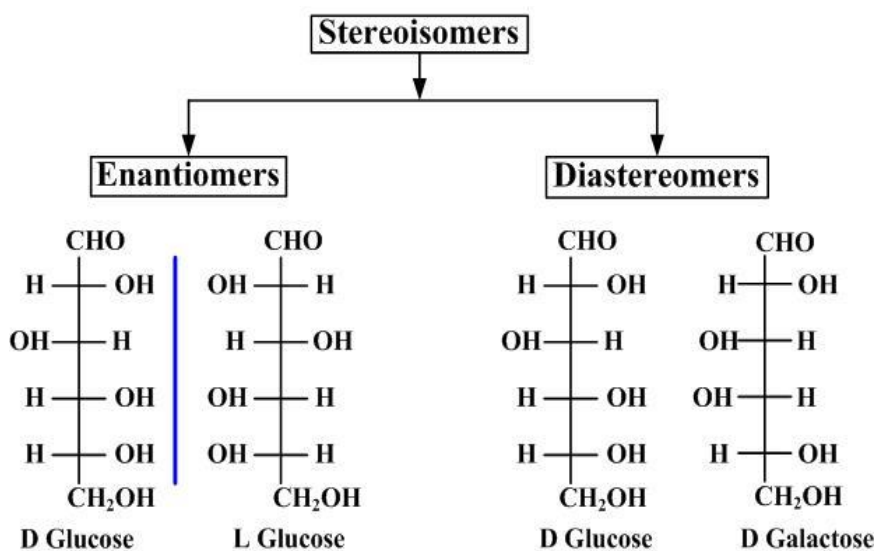


Figure 1.3: Types of Stereoisomers with example.

1.3 Discovery of Polarization

Light is a transverse electromagnetic wave where the electric and magnetic vectors oscillate in all directions in a plane perpendicular to the direction of propagation (unpolarized light) and

when it is allowed to pass through a polarizer its electric field vector is confined to a single direction (plane polarized light). If the electric field vector of light rotates in a circular path at a constant rate with a constant magnitude, it is called as circularly polarized light. It is called as right circularly polarized light (clockwise) or left circularly polarized light (anticlockwise) depending on the direction of rotation of electric field vector. When chiral samples interact with plane polarized light due to differences in electric field interaction, they rotate their plane of polarization.

Polarization is one of the specific properties of transverse waves and defines orientation of electric field shown in Figure 1.4. In 1669, Erasmus Bartholinus observed the phenomenon of double refraction in birefringent crystals (Iceland spar), although he was not aware of the phenomenon of polarization. In the year 1690, Dutch astronomer, mathematician, and physicist Christiaan Huygens analyzed double refraction and discovered plane-polarized light (PPL). (Subrahmanyam et al., 2010) Plane polarized light was discovered in the 17th century, and in Louis Pasteur observed molecular chirality in 1848.

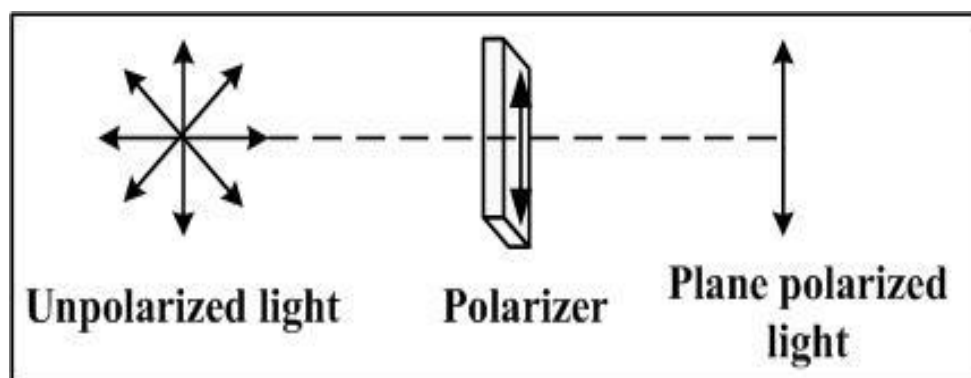


Figure 1.4: Unpolarized light travels through a polarizer, which converts it into PPL.

1.3.1 Plane Polarized Light (Linearly Polarized Light)

For a plane polarized light, oscillations of electric field vector are confined to a single direction, lying in the plane perpendicular to the direction of propagation. Plane polarized oscillations can be horizontally polarized (left and right) or vertically polarized (up and down) as shown in Figure 1.5. (Subrahmanyam et al., 2010) (Subrahmanyam et al., 2010)

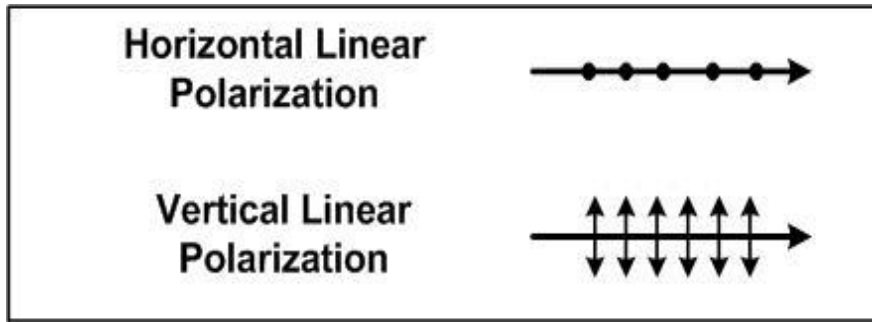
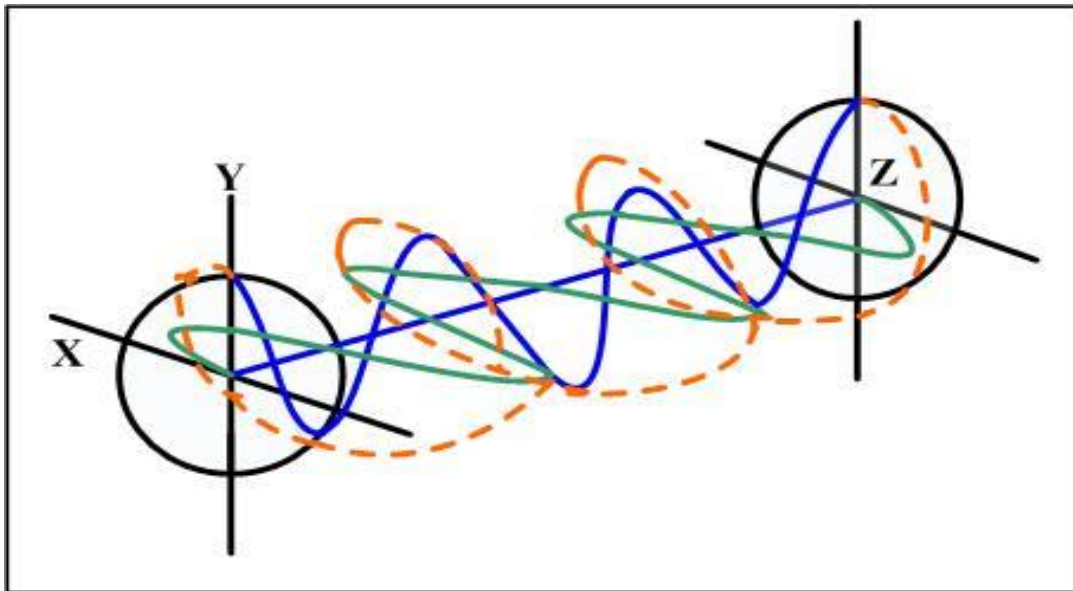


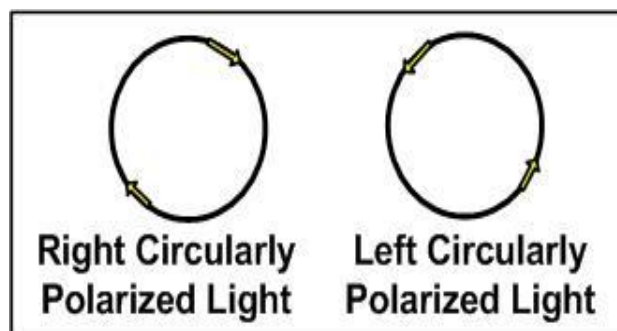
Figure 1.5: Horizontal and Vertical Polarization representation.

1.3.2 Circularly Polarized Light

Circularly polarized light is a resultant of two coherent linearly polarized light waves of equal amplitude, oscillating in perpendicular planes, and having a phase difference of 90° . When these rays superimpose, circularly polarized light is generated as shown in Figure 1.6a. In circularly polarized light, the magnitude of the electric field vector is constant, and it rotates with a constant rate around the direction of propagation and completes one rotation in one wavelength (λ). (Subrahmanyam et al., 2010)



(a)



(b)

Figure 1.6: (a) Schematic representation of circularly polarized light. (b) Schematic of right and left circularly polarized light.

Circular polarization direction is dependent on the rotation direction of the electric field vector as shown in Figure 1.6b. If electric field vector rotates in a clockwise direction, it is right circularly polarized light, and if it rotates in an anticlockwise direction, it is left circularly polarized light.

1.3.3 Elliptically Polarized Light

Elliptical polarization was explained by Augustin-Jean Fresnel in 1822. Elliptically polarized light is a resultant of two coherent linearly polarized light waves of different amplitudes oscillating in perpendicular planes and having a phase difference of 90° . When these rays superimpose, elliptically polarized light is generated. Its electric field vector rotates in an elliptical path about the direction of propagation. With respect to propagation direction, it is right-elliptically polarized light if the electric field vector rotates in a clockwise direction and left elliptically polarized light if it rotates in an anticlockwise direction.

1.4 Development in the Study of Chiral Molecules

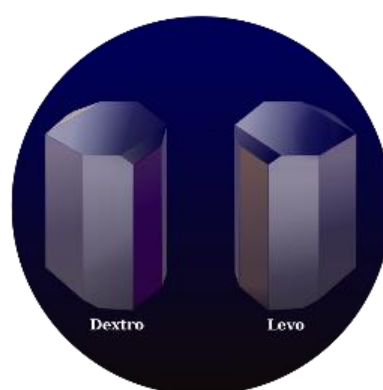
In 1811, Arago observed the rotation of plane-polarized light through a quartz crystal. Later in the year 1815, French scientist Jean Baptiste Biot observed the rotation of plane-polarized light through organic liquids such as sugar solutions and turpentine. These compounds having the ability to rotate plane-polarized light were termed as optically active compounds. When plane polarized light travels through the chiral materials, it causes **optical rotation (OR)**, either clockwise or in an anticlockwise direction, known as **optical activity (OA)**. The amount of rotation is the characteristic property of that particular organic compound.

Later, optical rotation of plane polarized light in opposite directions (clockwise or anticlockwise) was studied by English astronomer Sir John F. W. Herschel in 1822 on quartz crystals. In 1824, Fresnel proposed the theory to explain optical activity. He proposed that plane (linearly) polarized light is a combination of **50%** left circularly polarized and **50%** right circularly polarized light. When plane-polarized light is allowed to pass through chiral samples, both circularly polarized light components travel at different speeds and are absorbed within the chiral sample in different proportions. Due to this one circularly polarized component is absorbed more compared to other circularly polarized component; this difference in absorption is termed **circular birefringence**. Circular birefringence is observed in circularly polarized lights due to their chiral nature.

Louis Pasteur, a French physician and chemist, is recognized as the father of molecular chirality, shown in Figure 1.7a. In 1848 he linked optical activity and the dissymmetric arrangement (lack of some symmetry) of atoms and supported Jean Biot's theory of optical activity. He investigated the optical activity of tartaric acid crystals (sodium ammonium tartrate) found in old wine barrels. He analyzed the crystal under a microscope and was able to separate two different types of crystals from it that were found to be mirror images of each other as shown in Figure 1.7b. Then he dissolved crystals in a solvent, and two separate solutions were prepared. These solutions were studied using plane-polarized light (PPL). It was observed that the solution made up of one set of crystals rotated PPL clockwise and the solution made up of other set of crystals rotated light anticlockwise.



(a)



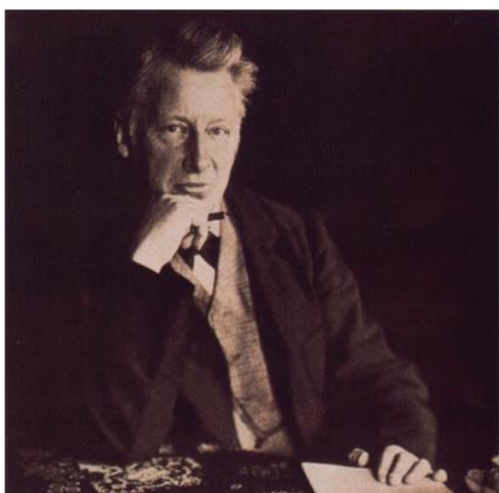
(b)

Figure 1.7: (a) Photograph of Louis Pasteur (Gal, 2011) (b) Enantiomeric crystals of tartaric acid separated by Louis Pasteur. (*Tartaric Acid*.)

Pasteur proposed that both crystals were made of the same molecules but had differences in symmetries, which rotated light in different (opposite) directions. (Derewenda, 2008; *Tartaric*

Acid.) This discovery led to the beginning of three-dimensional chemistry. Researchers believe separation of D-tartrate and L-tartrate crystals by Louis Pasteur in 1948 was the beginning of stereochemistry. (Derewenda, 2008)

In 1874, Jacobus H. van't Hoff, (Van't Hoff, 1874) a Dutch scientist, shown in Figure 1.8a, and Joseph A. Le Bel, (Bel, 1874) a French chemist, shown in Figure 1.8b, individually proposed that if a molecule has a carbon atom attached to four different groups, it exists in two stereoisomeric forms. They attempted to explain chiral molecules using tetrahedral geometry. An asymmetric carbon atom, also known as a chiral carbon or chiral center, is one that is attached to four different groups. Van't Hoff and Le Bel hypothesized that if a molecule contains n chiral carbons, the total number of stereoisomers will be 2^n . (Wisniak, 2002)



(a)



(b)

Figure 1.8: (a) Photograph of Jacobus H. van't Hoff (Krätz, 1974) (b) Joseph A. Le Bel (Encyclopaedia, 2023)

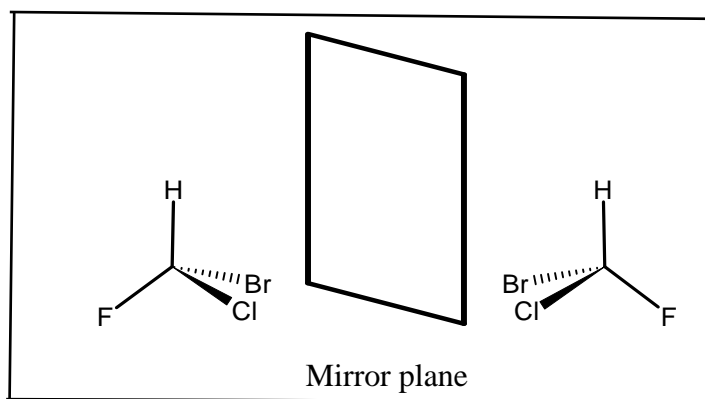


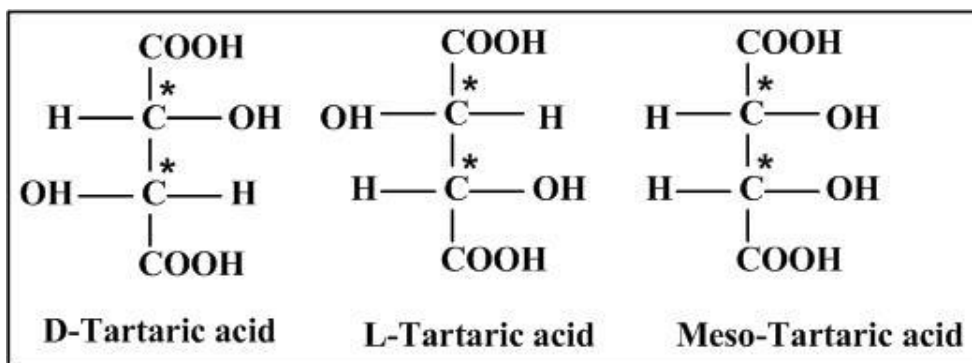
Figure 1.9: An example of a chiral molecule (bromo-chloro-fluoro methane) where the central carbon atom is the chiral center attached to four different groups. It exists in two stereoisomeric forms, both mirror images of each other.

In Figure 1.9, bromo-chloro-fluoro-methane is shown as an example of a chiral molecule, where four different atoms are attached to the central carbon atom (chiral center), and it exists in two stereoisomeric forms that are mirror images of each other.

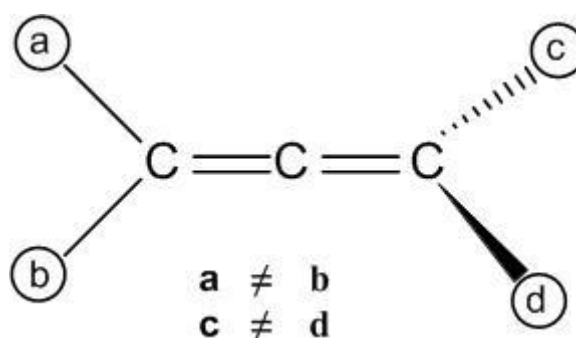
1.5 What is Chirality and how does it get into Molecules?

In terms of optical activity (OA), chirality means a molecule has the property to rotate plane-polarized light. The scientists Jacobus H. van't Hoff and Joseph A. Le Bel introduced the chiral carbon center (asymmetric carbon): a carbon attached to four different groups. Both enantiomers of chiral compounds consist of asymmetric carbon, and due to the presence of it, molecules lose their symmetry. Due to their lack of symmetry, molecules are not superimposable on their mirror images by any set of rotations or translations.

Is the absence of reflection symmetry an essential condition for a molecule to be chiral? Can multiple chiral centers be present in chiral molecules? What causes the rotation of plane-polarized light inside these molecules? Eventually, it was shown that some compounds can be achiral even though they have more than one chiral center. One such example is meso form of tartaric acid. All stereoisomeric forms of tartaric acid have two chiral centers as shown in Figure 1.10a.



(a)



Chiral allene)

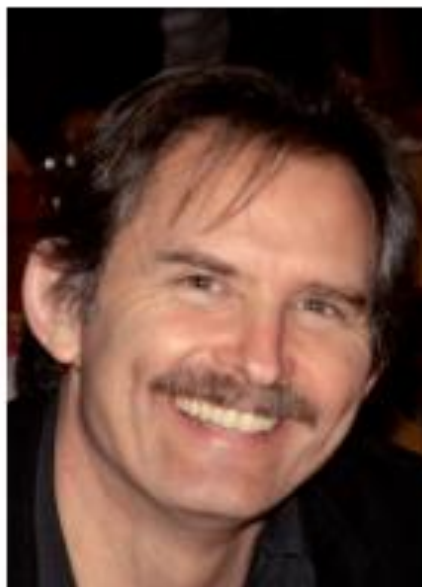
(b)

Figure 1.10: (a) Stereoisomeric structures of tartaric acid. D-tartaric acid and L-tartaric acid (optically active) and meso Tartaric acid (optically inactive). (b) Chiral allenes have two different groups attached on terminal carbon atoms.

The two forms of tartaric acid, D-tartaric acid and L-tartaric acid, are optically active (chiral), but meso-tartaric acid is optically inactive (achiral), despite the fact that each stereoisomeric structure has two chiral centers giving answer to the above query that multiple chiral centers can be present in chiral molecules. In the case of meso-tartaric acid, two chiral carbons are connected to the same group of atoms, while mirror symmetry is present as shown in Figure 1.10a, and allenes are chiral even though they don't have chiral centers. Chiral allene is shown in Figure 1.10b. Due to these exceptions, the chirality definition was modified by Kurt Mislow (Buda et al., 1992) shown in Figure 1.11a and Jay Siegel in 1984 as shown in Figure 1.11b. They correlated chirality with the molecule's symmetrical properties and modified the definition of chirality. (Mislow & Siegel, 1984)



(a)



(b)

Figure 1.11: (a) Photograph of Kurt Mislow (Buda et al., 1992) (b) Jay Siegel (S.Siegel, 2009) According to Mislow and Siegel, for a molecule to be chiral, three symmetry elements - the plane of symmetry (σ), the centre of symmetry (i), and the improper axis of symmetry (S_n) - must be absent in the molecule. The simple axis of symmetry (C_n) may or may not be present in the molecule. (Mislow & Siegel, 1984) As in meso-tartaric acid, mirror symmetry is present, and its presence signifies that it is not chiral.

1.6 Different Types of Chirality Present in the Molecule

Asymmetric molecules have no symmetries, while dissymmetric molecules are those that lack the plane of symmetry (σ), the centre of symmetry (i), and the improper axis of symmetry (S_n). Chiral molecules can be asymmetric or dissymmetric, but the necessary condition for a molecule to be chiral is that it should be at least dissymmetric. (Saha & Chakraborty, 2012) In addition to the chiral center, other chiral elements such as the chiral helix, chiral plane, and chiral axis also influence the molecule and make molecules chiral. Molecules can exhibit different types of chirality.

1.6.1 Chiral Center

A molecule is chiral if its central carbon atom is attached to four different groups. These four different groups are arranged as if they are placed at the four corners of a tetrahedron. The chiral carbon atom is also known as an asymmetric carbon atom or a stereogenic chiral center.

Bromo-chloro-fluoro-methane is a chiral compound with one chiral center, while 2-bromo-3-chlorobutane has two chiral centers are shown in Figure 1.12.

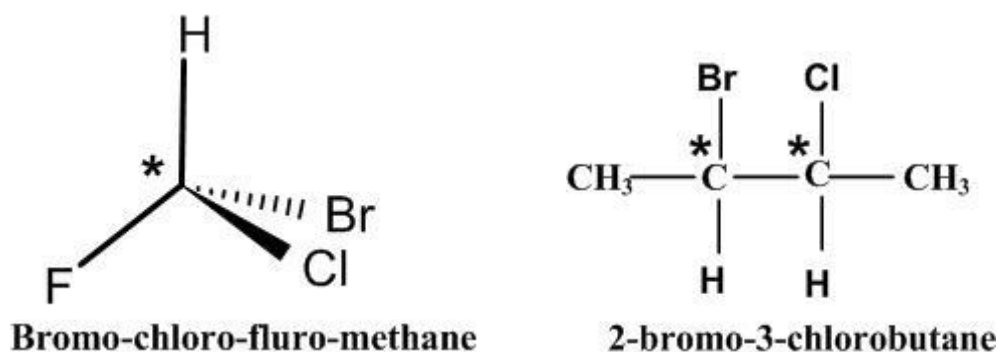


Figure 1.12: Bromo-chloro-fluoro-methane has one chiral center, while 2-bromo-3-chlorobutane has two chiral centers.

1.6.2 Helical Chirality

Helical chirality, also termed “spiral chirality” is present in helical molecules. It is a 3-dimensional chirality and is not superimposable on its mirror image. It consists of two enantiomeric forms: right handed and left handed. Helicity arises in molecules due to the overcrowding of molecules. Due to overcrowding, repulsion takes place between molecules and they do not lie on the same plane. Some molecules move above the plane and some move below the plane. If helix describes the clockwise direction, it is designated P+ and if it describes the anti-clockwise direction, it is designated M-. Helicene is an example of a helical chiral molecule shown in Figure 1.13. (Rice et al., 2018)

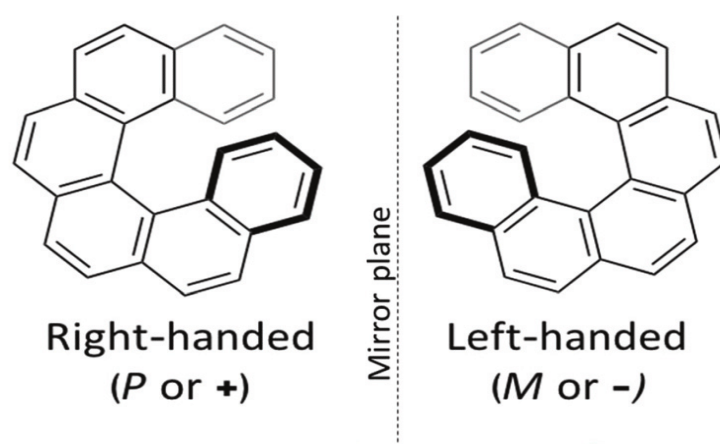


Figure 1.13: Enantiomers of helical chiral molecule.(Rice et al., 2018)

1.6.3 Planar Chirality

It develops as a result of the difference on both sides of the plane, also termed as 2-dimensional chirality. (López & Palomo, 2021) Ferrocene shown in Figure 1.14 is an organometallic compounds and an example of a planar chiral molecule, wherein the central iron (Fe) atom is bonded to two organic rings.

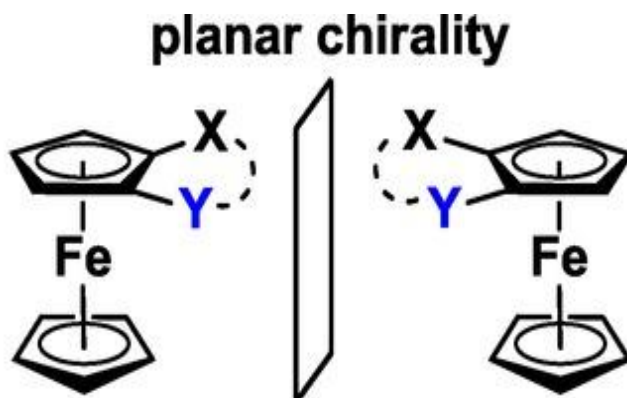


Figure 1.14: Planar chiral Ferrocenes. (Zhu et al., 2016)

1.6.4 Axial Chirality

Axial chiral molecules consist of a chiral axis about which groups are attached in a nonplanar arrangement. These molecules are not superimposable on their mirror image. Allenes (which consist of two or more double bonds) and atropisomers (BINOL) show axial chirality. Enantiomers of axial chiral molecule BINOL are shown in Figure 1.15.

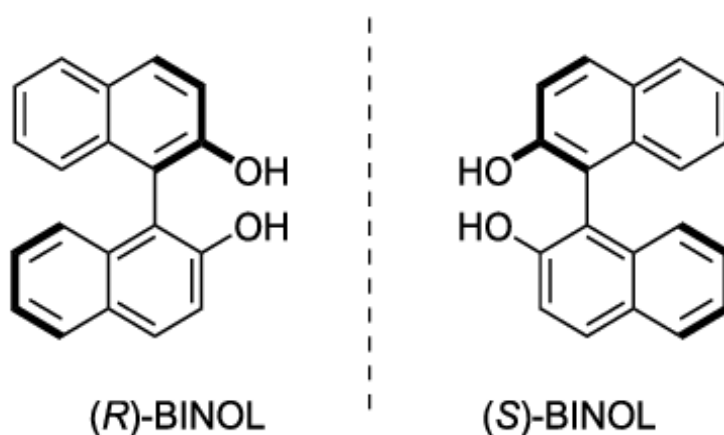


Figure 1.15: Enantiomers of axially chiral molecule BINOL. (Krajnc & Niemeyer, 2022)

1.7 Optically Active and Optically Inactive Enantiomers

The rotation of the plane of plane-polarized light is a very significant characteristic of a chiral molecule. If the plane of polarization after passing through the optical active sample is rotated in a clockwise sense, it is a dextrorotatory sample, and if it rotates in an anti-clockwise sense, it is a levorotatory sample. Schematic diagram is shown in Figure 16. (Brakel, 2012) A **racemic mixture** is a mixture of two opposite-handed enantiomers mixed in equal quantity. These mixtures contain 50% right-handed enantiomers and 50% left-handed enantiomers. When plane-polarized light falls on such samples, no optical rotation is observed. Equal proportions of right- and left-handed enantiomers cancel each other's optical rotation and are optically inactive as shown in Figure 1.16. (Brakel, 2012)

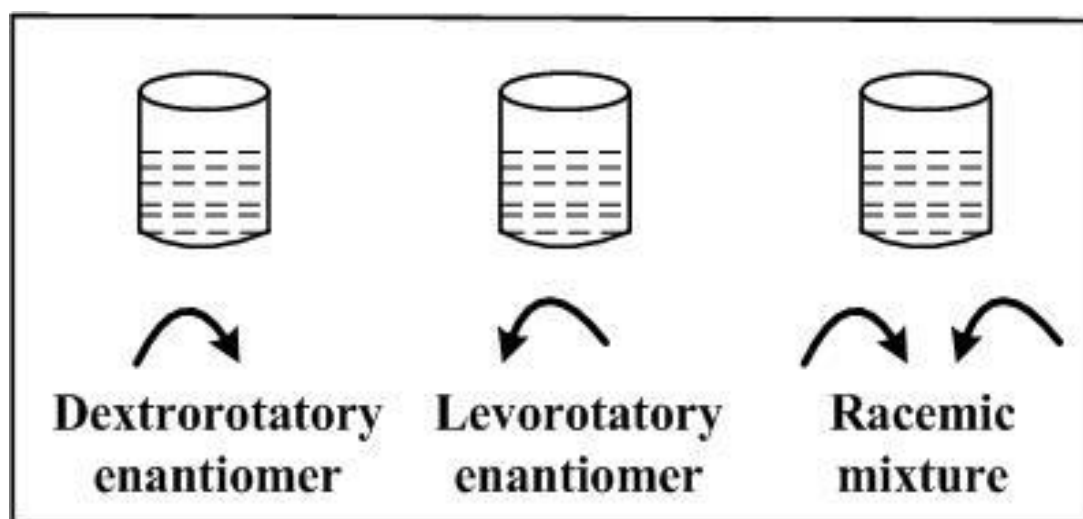


Figure 1.16: The rotation of plane polarized light through dextrorotatory enantiomer is clockwise, levorotatory is anticlockwise, and optical rotation is cancelled in racemic mixture.

1.8 Why Chiral Molecules show Optical Rotation?

Optical rotation is a significant property of a chiral molecule. When plane-polarized light falls on chiral materials plane of polarization is rotated, it is termed optical rotation as shown in Figure 1.17, chiral molecules are also known as optically active molecules. The ability to rotate the plane of polarization by an optically active substance is called optical activity. (Subrahmanyam et al., 2010)

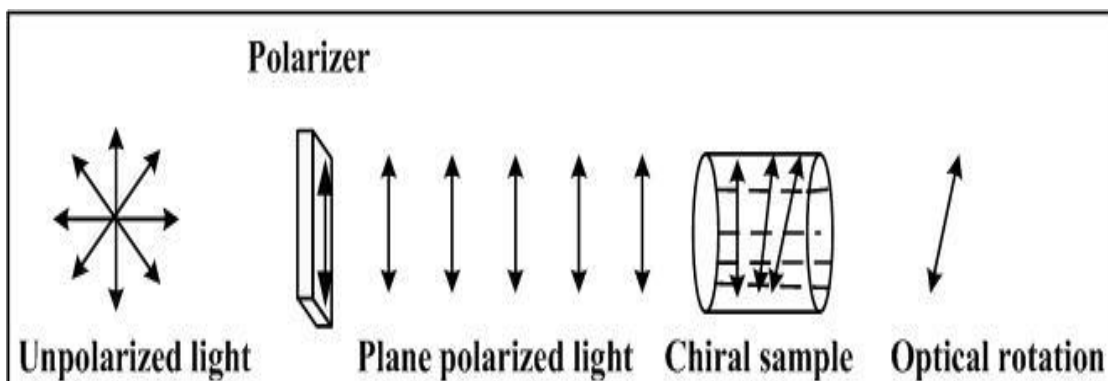


Figure 1.17: Rotation of plane of polarization through optically active sample.

1.9 The Enantiomeric Purity

Enantiomeric excess (ee) is an important parameter as it indicates optical purity. The purity of chiral molecules is quantified by using enantiomeric excess (ee). It is represented with a percentage. Right-handed or left-handed, pure enantiomers have a 100 % enantiomeric excess (ee), which denotes high degrees of purity. In a racemic combination, enantiomeric excess (ee) is 0 %. Enantiomeric excess (ee) is calculated with Eq. 1.

$$\%ee = \frac{R - S}{R + S} \times 100 \quad (1)$$

1.10 Fresnel Theory of Optical Rotation

Fresnel explained why the plane of polarization rotates as it travels through an optically active medium. A linear polarization is equivalent to two oppositely oriented circular polarizations, a combination of 50% right circularly polarized (RCP) and 50% left circularly polarized (LCP) light. When linearly polarized light travels through an optically inactive substance (achiral), both circularly polarized components are absorbed equally and travel with the same velocities within the sample, as shown in Figure 1.17. Since RCP and LCP travel at the same speeds inside the optically inactive sample, no phase difference is developed between RCP and LCP. The polarization state remains unchanged, and its vibration direction is the same as the incident direction.

When the linearly polarized light travels through an optically active material (chiral), its one of the circularly polarized components travels faster than another, as both are absorbed in different proportions. As a result, RCP and LCP travel at different velocities within the

substance, a phase difference is developed. When both circularly polarized components emerge from chiral substance, RCP and LCP combine to form linearly polarized light, but their state of polarization is shifted compared to the incident polarization direction as it can be observed in Figure 1.18.

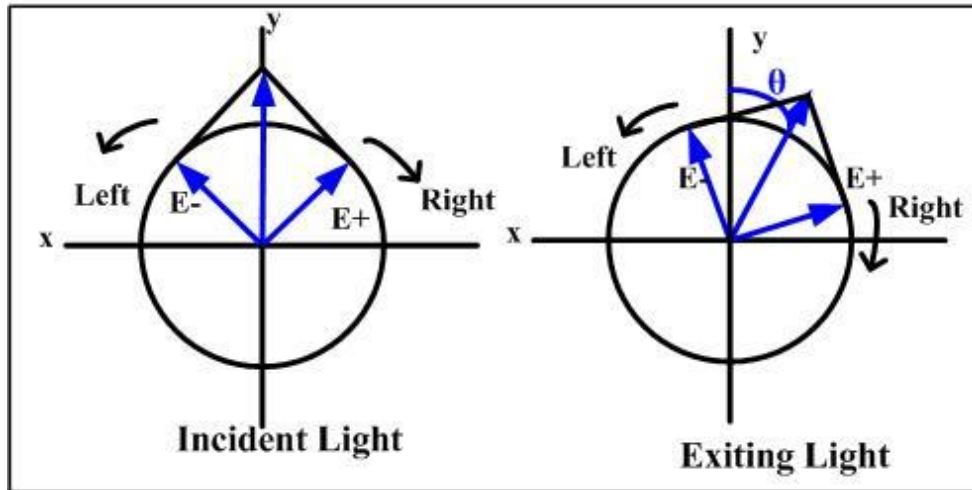


Figure 1.18: Rotation of plane of polarization due to difference in absorption of RCP and LCP.

Angle of polarization (θ) through an optically active sample is calculated by Eq. 2.

$$\theta = \frac{\pi}{\lambda}(\mu_R - \mu_L)d \quad (2)$$

d is the thickness of the optically active medium, λ is the incident wavelength, and μ_R and μ_L are refractive indices of right and left circularly polarized light, respectively.

1.10.1 Fresnel's Experimental Explanation of Optical Rotation

Fresnel gave an experimental demonstration to support optical rotation theory and developed an experimental setup that separated circularly polarized components due to **circular birefringence**. His experimental geometry consists of right-handed and left-handed quartz prisms arranged in alternate fashion in a form of rectangular box as shown in Figure 1.19. The optic axis of each prism was kept in the direction of the incident ray. When linearly polarized light enters such a geometry, it splits into RCP and LCP due to circular birefringence. RCP travels slower in the right-handed quartz prism while fast in the left-handed quartz prism, and opposite is the case for LCP. Due to this, one of the circularly polarized components has a higher refractive index in each prism.

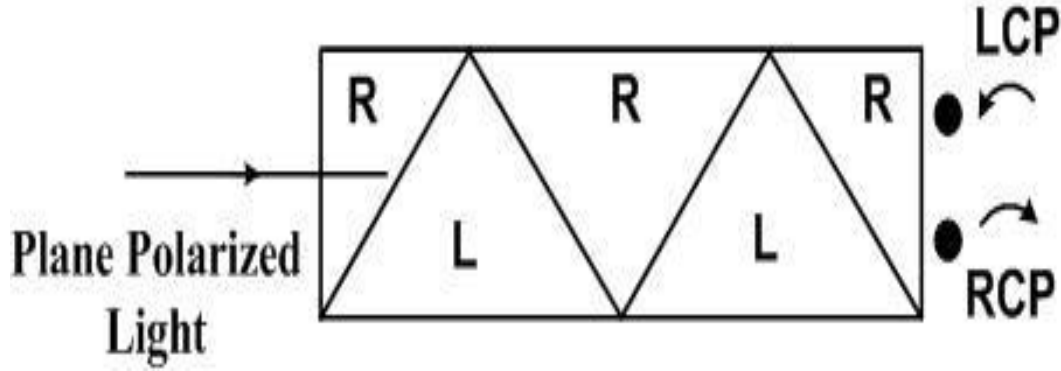


Figure 1.19: Successive alternate right-handed and left-handed quartz prisms geometry that separates RCP and LCP.

When linearly polarized light enters the first right-handed quartz prism, it splits into RCP and LCP. RCP travels slow compared to LCP in the first prism, which acts as a rarer medium for LCP, but the spatial difference is not observed at this stage because it is very small. Then both circularly polarized components enter the second left-handed quartz prism, where RCP travels fast and LCP gets absorbed more. Due to this difference in absorption, RCP bends more towards the base of the left handed quartz prism and LCP bends away from the base. With successive alternate right-handed and left-handed quartz prism arrangements, the spatial difference between RCP and LCP increases and two well separated spots can be observed. Spots are well separated and correspond to the circularly polarized components that are oriented in opposite directions. (Ghatak, 2017; Subrahmanyam et al., 2010)

1.10.2 Specific rotation

The specific rotation is a rotation of plane-polarized light when it travels through a one-decimeter-long column of optically active solution with a concentration of 1 gm/ml for a particular wavelength of light at a particular temperature. Specific rotation is calculated with Eq. 3.

$$[\alpha]_{\lambda}^T = \frac{\alpha}{c \times l} \quad (3)$$

Where $[\alpha]_{\lambda}^T$ is specific rotation of the sample, α is observed optical rotation, c is concentration in g/ml and l is the path length in dm. (Ghatak, 2017; Subrahmanyam et al., 2010)

1.11 The Significance of one Enantiomer

It is important to know the difference in chirality between two enantiomers as they are used in pharmaceuticals. Even though the physicochemical properties of enantiomers are identical, their biological activities differ. Due to differences in biological activities, they show differences in pharmacokinetics, pharmacodynamics, and toxicity. (Sanganyado et al., 2017) Some of the examples are discussed to show why it is important to understand the chirality of the enantiomer.

1.11.1 Thalidomide Tragedy

The thalidomide drug was synthesized by Kunz in 1954, a chemist at Chemie Grunenthal GmbH. (Eriksson et al., 2001) It was prescribed to pregnant women in the first trimester between 1957 and 1962 for its benefits in reducing morning sickness. But adverse effects were reported all around the world. It reduced the growth of the fetus, and thousands of children were born with severe birth defects (limb malformations).

In a study, it was found that thalidomide was marketed in racemate form and its R-enantiomer was found to have sedative effects while the S-enantiomer was found to be teratogenic and responsible for thousands of cases of defects in children. It took time to recognise the adverse effects of S- enantiomers and till then thousands of children had become victims of tragedy. (Ali & Aboul-Enein, 2007; Eriksson et al., 2001; Kallenborn & Hühnerfuss, 2001; Kim & Scialli, 2011; Tokunaga et al., 2018; Woolf, 2022; W. Zhang et al., 2019) Later it was banned, but victims of this tragedy were already pushed to live with musculoskeletal problems for the rest of their lives. (Newbronner & Karl Atkin, 2017)

1.11.2 Dopamine to treat Parkinson's

Dopamine exists in two enantiomeric forms: L-dopamine and D-dopamine. L-dopamine is synthesized in the brain. In the brain, it acts as a neurotransmitter. A lack of it causes the Parkinson's disease, in which the patient loses control of the nervous system, which affects memory and body movement. So, levodopa is given to such patients as it gets absorbed by the nerve cells in the brain and is turned into L-dopamine. While D-Dopamine is toxic and can cause severe fever with thrombocytopenia syndrome virus infection (low platelet level). (Gandhi & Saadabadi, 2022; Hu et al., 2018; Husain & Rao, 1998; Ogawa et al., 2021; Segura-Aguilar et al., 2014)

1.11.3 Penicillamine to treat Rheumatoid Arthritis

D-Penicillamine is used to cure rheumatoid arthritis, in which the immune system is disordered and it affects joints and other parts of the body. (A.Joyce, 1990; Suarez-Almazor et al., 2000) while L-Penicillamine is highly toxic.(Wilson & Vigneaud, 1948)

1.11.4 Aspartame for Diabetic People

Aspartame exists in two stereoisomeric forms. L-aspartame tastes sweet and is widely used in the food and beverage industries. It is used by diabetics people as an artificial sweetener.(Chattopadhyay et al., 2014; A. Sharma et al., 2016) It is a non-nutritive sweetener, while D-aspartame has no taste.

1.12 Different Methods to Study Chiral Materials

1.12.1 Polarimetry

E. L. Malus made the first polarimetric measurement in 1808. It is a measurement technique to measure the optical rotation of optically active solutions. It consists of a monochromatic light source, a polarizer, and an analyzer. A cylindrical tube known as a polarimeter cell is filled with the optically active sample and is placed between the polarizer and analyzer that are kept in the crossed position. For quality and purity, polarimetry is widely used in the pharmaceutical industry, the food and beverage industry, and chemical industries.(Ghatak, 2017; Nesse, 1991; Subrahmanyam et al., 2010)

Working of Polarimeter

In a polarimeter, the polarizer and analyzer's transmission axis are kept perpendicular to each other, and if the polarimeter cell is empty or an achiral sample is filled in it, no optical rotation (α) is observed. The field of view appears completely dark. But if a chiral or optically active sample is filled in the polarimeter cell, the field of view appears bright, due to the rotation of the polarization plane. This is known as the optical rotation of the chiral sample. In optical rotation, the light coming out of the polarimeter cell remains plane polarized but its plane of polarization is rotated. The amount of optical rotation depends on the concentration of the sample and path length travelled by plane polarized light, but the direction of optical rotation solely depends on the handedness of the chiral sample. Schematic of polarimeter is shown in Figure 1.20.

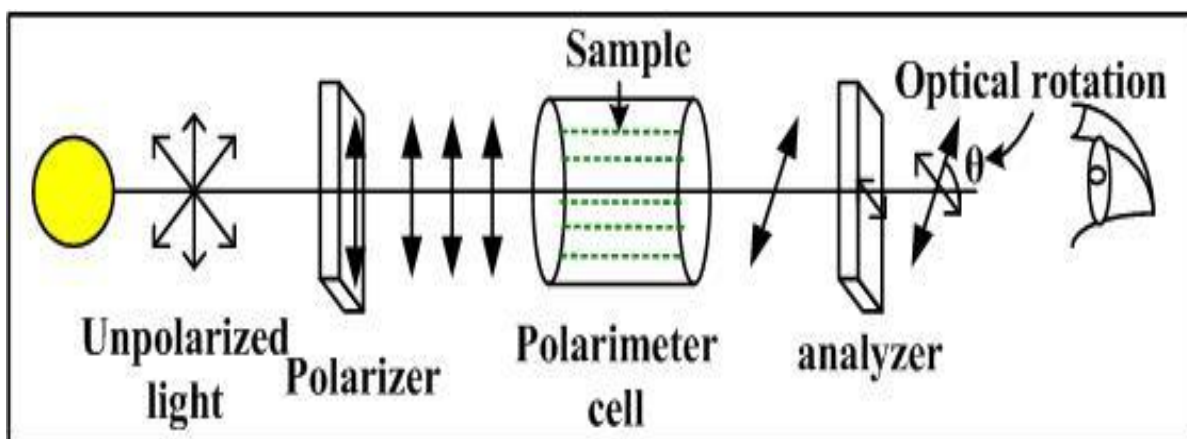


Figure 1.20: Schematic and working of polarimeter.

If the dextrorotatory sample is filled in a cell, it rotates plane-polarized light in the clockwise direction, and if the levorotatory sample is filled in it, it rotates light in the anticlockwise direction. To calculate optical rotation, the analyzer has to be rotated either in a clockwise or anti clockwise direction to make the field of view dark again. The amount of rotation and rotation direction are employed to calculate the optical rotation for an optically active sample. Once the optical rotation value is calculated, the sample's specific rotation $[\alpha]_{\lambda}^T$ can be calculated. (Gribble & Hall, 1993; Nesse, 1991; Subrahmanyam et al., 2010)

1.12.2 Types of Polarimeter

The amount of rotation of plane-polarized light through optically active samples is measured with a scientific polarimeter available on the market. Some of the polarimeters are discussed below. All the polarimeters mentioned have a sample cell positioned between the polarizer and analyzer. The polarizer and analyzer are kept in a crossed position. To enhance the polarimeter's working, some other optical elements are included in the optical path for precise measurement.

1.12.1.1 Laurent's half-shade polarimeter

1.12.1.2 Biquartz polarimeter

1.12.1.3 Lippich polarimeter

1.12.1.1 Laurent's Half-Shade Polarimeter

In Laurent's half-shade polarimeter, after the polarizer half shade plate (HSP) is placed. It is made up of a semicircular half-wave plate ($\Delta = \frac{\lambda}{2}$, $\delta = \pi$) cemented with a semicircular glass plate. Half of the incident light passes through the half-wave plate and the other half through the glass plate. In this combination, a semicircular half-wave plate turns the plane of polarization of the incident light by 2θ . (half wave plate rotate incident plane of polarization by 2θ) This geometry is used to measure optical rotation values precisely. (Subrahmanyam et al., 2010) Schematic of half shade plate used in Laurent's polarimeter is shown in Figure 1.21.

A monochromatic source is used, the polarizer and analyzer are placed in crossed positions, and a chiral sample is filled in the sample chamber. Just before the sample chamber, half shade plate (HSP) is placed, and the optic axis of the half wave plate is parallel to the refracting edge in the direction AB. Light vibrations that pass through the glass plate are along OD, while vibrations in HWP are divided along OA (e-ray) and ON (o-ray). HWP introduces a phase difference of π and o-ray vibrations are confined in the direction OM. Resultant vibrations from HWP are along OC, and vibrations from glass are along OD.

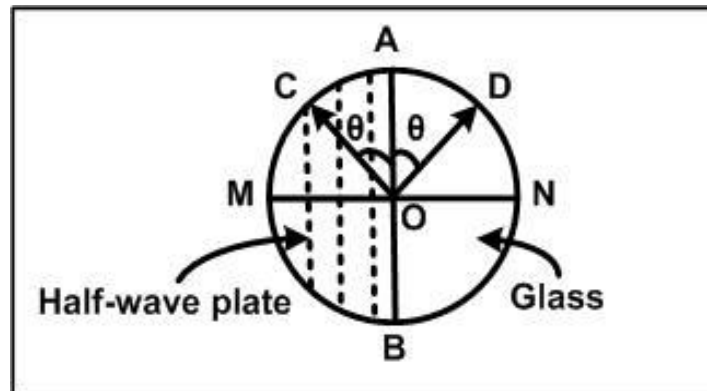


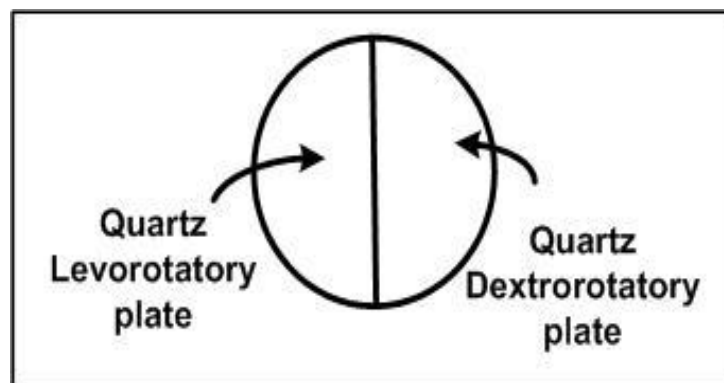
Figure 1.21: Schematic of half shade plate used in Laurent's polarimeter.

If the analyzer transmission axis is parallel to OC, HWP is illuminated, and the glass plate appears dark. When analyzer's transmission axis is parallel to OD, glass plate is illuminated, and the HWP plate appears dark. When a chiral sample is filled in the sample chamber, HSP is illuminated partially, and the analyzer is rotated to observe complete darkness in both halves, and the optical rotation of the sample is measured precisely. (Subrahmanyam et al., 2010)

1.12.1.2 Biquartz Polarimeter

In these polarimeters, a biquartz plate is used for the precise measurement of optical rotation. It is a combination of two semicircular plates of quartz; one plate is dextrorotatory and the other is levorotatory. Optic axis of the quartz plate is perpendicular to the refracting edge. A white light source is used in a biquartz polarimeter. The thickness of each semicircular plate is kept 3.75 mm so that it can rotate the yellow light to 90° . A schematic of a biquartz plate is shown in Figure 1.22a. Vertically polarized light is allowed to fall on the biquartz plate (optically active plates),

Both quartz plates rotate PPL in opposite directions as the angle of rotation of light is inversely proportional to the square of wavelength. Hence, the red light is rotated less compared to the blue light, as shown in Figure 1.22b. Rotated light is allowed to pass through the sample. The analyzer is placed after the sample chamber and its transmission axis is rotated. When the analyzer's transmission axis is perpendicular to the polarizer axis, yellow light is not allowed to pass through the analyzer and darkness is observed. If its transmission axis is rotated at one particular orientation, half plate appears blue and half appears red. When the analyzer transmission axis is oriented in the opposite direction, colours are reversed. The analyzer transmission axis is rotated to observe equally bright colours, and the optical rotation of the sample is assigned precisely. (Subrahmanyam et al., 2010).



(a)

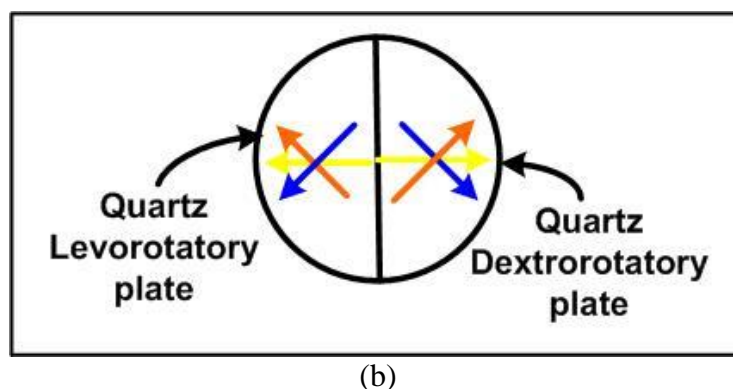


Figure 1.22: (a) Schematic of biquartz plate used in biquartz polarimeter. (b) Colours observed through biquartz plate.

a) 1.12.1.3 Lippich Polarimeter

Lippich polarimeters work for multiple wavelengths, so they have wide use in polarimetry. It consists of two small nicol prisms, A and B, and a third nicol prism C, half the size of A. After Nicol prism A, Nicol prism C is placed, covering half field of view of the polarizer. The polarizer (A) and small Nicol prism's (C) plane of vibrations are kept at a certain angle, followed by the sample cell and analyzer. Sample cell containing an optically active sample. Schematic of Lippich polarimeter is shown in Figure 1.23.

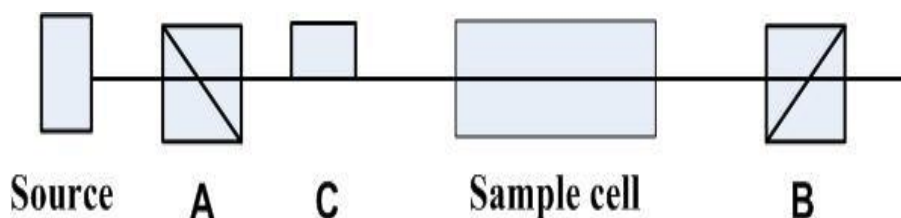


Figure 1.23: Schematic of Lippich polarimeter.

When the analyzer is rotated, the field of view shows differences in illumination in the right and left halves due to the orientation of Nicol prisms A and C. The analyzer is rotated precisely so that both halves can be observed equally illuminated, and optical rotation is measured. (Subrahmanyam et al., 2010)

1.12.3 Optical Rotatory Dispersion (ORD)

Optical rotatory dispersion (ORD) is the measurement of optical rotation as a function of wavelength. It is a spectroscopic method that is useful to determine the structure and absolute configuration of the optically active chiral molecule. It involves a sample placed between two crossed polarizers, and a device that measures optical rotation versus wavelength is called a spectropolarimeter.

When this optical rotation is measured with respect to wavelength, it is called optical rotatory dispersion (ORD). It depends on certain parameters: the nature of the sample, the length of the sample cell, the concentration of the sample, the temperature, and the wavelength of the incident light. ORD is useful to determine optically active compounds, analyze them quantitatively, and measure absolute configurations. Dextrorotatory and levorotatory samples are first assigned by looking at the ORD curves of the samples. After that, optical rotatory curves are correlated to a known configuration, and the absolute configuration of the chiral sample is identified. (Castiglioni et al., 2011; Eyring et al., 1968; Snatzke, 1968; Yang et al., 2010) Curves observed in optical rotatory dispersion are explained with positive and negative cotton effect curves. If the sample is dextrorotatory, the ORD curve rises to a maximum, falls rapidly to a minimum and then slowly rises while opposite is the effect when levorotatory sample is filled in sample chamber. (Jirgensons, 1965)

Circular dichroism (CD) is an absorption spectroscopy that uses circularly polarized light to study the structures of optically active chiral molecules. CD working mechanism depends upon differential absorption of left circularly polarized (LCP) and right circularly polarized (RCP) light. Ultraviolet circular dichroism UV (CD) is used to investigate secondary and tertiary structures of proteins and UV/Vis is used to investigate charge transfer transitions Schematic of circular dichroism spectroscopy is shown in Figure 1.24.

In Circular Dichroism (CD) electronic transitions are probed. (V. Sharma et al., 2009) this difference in absorption as a function of wavelength can be measured with a circular dichroism spectropolarimeter. Circular dichroism (CD) absorption spectroscopy is used to investigate the internal structure of chiral molecules and the secondary and tertiary structure of proteins as LCP and RCP light are absorbed to different extents in optically active compounds and they travel through chiral samples at different speeds.(Eyring et al., 1968; Snatzke, 1968)

It consists of a monochromatic light source that is allowed to pass through a polarizer. The voltage modulation of the electro optic modulator (EOM) is done through a high voltage amplifier, and alternately linearly polarized light is modulated to right and left circularly polarized light. When incident light switches between RCP and LCP, there is a difference in absorption as both are absorbed to a different extent through the sample. Then the light transmitted through the sample is allowed to fall on the detector.

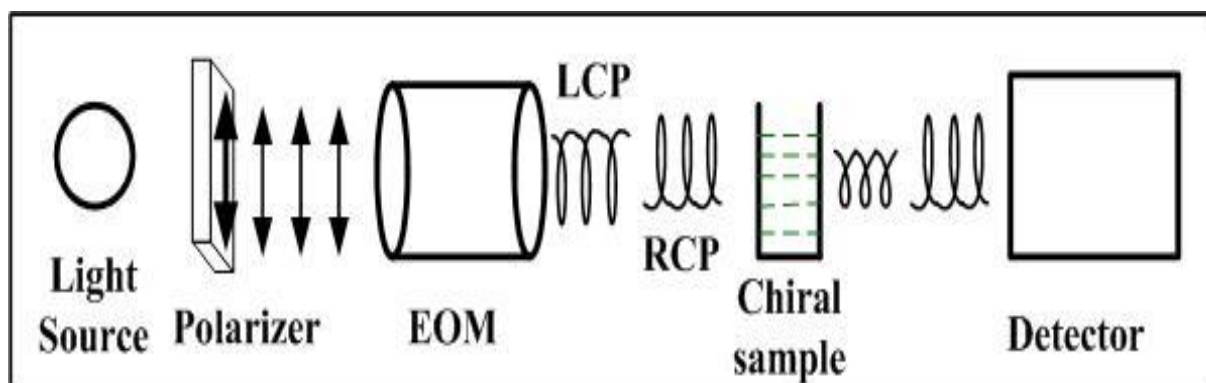


Figure 1.24: Schematic of circular dichroism spectropolarimeter. The differential absorption of RCP and LCP through a chiral sample helps to investigate structure of a molecule.

Working Mechanism of Circular Dichroism Spectropolarimeter

It works on the concept of absorption and follows the Beer-Lambert Law proposed by August Beer and Johann Heinrich Lambert. It states the relationship between absorbance and concentration of optically active materials. According to Beer-Lambert-Law, absorbance through the material when light travels through it is calculated with Eq. 4.

$$A = \epsilon cl \quad (4)$$

In Eq. 4, A is the absorbance amount of light, ϵ is the molar extinction coefficient, c is the concentration of the sample, and l is the path length through which light travels. The change in polarization is measured with θ , which is equal to Eq. 5.

$$\theta = \frac{2.303}{4} (A_L - A_R) \cdot \frac{180}{\pi} \text{ degrees} \quad (5)$$

Where A_L and A_R are absorbance for left and right handed circularly polarized light through optically active sample.

1.13 Raman Spectroscopy

The Raman effect is the inelastic scattering of photons observed by Professor C. V. Raman and his research scholar K. S. Krishnan in 1928. (Raman & Krishnan, 1928) He was honoured with the Nobel Prize in 1930 for his incredible work. A. Smekal explained the Raman effect theoretically in 1923.(Smekal, 1923) According to the Raman effect, the frequency of scattered photons after interaction with matter differs from the frequency of the incident photons. When an incident photon of a certain frequency interacts with matter, scattered radiation consists of frequencies that are lower and higher in frequency with respect to the incident photon, and the direction of the scattered photons is different from the direction of the incident photon.(Banwell & Mccash, 2017; Landsberg & Mandelstam, 1928) Raman spectroscopy is a molecular spectroscopic technique that probes vibrational energy levels of a molecule and provide information about molecule.

If the energy of the incident photons after scattering doesn't change, this is termed "elastic scattering" and is known as Rayleigh scattering. Raman scattering is observed when a static electric field from light in the form of electromagnetic wave is applied to a molecule, it becomes deformed (distorted) because the positively charged nuclei of an atom are attracted to the negative pole of the electric field, whereas electrons are attracted to the positive pole of the electric field. The electric dipole moment (μ) is induced due to this distortion and separation between nuclei and electrons. The value of the induced dipole moment (μ) is affected by the applied electric field E and its value is equal to Eq. 6.

$$\mu = \alpha E \quad (6)$$

In Eq. 6, α is the molecule's polarizability and E is applied electric field. If a molecule vibrates with natural vibrational frequency ν_{vib} and an electric field E of frequency ν_1 is incident on it. The value of the induced dipole moment (μ) is shown in Eq. 7.

$$\mu = \alpha_0 E_0 \sin(2\pi \nu_1 t) + \frac{1}{2} Q_0 E_0 \left(\frac{\partial \alpha}{\partial Q}\right) [\cos(2\pi (\nu_1 - \nu_{vib})t) + \cos 2\pi (\nu_1 + \nu_{vib})t] \quad (7)$$

The first term of Eq. 7 is for Rayleigh scattering. The second and third terms of the equation are Raman scattering and represent Stokes and anti-Stokes scattering, respectively. Anti-Stokes scattering has a higher frequency, while the frequency of Stokes scattering is lower compared

to the frequency of the incident photon. The schematic of Rayleigh and Raman scattering process is shown in Figure 1.25.

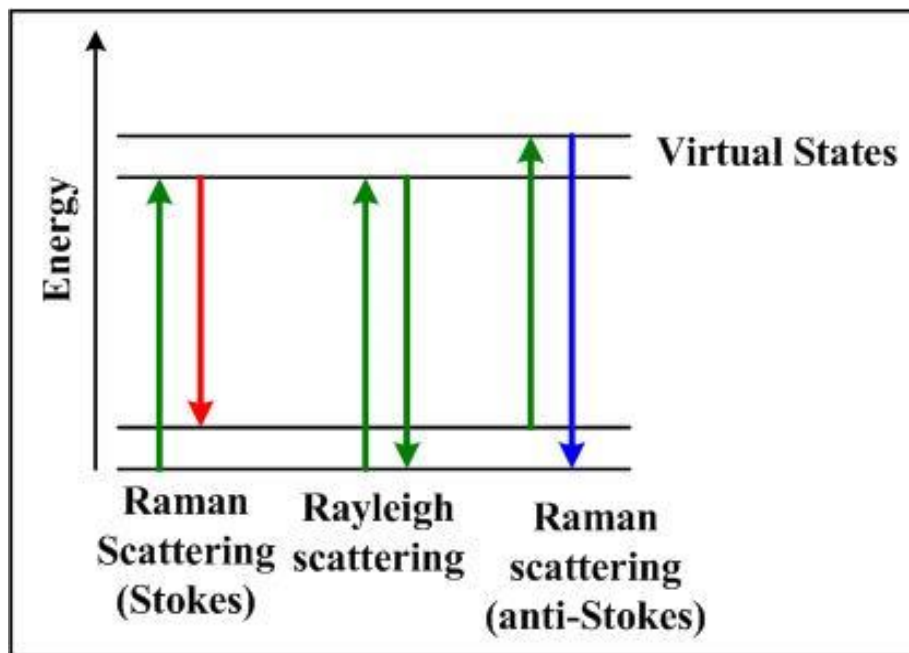


Figure 1.25: Schematic diagram of the Rayleigh (elastic scattering) and Raman scattering (stokes and anti-stokes inelastic scattering).

The total number of vibrational modes of a molecule is influenced by the symmetry of the molecule. If a molecule is linear, such as HCl or CO₂, their number of vibrational modes is $3N-5$, and for non-linear molecules, the number of vibrational modes is $3N-6$. (Banwell & Mccash, 2017)

1.13.1 Essential Condition for a Molecule to be Raman Active

Raman activity in a molecule is correlated with the polarizability ellipsoid shown in Figure 1.26. It is a three-dimensional surface whose distance from the elliptical center is proportional to $\frac{1}{\sqrt{a_i}}$. Where a_i is the polarizability along the path that connects position i on the ellipsoid with the electrical center. (Banwell & Mccash, 2017; Vandenabeele, 2013; Vasilescu & Adrian-Scotto, 2010)

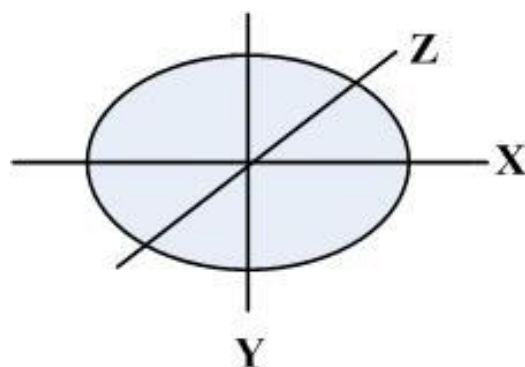


Figure 1.26: Polarizability ellipsoid. (Vasilescu & Adrian-Scotto, 2010)

For a molecule to be Raman active, its rotation and vibration should change molecule's polarizability. An essential condition for a molecule to be Raman active is that if either of its parameters (magnitude, shape, or orientation of the polarizability ellipsoid) changes, the molecule is Raman active. (Banwell & Mccash, 2017; Wong, 1994) The polarizability ellipsoid of the water (H_2O) molecule is shown in Figure. 1.27. Water molecules have three vibrational modes. Symmetric stretching mode, bending mode, and asymmetric stretching mode.

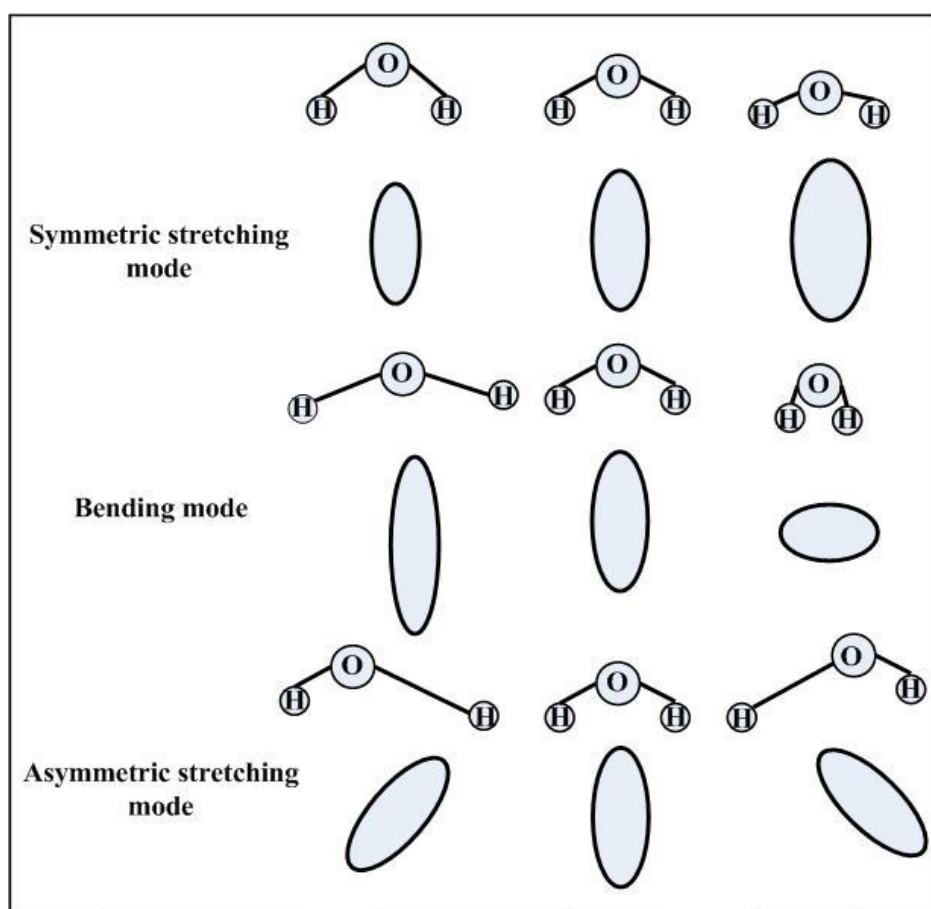


Figure 1.27: Polarizability ellipsoid of water molecule for different vibrational modes.

All modes of the water molecule are Raman active. The polarizability ellipsoid's size changes in symmetric stretching and bending modes, and both modes are Raman active. While in asymmetric stretching mode, the direction of the polarizability ellipsoid changes and is Raman active. (Banwell & Mccash, 2017)

1.13.2 Requirements of Raman Spectroscopy

In Raman spectroscopy, peak position, peak shift, bandwidth, and intensity are essential parameters that give detailed information and the fingerprint of the molecule. Vibrational frequency depends on the symmetry and the structure of the molecule. The peak position in a Raman spectrum is correlated to the bond length/strength of the molecule. Peak intensity is determined by the transitions that occur according to the selection rule. (Banwell & Mccash, 2017; Vandenabeele, 2013)

1.13.3 Relation between Symmetry of Molecule and Raman Activity

Two spectroscopic techniques to probe vibrational transitions in a molecular system are Raman spectroscopy and Infrared spectroscopy. Infrared spectroscopy is an absorption spectroscopy and a complementary technique to Raman spectroscopy. The symmetry of the molecule affects Raman activity and helps to determine whether the molecule is Raman active or not. The rule that establishes a correlation between the symmetry of the molecule and their Raman activity and infrared activity is termed the "*rule of mutual exclusion.*" According to the rule, vibrations that are Raman active will be inactive in infrared if a molecule has a center of symmetry, and some of the vibrations will be active in both Raman and infrared if the molecule has no center of symmetry.

1.13.4 Applications of Raman spectroscopy

Raman spectroscopy can be performed in all ranges of wavelengths and is widely used in pharmaceuticals to determine the active pharmaceutical ingredient (API) concentration present in drugs, to check raw material verification, to check purity, to identify contaminants, and in combinational chemistry. It is widely used in geology and mineralogy to identify gemstones and minerals, to identify mineral and phase distribution in minerals. To know the behaviour of materials in extreme conditions. It is a very useful tool to check for toxic materials present in the environment. (Rostron et al., 2016)

1.14 Vibrational Optical activity (VOA)

Vibrational Optical activity is a spectroscopic technique that deals with optically active chiral molecules. It works on the principle of differential interactions of chiral molecules with RCP and LCP light. This spectroscopic technique depends upon the vibrational transitions that take place in the molecules. Two types of vibrational optical activity are: **Raman Optical activity** in which intensity difference in RCP and LCP in scattered light is analyzed and **Vibrational circular dichroism** in which differential absorption of RCP and LCP is analyzed. (L. A. Nafie et al., 1976; Laurence A Nafie, 2018) VOA flow chart is shown in Figure 1.28 and both vibrational spectroscopic techniques provide detailed structural information of the molecule. (Laurence A Nafie, 2018; Online & Bour, 2014)

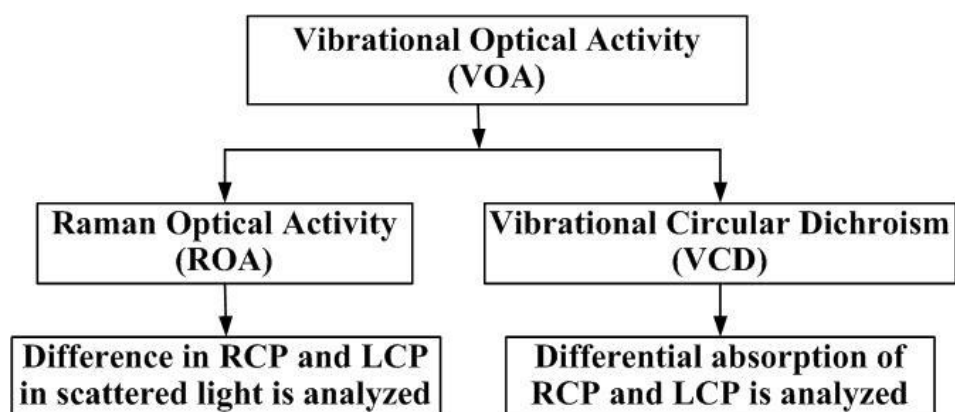


Figure 1.28: Types of Vibrational optical activity (VOA).

1.15 Raman Optical Activity (ROA)

Raman optical activity (ROA) is a chiroptical phenomenon and a powerful spectroscopic technique to study structural details of the optically active chiral molecules. In it, left and right circularly polarized light are allowed to fall on the chiral sample, and scattered light is analyzed for structural details. (Atkins & Barron, 1969) When LCP and RCP light scatters from an optically active chiral sample it contains circularly polarized components in it. These scattered circularly polarized components have a small difference in Raman intensities, and this difference is minutely reviewed to provide information on the absolute configuration of the organic compounds and biological chiral molecules such as proteins and viruses. (L D Barron et al., 2002)

When LCP and RCP light fall on one enantiomer of a chiral sample, ROA is observed due to a change in the polarizability and optical activity tensors of the molecule. When scattered light is collected, it provides information about that enantiomer, and when ROA spectra of other enantiomer is collected, it is completely mirror image (opposite) of the previous enantiomer. In Figure 1.29, different types of ROA experimental configurations are shown, first type of configuration is Incident Circular Polarization Raman optical activity ICP(ROA), in which small difference in scattered intensities is observed when right and left circularly polarized light is incident on the sample. Second is Scattered Circular Polarization Raman optical activity SCP(ROA), in which difference in scattered circular polarization is determined when unpolarized or linearly polarized light falls on the sample. and the third is Dual Circular Polarization DCP (ROA), in which both incident and scattered intensities are circularly polarized. ICP(ROA) and SCP(ROA) are combined in DCP. and when incident and scattered modulations are in phase it is DCP_I(ROA); when incident and scattered modulations are out of phase, it is DCP_{II}(ROA). (Laurence A. Nafie et al., 1991)

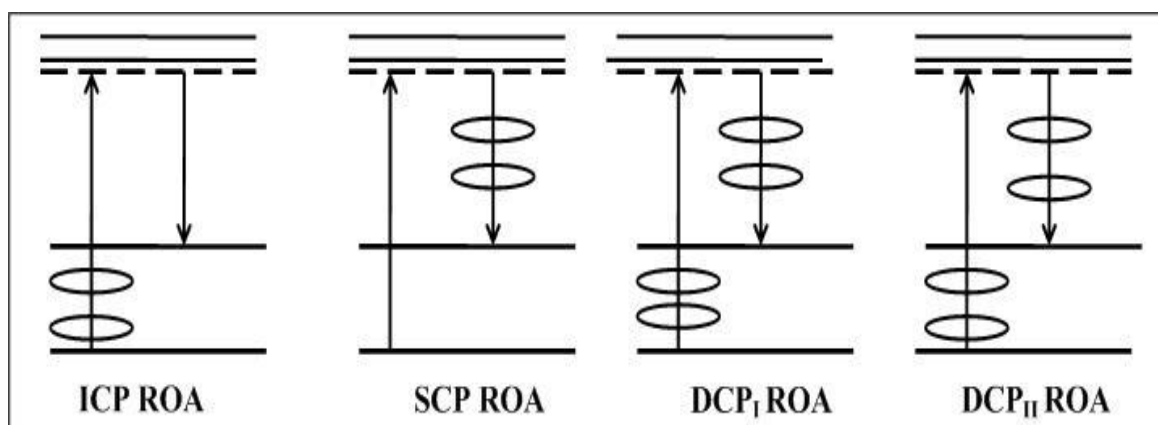


Figure 1.29: Energy level diagrams of different forms of ROA. (Laurence A Nafie et al., 1991) This small intensity difference in scattered light was first observed by P.W. Atkins and L.D. Barron in 1969 at the University of Oxford, and to find the difference between scattered intensities, Barron & Buckingham (1971) introduced the dimensionless circular intensity difference (CID) parameter. (Laurence D. Barron & Buckingham, 1971)

$$\Delta = \frac{I_R - I_L}{I_R + I_L} \times 100 \quad (8)$$

The circular intensity difference is calculated with Eq. 8. Where I_R and I_L are scattered intensities when RCP and LCP light is incident on the sample. Lutz Hecht and Barron at the

University of Glasgow made several developments in Raman optical activity setups and recorded the optical activity spectra of several samples.(Hecht & Barron, 1994)

Werner Hug of the University of Fribourg introduced the ROA geometry that observes differences in scattered intensities that contain right and left circularly polarized (SCP) components. When plane polarized or unpolarized light falls on the chiral sample. (Werner Hug, 2006; Werner Hug & Hangartner, 1999) Later, BioTools, Inc. manufactured the ChiralRAMAN equipment to observe ROA; it was based on SCP. In 2003, Werner Hug modified the optical setup with a half wave plate and introduced the novel concept of virtual enantiomer of the chiral molecule. (W Hug, 2003)

In ROA new polarization effect is observed due to the interference mechanism between light waves scattered due to the polarizability and optical activity property tensors.(Laurence D. Barron, 2015; Laurence D Barron et al., 2007) Ab initio simulations of ROA spectra provide three dimensional structure and orientation details of the groups attached to the molecule.

In recent years, ROA, an analogue of VCD, has emerged as a powerful spectroscopic technique that measures a difference in the Raman scattering intensities of left and right circularly polarized light and provides detailed stereochemical information about a chiral molecule.(Yamamoto & Watarai, 2010) Raman spectroscopy is a powerful spectroscopic technique and gives detailed vibrational information of molecule while Raman Optical Activity is sensitive to optically active chiral molecules and varies due to the vibrational coordinates of an optically active molecule.(Laurence D. Barron & Buckingham, 1971)

1.16 Vibrational Circular Dichroism (VCD)

Vibrational circular dichroism (VCD) is an extension of circular dichroism spectroscopy (CD) and a complementary spectroscopic technique to Raman optical activity (ROA). It works in the infrared and near-IR regions and vibrational transitions are observed. It measures the difference in absorbance of right and left circularly polarized light when it passes through an optically active chiral sample as shown in Figure 1.30. This technique provides absolute stereochemical configuration and structural details of the chiral molecules and proteins. (L. A. Nafie et al., 1976; Sato, 2020) These molecules can be studied in both solid as well as in solution forms.

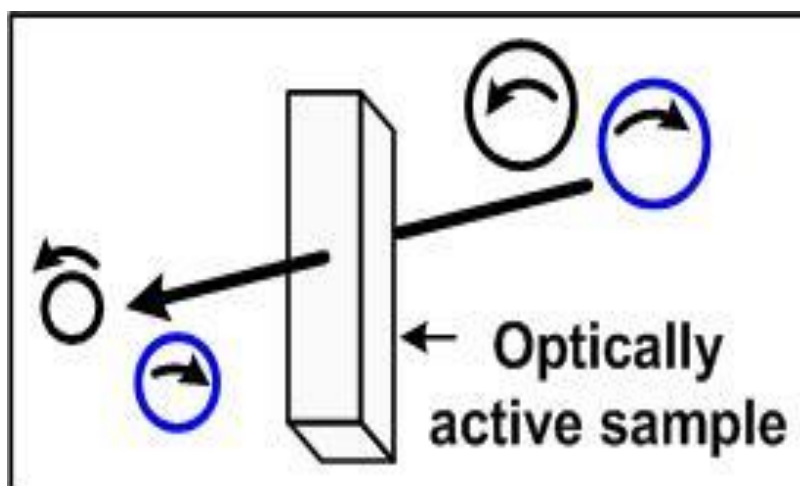


Figure 1.30: Vibrational circular dichroism (VCD) measures difference in absorbance of right and left circularly polarized light.

1.17 Negative Refractive Index Metamaterials (NIMs)

Negative refractive index metamaterials (NIMs) are artificially made structures. The word "metamaterial" is a Greek word where "meta" implies "something beyond the real." They exhibit unique electromagnetic properties that are not present in natural materials. When light falls on such metamaterials, it bends light in the opposite direction from the conventional materials as shown in Figure 1.31. These materials were theoretically proposed by Veselago in 1968 and brought into practical realization by J.B. Pendry. Transmission of electromagnetic radiation can be altered by fabricating structures in such a way that their permeability (μ) and permittivity (ϵ) can be made negative simultaneously, as both parameters affect the refractive index (R.I) of the material and its R.I value can be made negative. These metamaterials are used in making invisibility cloaks, nonlinear optics, perfect lenses, and antennas.

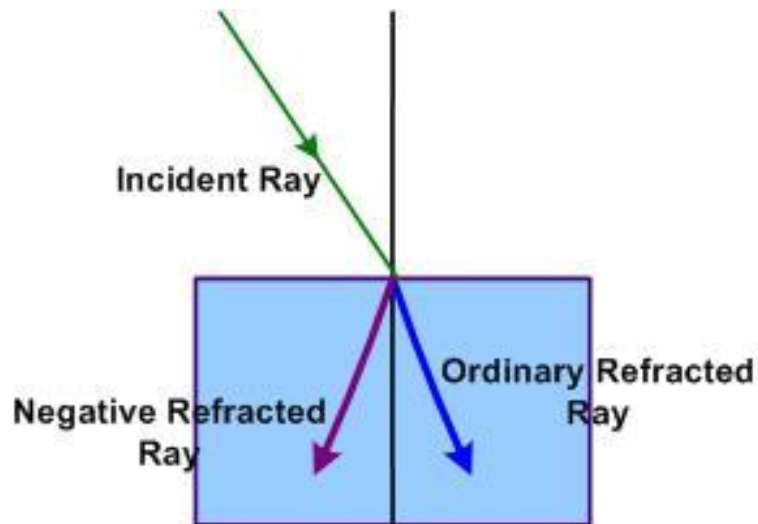


Figure 1.31: Negative refractive index metamaterials bend light in the opposite direction compared to conventional positive refractive index materials.

1.18 Chiral Metamaterials (CMMs)

Chiral metamaterials are asymmetric structures that have been artificially manufactured in the lab to have properties that aren't present in nature. Asymmetric unit cells lack mirror symmetry. (Plum et al., 2008; Zhao et al., 2010) Chiral metamaterials specific designs make them work at different frequency ranges (S. Zhang et al., 2009; Zhou et al., 2009) and can control the optical activity of the medium and show circular dichroism. Chiral metamaterials exhibit their own properties and can also act as NIMs was proposed by Tretykov et al. (S. Tretyakov, I. Nefedov, A. Sihvola & C.Simovski, 2003) with no requirement of negative permittivity (ϵ) and negative permeability (μ).