

Chapter 1

Introduction

1.1 The Importance of the Environment

The growth, health and persistence of human beings and other organisms all depend on the quality of the environment. Therefore, conservation and protection of the environment are essential in the present-day industrialized and developing world. Unfortunately, pollution of the environment is one of the most pressing problems of our age. The problem of the environment has now reached a level that poses a potential threat not only to health but also to entire populations. The quality of our environment is deteriorating day by day, due to the continuous discharge of undesirable constituents. The main sources of the contamination are the geometric increase in the global population, industrialization, domestic and agricultural activities, atomic explosions, and other environmental and global changes. If they are not properly controlled, these activities and changes can destroy the quality of our environment. Broadly, the environment is divided into three parts: the atmosphere, including the air sphere around the Earth; the lithosphere, which consists of the Earth itself; and the hydrosphere – all the water bodies, including the oceans and the surface and ground water. The hydrosphere and atmosphere components of the environment are directly and readily available for contamination by pollutants. Therefore, the quality of these components of the environment is deteriorating continuously, which is a matter of great concern. Again, the notorious pollutants find their way easily through water bodies and reach various levels in the food chain. The

atmosphere is only being contaminated by some gases and volatile organic pollutants. Furthermore, the ground and surface water in many places are not suitable for drinking purposes due to the presence of aesthetic and toxic pollutants. The air quality of some metropolitan cities is not safe according to minimum health requirements. Many toxic gases and organic pollutants, including lethal pesticides, phenols, plasticizers and so on, have been reported in the air. Briefly, the quality of water, air and edible foodstuffs is not safe in some places, and this poses a threat to human beings and other animals. Therefore, the conservation and improvement of the environment is essential and urgent [1–3]. In view of this, environmental authorities are seeking data and information on pollution levels and improvement measures in order to control the contamination of the environment.

1.2 Environmental Pollutants

Any undesirable and toxic chemical, commodity, organism or other object present in the environment may be considered as an environmental pollutant. The pollutant may be present in the form of a solid, a liquid or a gas. Among these, the presence of toxic pollutants poses a serious threat to human beings and other useful organisms. In general, environmental pollutants may be categorized into chemical and biological classes. The chemical pollutants are organic and inorganic compounds, while the biological contaminants are toxic microbes. Among the various organic environmental pollutants, pesticides, phenols, plasticizers and polynuclear aromatic hydrocarbons are the most toxic, while the toxic inorganic pollutants consist of some metal ions and their complexes. These organic and inorganic pollutants are considered to be the most toxic as they are carcinogenic in nature [4–12]. Most of these environmental pollutants enter into the human body through water and other foodstuffs. Therefore, the monitoring of pollutants in water bodies is essential. Prior to supplying water for drinking, bathing, agriculture and other purposes, it is important to determine the concentrations of these pollutants, if they are present. Of course, analysis of the total concentrations of the toxic pollutants is required and essential. There are many reports available in the literature on the analysis of the organic and inorganic pollutants present in various water bodies and the atmosphere, but the data presented are not reliable. This is due to the fact that some of the organic pollutants are chiral, and the data do not distinguish which mirror images of certain pollutants are present and which are harmful [13–15]. Because of this, knowledge about chirality, chiral pollutants and their methods of analysis is essential for environmental and industrial chemists, and for

scientists working in other analytical laboratories. In view of these facts, in the following sections, an attempt has been made to explain the meaning of chirality and chiral pollutants, along with the methods of analysis of chiral pollutants.

1.3 Chirality and its Occurrence

The term ‘chirality’ is derived from the Greek word *kheir*, meaning ‘handedness’ [13]. Any object that lacks the three elements of symmetry – that is, a plane of symmetry, a centre of symmetry and an axis of symmetry – exists in more than one form. These forms are nonsuperimposable mirror images of each other and are known as chiral objects (enantiomers) and this property termed optical activity. This optical activity results from the refraction of right and left circularly polarized light to different extents by chiral molecules (pollutants). The source of the rotation, and hence also the optical rotatory dispersion, is birefringence; that is, the unequal slowing down of right (R) and left (L) circularly polarized light ($n_R \neq n_L$, where n is the refractive index) as the light passes through the sample. On the contrary, ‘circular dichroism’ is the consequence of the difference in absorption of right and left circularly polarized light (*cpl*) ($\epsilon_R \neq \epsilon_L$, where ϵ is the molar absorption coefficient) [16, 17]. The rotation of polarized light is measured by a polarimeter and the angle of rotation (α) measured is expressed as follows:

$$[\alpha]_D^t = \alpha_{\text{obs}}/lc$$

where $[\alpha]_D^t$ denotes the specific rotation determined at t °C and using the D-line of sodium light, and α_{obs} is the observed angle of rotation, l is the length of the solution medium, in decimetres, and c is the concentration of the chiral pollutant, in g ml^{-1} . The value of $[\alpha]_D^t$ may be positive or negative, depending on the direction of rotation of the angle.

In radiation, the electric field associated with the light waves oscillates in all directions perpendicular to the direction of propagation, but in plane polarized light the electric field only oscillates in one direction, which is achieved by passing ordinary radiation through a Nicol prism. The electric field (E) and the magnetic field vector (H) oscillate at right angles to one another. The circularly polarized light (*cpl*) is described by examining the movement of the electric field only. The linearly polarized light is represented mathematically and graphically as a combination of left and right coherent rotating beams of *cpl*. In an isotropic medium, the two components travel at the same velocity but in opposite directions. In

1894, Curie [18] showed that both the electrical and the magnetic fields have individual mirror planes of symmetry. These planes are eliminated in collinear combination of the two fields. Two enantiomorphous combinations are possible; one in which the electric and magnetic fields are parallel, and another in which the component fields are antiparallel. If both fields oscillate at the same frequency, the two chiral combinations represent right- and left-handed circularly polarized electromagnetic radiation. Drude [19] proposed that the interaction of a chiral molecule with an electromagnetic field gives rise to a helical charge displacement in the molecule, and hence that an oscillatory charge displacement has a right-handed helical form in one optically active isomer and a left-handed form in its antipode. The electric and magnetic dipole moments that develop in a chiral molecule are parallel for the right-handed helical charge displacement and antiparallel for the other antipode, which results in positive or negative circular dichroic light absorption. In 1935, Lowry [20] observed circular dichroism in quartz crystals.

From elementary particles to humans, chirality is found in a wide range of objects [21]. This observation leads to the interpretation that chirality plays a very important and essential role in the existence of the universe, which is still a mystery. There are several examples that indicate the presence of chirality in our environment. In the old kingdoms of Upper and Lower Egypt, many examples of burial chamber mural paintings depict significant events in our modern view of chirality [13]. Additionally, out of the 1168 galaxies listed in the *Carnegie Atlas of Galaxies*, 540 are chiral when coupled with the direction of their recession velocities [22]. The influence of chirality can be observed in plants and animals, where numerous examples of asymmetric structures can be observed. For example, the helical structures of plants and animals make them asymmetric in nature. Briefly, chirality exists almost everywhere in the universe and is associated with the origin of the Earth [23].

1.4 The Chemical Evolution of Chirality

The chemical evolution of chirality began in 1809 with the discovery of Haüy [24] who postulated, from crystal cleavage observations, that a crystal and each constituent space-filling molecule are images of each other in overall shape. In 1819, Mitscherlich [25] postulated a law of isomorphisms which describes the similarity of crystal shape to an equivalent stoichiometry in chemical composition. In 1822, Herschel [26] made the connection

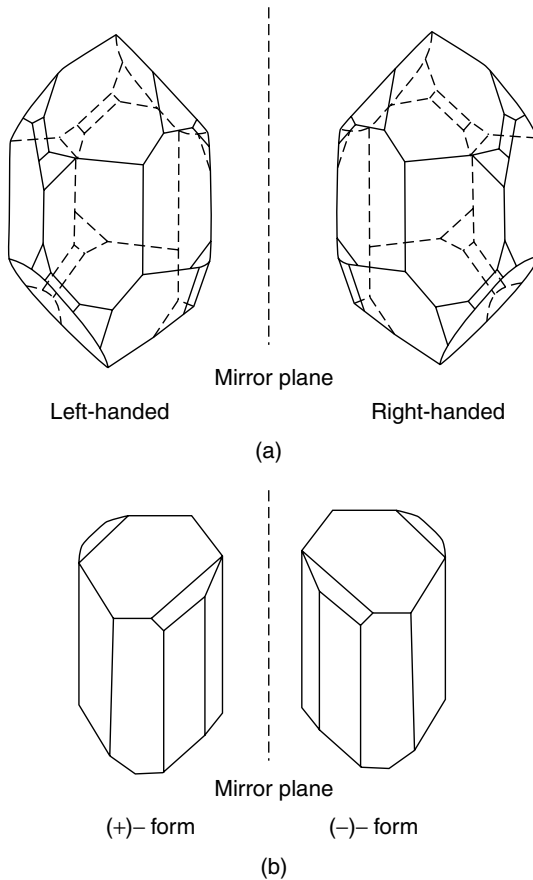


Figure 1.1 The stereostructures of (a) quartz and (b) sodium ammonium tartarate crystals.

between the morphological handedness of quartz crystals and the sign of the optical activity of the crystals. Herschel observed two types of quartz crystals, distinguished by the right- and left-handed screw sets of hemihedral facets, which reduce the crystal symmetry from hexagonal to trigonal. Furthermore, the author found that all of the crystals of the left-handed morphological set were levorotatory, while those of the right-handed set were dextrorotatory, producing opposition rotation of the plane polarized light; that is, clockwise and anticlockwise rotations (Figure 1.1). At that time, Herschel supposed that the morphological chirality of quartz crystals and their signs of rotation had a common molecular basis, but in 1824 Fresnel [27] determined that the optical activity in these crystals has a circular

birefringence $n_L - n_R$, which is positive for dextrorotatory and negative for levorotatory media, where n_L and n_R are the refractive indices for left- and right-handed circularly polarized light, respectively. Furthermore, he proposed that such types of structures (molecules) have both left- or right-handed helical forms or arrangements. In 1844, Mitscherlich [28] reported that sodium ammonium salts of tartaric acid showed different activities with respect to *Penicillium glaucum*, but he could not explain this behaviour scientifically. Later on, in 1848, Pasteur [29] reported the differing destruction rates for *dextro* and *levo* ammonium tartarate by the mould *Penicillium glaucum*, by considering the work of Herschel and Fresnel. Pasteur repeated the crystallization of the sodium ammonium salt of tartaric acid and separated two types of crystals that had enantiomorphous crystal facets. Using Haüy's morphological principle, he inferred that the individual molecules of (+)- and (-)-tartaric acid were stereochemically dissymmetric in nature and related in the form of nonsuperimposable mirror images. In spite of these findings, these observations could not be explained properly at that time, due to the lack of extensive scientific knowledge on this issue. In 1874, Le Bel [30] and van't Hoff [31] independently proposed that the four valences of the carbon atom remain directed towards the vertices of an atom-centred tetrahedron. This finding led to the development of the theory of three-dimensional molecular structures of molecules, by which the phenomenon of chirality and Pasteur's discovery could be explained scientifically. Later on, the different biological properties of the enantiomers were explained as being due to their three-dimensional structures (configurations).

1.5 The Electronic Theory of Chirality

Enantiomers differ only in the spatial arrangement of the atom, and the electro-optical activity of the constituent atoms produces an optical molecular inequivalence by adding to the molecular optical activity of one enantiomer and subtracting from that of the other. The electronic interaction discriminates between the binding energies of the corresponding electronic states – stationary or transitional – of the two mirror images of a chiral molecule. Therefore, an electro-energy increment is added to the binding energy of a given electronic state in one enantiomer and subtracted from its counterpart enantiomer, resulting in an electro-energy difference between the two enantiomers. Therefore, L-amino acids in a preferred conformation are more stable than D-amino acids. Similarly, D-aldotriose in a preferred conformation is more stable than its antipode.

1.6 The Importance of Chirality

As discussed above, chirality exists everywhere in the universe and hence it plays a vital part in some aspect of our lives. The consideration of chirality aspect is very important in the environment and some industries, particularly the pharmaceutical, agrochemical, food and beverages, and petrochemical industries. We have already discussed the importance of chirality in environmental pollutants, as the different enantiomers of the pollutants have different toxicities. In the pharmaceutical and drug industries, the existence of chirality became particularly important in the wake of the thalidomide tragedy in the 1960s.

Thalidomide was put on the market in the late 1950s as a sedative, in its racemic form. Even when applied in the therapeutic and harmless (+)-form, the *in vivo* interconversion into the harmful (–)-isomer was shown to be responsible for the disastrous malformations of embryos when thalidomide was administered to women during pregnancy [32–34]. In addition to creating a general awareness, the thalidomide tragedy resulted in stricter controls and reconsideration of the approval guidelines for newly developed drugs. To protect patients from unwanted and harmful enantiomers [35] and side effects, the possibility of different actions of the individual enantiomers with regard to pharmacology and toxicology had to be taken into account. In spite of the fact that the optical isomers of a racemic drug can exhibit different pharmacological activities in living systems [35–41], the bioactive synthetic compounds, most of which are chiral drugs, are administered as racemates [42].

A similar situation also pertains in the agrochemical industry, as many pesticides and other agrochemicals are chiral in nature. In 1981, Spencer [43] reported that out of 550 pesticides, 98 % were synthetic in nature with 17 % chiral molecules and less than 8 % have been marketed as single isomers. Lewis *et al.* [44] also reported that 25 % of pesticides are chiral in nature. Recently, Vetter [45] reviewed the enantioselective fate of chiral chlorinated hydrocarbons and their metabolites in environmental samples. Not all of the aspects of chirality in the agrochemical industry have been fully explored yet, but investigations are under way. Therefore, knowledge about chirality in the agricultural industry is also very important.

Chirality is also important in the food and beverage industries, as many food products contain several chiral substances. Control of the fermentation process and of storage affects when a single isomer is converted into a racemic mixture as time proceeds. The *S*-enantiomer of asparagine is bitter, while the *R*-antipode is sweet. Amino acids, which are essential for animal

growth, are all chiral except for glycine. The chemical products used to produce flavours and fragrances are highly dependent on enantioseparation for their properties. The terpenes carvone and limonene are other chiral molecules that are used in the food and beverage industries.

Many hydrocarbons are by-products of the petroleum industry and some of them are chiral in nature. The most important chiral hydrocarbons are halogenated, polyalkane and so on. These hydrocarbons are also used as precursors for many synthetic formulations and products, in which chiral products are produced during the synthesis processes. The different oilfields have been produced from various materials at different times, and hence different ratios of stereoisomers can be found in samples from adjacent fields. Briefly, chirality is of considerable importance in the petrochemical industry.

1.7 Nomenclature for Chiral Pollutants

The nomenclature for the enantiomers can be explained as follows. Initially, the optical isomers were distinguished using (+) and (−) signs or *d* (*dextro*) and *l* (*levo*), indicating the direction in which the enantiomers rotate the plane of the polarized light. (+) or *d* stands for a rotation to the right (clockwise), whereas (−) or *l* indicates a rotation to the left (anticlockwise). The main drawback of such an assignment is that one cannot derive the number of chiral centres from it. This is possible when applying the *R/S* notation, which describes the absolute configuration (the spatial arrangement of the substituents) around the asymmetric carbon atom of the pollutant (the molecule). This assignment is based on the Cahn–Ingold–Prelog (CIP) convention [46]. It has almost replaced the older D/L notation, which correlates the configuration of a molecule with the configuration of D/L-glyceraldehyde according to the Fischer convention. Today, the latter nomenclature is predominantly restricted to amino acids and carbohydrates [47]. The assignment of *R* or *S* according to CIP follows the sequence rule; that is, the order of priority of the substituents on the centre of chirality. It can be determined on the basis of the decrease in the atomic number of the atoms directly bonded to the centre of chirality. In the case in which two or more of these atoms are identical, the next bonded atoms have to be considered, and then eventually the third bonded atoms and so on. In so doing, the branch containing the atoms with the highest atomic numbers attains the highest priority. If double or triple bonds connect the atoms, their weight is higher than that of two

or three singly bonded atoms [47]. When isotopes of atoms are involved, the order of priority can be determined by putting in order the decline in their mass numbers. It is very interesting to observe that in closely related structures the nomenclature of the absolute configuration may change, whereas the spatial arrangement of the substituents is maintained. Other consequences of chirality are concerned with the metabolic processes. Several transformations, such as prochiral to chiral, chiral to chiral, chiral to diastereoisomer, chiral to nonchiral and chiral inversion, can occur [35, 48].

In general, the phenomenon of chirality exists in organic pollutants (at the molecular level). However, it is also found in some inorganic pollutants. In some pollutants, the carbon atoms remain attached to four different atoms or groups. This arrangement makes the whole pollutant (molecule) asymmetric in structure. This type of pollutant differs in three-dimensional configurations and exists into two forms, which are mirror images of each other. No matter what symmetry operation is applied to this sort of pollutant, one will never be able to superimpose the two mirror images upon each other. These mirror images are called optical isomers (since they have the capacity to rotate the plane polarized light), or stereoisomers, enantiomers, enantiomorphs, antipodes or chiral molecules. The phenomenon of the existence of these different enantiomers is called stereoisomerism or chirality. A 50 : 50 ratio of the enantiomers is called a racemic mixture, and does not rotate plane polarized light. The absence of rotation of plane polarized light is due to the equal and opposite rotation of the two enantiomers (50 : 50) and hence this phenomenon is called external compensation. Some enantiomers contain a plane of symmetry and hence are unable to rotate plane polarized light. This type of enantiomer are called the *meso*-form. The optical inactivity of the *meso*-form is due to the opposite signs of the rotation of its two halves, and hence the phenomenon of optical inactivity is called internal compensation. In addition to the central chirality, axial chirality can occur in allenes and cumulenes. In the former class, the substituents do not necessarily have to be different, since the second double bond causes the loss of the C_3 rotational symmetry element. In the latter class, only the members with an odd number of cumulated carbon atoms are potentially chiral, whereas an even number of carbon atoms results in *E*-/*Z*-isomerism (geometric isomerism) [27]. Another type of axial chirality is represented by atropisomers, which possess conformational chirality. As long as the *ortho*-substituents in tetra-substituted biaryls are large enough, the rotation around a C–C single bond will be hindered and will prevent the two forms from interconverting. Finally, there exists a planar chirality,

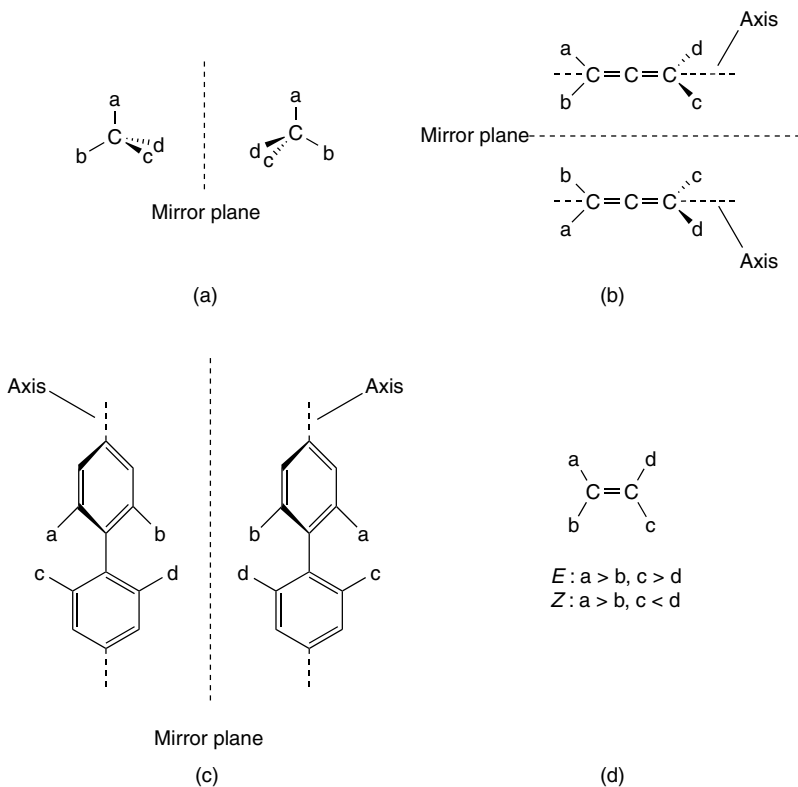


Figure 1.2 The different type of stereoisomerisms: (a) central chirality, (b) axial chirality, (c) atropisomerism and (d) *E*-/*Z*-isomerism (geometric).

which arises from the arrangement of atoms or groups of atoms relative to a stereogenic plane. However, this form of chirality is rather rare [32]. Helicity is a special form of chirality and often occurs in macromolecules such as biopolymers, proteins and polysaccharides [47]. A helix is always chiral due to its right-handed (clockwise) or left-handed (anticlockwise) arrangement. In the case in which a stereoisomer has more than one stereogenic centre, the number n of theoretically possible enantiomers can be derived from the formula 2^n . The phenomenon of stereoisomerism in different types of molecules (pollutants) is shown in Figure 1.2.

1.8 Chirality in Environmental Pollutants

It has recently been observed that one of the two enantiomers of a chiral pollutant/xenobiotic may be more toxic than the other [49]. This is important

information for the environmental chemist when performing an environmental analysis. The biological transformation of chiral pollutants can be stereoselective, and so the uptake, metabolism and excretion of enantiomers may thus be very different [49, 50]. Therefore, the enantiomeric composition of chiral pollutants may be changed by these processes. Metabolites of chiral compounds are often chiral. Thus, to obtain information on the toxicity and biotransformation of chiral pollutants, it is necessary to explain chirality in environmental pollutants.

As discussed earlier, the number of the enantiomers of a pollutant depends on the number of chiral centres present in the pollutant. A simple example of this type of chirality is presented in Figure 1.3(a), which shows the enantiomers of [1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)] (DDT) with one stereogenic centre. There are several pollutants that contain two stereogenic centres and exist as four stereoisomers. The four stereoisomers make up the two pairs of enantiomers. It is important to mention here that the stereoisomers, which are not mirror images of each other, are called diastereoisomers and, unlike enantiomers, they have different physical and chemical properties. Examples of these types of pollutant include the 1,3,4,6,7-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[*g*]-2-benzopyran-1-one (HHCB) metabolite of galaxolide, bis(2,3-dichloro-1-propyl) ether (*meso*- form) and so on (Figure 1.3(b)).

Some pollutants do not contain a chiral centre but are still chiral due to their overall chiral structures. The best examples of this type of chirality are found in biphenyls and cyclic pollutants. Biphenyls that contain four large groups in *ortho*- positions cannot freely rotate about the central single bond because of steric hindrance. In such pollutants, the two ring systems are oriented in perpendicular planes, or in any plane between angles 0 and 90°. The example of chirality in polychlorinated biphenyls (PCBs) is shown in Figure 1.3(c). It is not necessary that all four groups are responsible for the existence of chirality. Three or even two large groups, if placed properly, can also hinder free rotation, as required for the existence of chirality. Basically, in PCB the existence of chirality is controlled by the energy of free rotation of the central bond. The groups led by Schurig [51], König [52, 53] and Harju [54] have carried out detailed studies on the free rotation of PCBs. Interested readers should consult these publications. The chirality in cyclic pollutants can be explained easily by citing the example of hexachlorocyclohexane (HCH) pesticide. It is very interesting to note that out of eight isomers of HCH, only α -HCH is chiral. The other isomers of HCH are achiral due to the presence of some symmetry elements (a centre

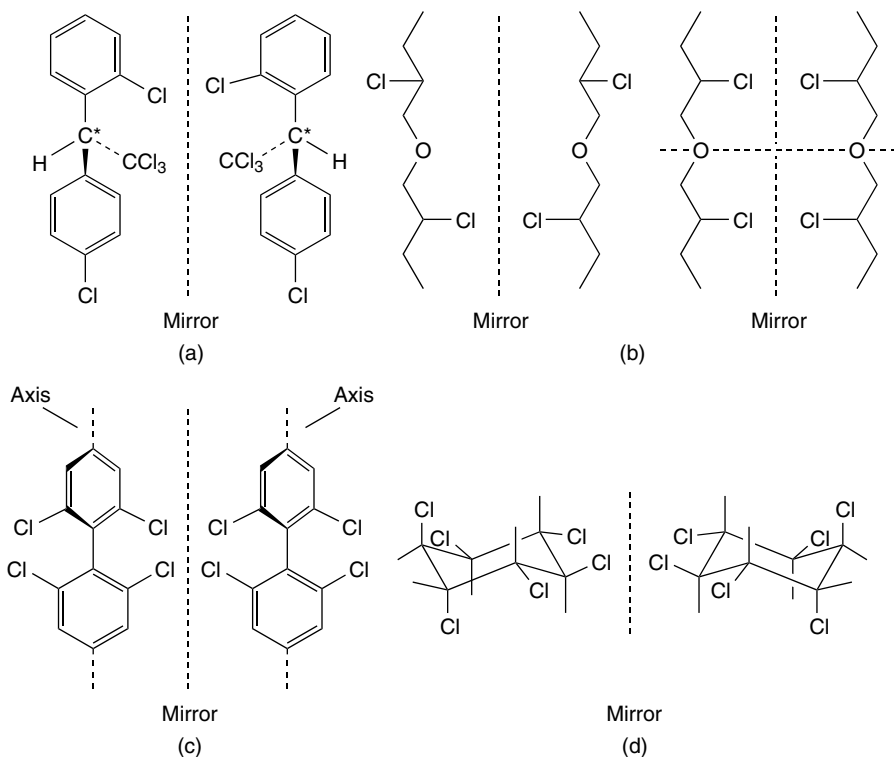


Figure 1.3 The enantiomers of some chiral pollutants: (a) DDT, (b) bis(2,3-dichloro-1-propyl)ether, (c) PCB and (d) α -HCH.

of symmetry, an axis of symmetry or a plane of symmetry). The structure of α -HCH is shown again in Figure 1.3(d).

1.9 Chirality and its Consequences in the Environment

Many xenobiotics and pollutants are chiral in nature and the two enantiomers of these pollutants may have different toxicities [13]. Additionally, the degradation of some chiral pollutants is stereospecific in the environment, and the degradation of some achiral pollutants may result in chiral toxic metabolites. Moreover, it has also been reported that enantiomers may react at different rates with achiral molecules in the presence of a chiral catalyst [13]. It is also obvious that most of the identities and the structures in nature are chiral and, therefore, that there is a greater chance that the environmental pollutants will react at different rates. Therefore,

to predict the exact toxicities of pollutants, determination of the concentrations of both of the enantiomers is not just required but essential. In 1991, Kallenborn *et al.* [55] reported the enantioselective metabolism of α -hexachlorocyclohexane in the organs of eider duck, while in the same year Faller *et al.* [56] reported the degradation of α -hexachlorocyclohexane enantioselectively by marine bacteria. Therefore, environmental chemists are also looking for the optimum technique with which to determine the chiral ratio of xenobiotics in the environment. Furthermore, diverse groups of people, ranging from regulators to the materials industries, clinicians and nutritional experts, agriculturalists and environmentalists, are now demanding data on the ratio of the enantiomers, rather than the total concentrations of the racemic pollutants.

1.10 The Enantiomeric Ratio and Fractions of Chiral Pollutants

About 25 % of agrochemicals – including pesticides – are chiral in nature and, therefore, many of these chiral agrochemicals are applied in agricultural and forestry activities in the form of their racemates. The task of the environmental chemist involves the study of the conversion of enantiomers by biological processes and of their compositions. Several terms have been used to describe the extent of deviations from the racemic compositions. The most commonly used terms are the enantiomeric ratio (ER) and the enantiomeric fraction (EF) [57]. ERs are defined by the ratio of the enantiomers, which is directly obtained by integration of the analysis methods. If the directions of the rotation of plane polarized light by the enantiomers are known, the ER is formed by the quotient of the dextro- and levorotatory enantiomers (ER_{\pm}). In the case of a lack of standards for the enantiomers and no information on the directions of the plane polarized light, the ER is defined as the quotient of the first and the second eluted enantiomer ($ER_{1/2}$) in any chromatographic method, and is defined as follows:

$$ER = C_+/C_- \quad \text{or} \quad C_1/C_2 \quad (1.1)$$

where C is the concentration of the levo- (–), dextro- (+), first (1) or second (2) eluting enantiomer in the sample. A value for the absence of the second enantiomer ($C_2 = 0$) is mathematically not defined and has to be presented as the limit towards infinity. Enantiomeric ratios extend from infinity (only C_+ or C_1) to the limit $\rightarrow 0$ (only C_- or C_2), with ER equal

to 1 for the racemate. Furthermore, ERs are not based on a numerical but on a logarithmic scale, which may cause some problems; such as the fact that while the ER of a tenfold amount of the second eluted enantiomer is 0.1, the ER of a tenfold amount of the first enantiomer is 10. Hence the mean value will be $10^{(-1-1)/2} = 10^0 = 1.0$ (rather than the arithmetic mean 5.05). The reciprocal values of ER = 0.5 and 0.4 ($\Delta ER = 1.0$) are 2.0 and 2.5 ($\Delta ER = 0.5$), and an ER of 2.0 and 2.1 ($\Delta ER = 1.0$) corresponds to reciprocal values of 0.5 and 0.48 ($\Delta ER = 0.02$) [45]. Therefore, calculation of mean values of ERs in repetitive injections of standards or from different samples should be carried out after transfer to the log scale. Basically, the values of ERs should be greater than one, with an accuracy of ± 0.1 . For ERs less than one, it is often necessary to present two values after the decimal point.

Sometimes, the enantiomeric purity is expressed by the enantioexcess (ee), which indicates the excess of one enantiomer that has a higher concentration (C_H) over another that has a lower concentration (C_L), as follows:

$$ee = C_H - C_L / C_H + C_L \quad (1.2)$$

The value of ee varies from zero to one for the racemic and optically active pure enantiomers. In general, ee is expressed in terms of a percentage, as follows:

$$\% ee = 100(C_H - C_L / C_H + C_L) \quad (1.3)$$

The % ee is compatible with the law of mixing (mixing of samples of different ee) and % ee is equivalent to the optical purity:

$$\text{optical purity} = 100(\alpha_{\text{samp}} / \alpha_{\text{enan}}) \quad (1.4)$$

where α_{enan} is the specific rotation of the pure enantiomer and α_{samp} is the specific rotation of the sample [58]. The chromatographic purity or enantiopurity ee enantiofraction is described by the following equation:

$$\text{enantiopurity} = C_1 / (C_1 + C_2) \quad (1.5)$$

which has been used to express enantiomer purity in the pharmaceutical, agrochemical, environmental and other analytical sciences [59–61].

The enantiomeric ratio data are transferred into enantiomer fractions (EFs) as a standard descriptor [62] and the EF can be calculated from the ER using the following equation:

$$EF = ER / (ER + 1) \quad (1.6)$$

This descriptor provides a more meaningful representation of the graphical data than the ER, and is more easily employed in mathematical expressions [62, 63].

1.11 Methods for the Separation of Chiral Pollutants

The splitting of a chiral pollutant into its enantiomers is called separation. Various methodologies have been used for the separation of the enantiomers of drugs and pharmaceuticals. The basic principles of the enantiomeric separation of chiral pharmaceuticals and environmental pollutants are similar and, therefore, the approach used for the enantiomeric separation of pharmaceuticals may be used for the chiral separation of environmental pollutants. The different approaches applied for chiral separation of pharmaceuticals include: the classical approach, using enzymatic degradation of one of the enantiomers; preferential crystallization; and modern technologies, including spectroscopic, electrophoretic and chromatographic methods.

In the enzymatic method, the destruction of one form affects separation by biochemical processes. When certain micro-organisms, such as yeast, moulds and bacteria, are allowed to grow in the solution of racemic mixtures, they assimilated one form selectively, leaving the other one behind in solution. For example, if ordinary mould, *Penicillium glaucum*, is added to a racemic solution of ammonium tartarate, the solution becomes levorotatory due to the destruction of the *dextro*- form [29]. The principle of crystallization is based on the formation of diastereomeric salts by the two enantiomers with the optically pure compound, and these diastereoisomeric salts can easily be separated [21, 64]. In this process, the optically active resolving agent must be of high optical purity. In most cases, after the separation of the desired enantiomers from the diastereoisomeric salts, the resolving agent is recovered and made available for reuse [64–66]. Additionally, mechanical methods of separation (by needle etc.) have also been utilized for separation of the crystals of some racemic compounds, such as sodium ammonium tartarate and quartz, as the crystals of these compounds are mirror images of each other. These classical methods have not been able to achieve the status of routine laboratory practice due to certain drawbacks. The most important drawbacks associated with these methods are the degradation of one enantiomer in the enzymatic method, while the applications of the crystallization method are very limited. Nowadays, chromatographic, electrophoretic, spectroscopic, biosensing and membrane methods are the most common techniques applied in this

area [37–40, 67, 68], the chromatographic and electrophoretic methods being very sensitive, reproducible and reliable. Moreover, these methods can easily be used to determine the enantiomeric ratio of chiral pollutants in different matrices. Briefly, the chromatographic and electrophoretic methods for chiral separation are ideal and practical and, therefore, these methods will be discussed herein.

1.11.1 Chromatographic Methods

Nowadays, chromatographic and electrophoretic methods are the most popular techniques applied in this field of work. In chromatographic methods, two approaches are used, the indirect and the direct. The indirect chromatographic separation of racemic mixtures can be achieved by derivatization of the racemic pollutant with a chiral derivatizing agent (CDA), resulting in the formation of a diastereoisomeric complex/salt. Diastereoisomers that have differing physical and chemical properties can be separated from each other by an achiral chromatographic method. A precondition for a successful derivatization is the presence of suitable functional groups in the pollutant. Additionally, to increase the physicochemical differentiation, the derivatization should occur close to the chiral atom. Although the indirect chromatographic approach has the advantage of predetermining the elution order, which can be important for the determination of optical purities, there are some limitations to this technique. The derivatization procedure is tedious and time-consuming, due to the different reaction rates of the individual enantiomers, and a suitable chiral derivatizing agent in a pure form is sometimes poorly available. Moreover, this approach cannot be used easily with environmental samples.

On the other hand, the direct chromatographic approach involves the use of a chiral selector either in the mobile phase, where it is called a chiral mobile phase additive (CMPA), or in the stationary phase, called a chiral stationary phase (CSP). In the later case, the chiral selector is chemically bonded or coated, or allowed to be absorbed on some suitable solid support. Of course, the use of chiral selectors in the form of CMPAs still exists, but there are few publications in the literature on this approach. This is due to the high running cost of the experiment, as a greater amount of chiral selector is required for the preparation of the mobile phase. Besides, there is very little chance of recovery of the chiral selectors and hence a large amount of costly chiral selector is wasted. Contrary to this, CSPs have achieved a great reputation in the chiral separation of enantiomers by chromatography and, today, they are the tools of choice in almost all analytical, biochemical, pharmaceutical and pharmacological institutions

and industries. The most important and useful CSPs are available in the form of open and tubular columns. However, some chiral capillaries and thin layer plates are also available, for use in capillary electrophoresis and thin layer chromatography. Chiral columns and capillaries are packed with several chiral selectors.

The chromatographic methods involve the use of gas or liquid separately, as the mobile phases. Therefore, the former kind of chromatography is called gas chromatography (GC), while the later is termed liquid chromatography (LC). Due to some problems associated with gas chromatography, it cannot be accepted as the method of choice for chiral separation of racemic compounds. The major disadvantage of GC is its requirement of the conversion of the racemic compound into volatile species, which is carried out by a derivatization process. Therefore, LC is the best remaining technology for the chiral separation of a wide variety of racemates. The main advantage of LC is its ability to determine the enantiomers in environmental samples directly. Over the course of time, various types of liquid chromatographic approaches have been developed and used in this field of work, the most important methods being high performance liquid chromatography (HPLC), sub- and supercritical fluid chromatography (SFC), capillary electrochromatography (CEC) and thin layer chromatography (TLC). The different modes of chromatography used for the chiral separation of a variety of racemic drugs, pharmaceuticals and pollutants are shown in Figure 1.4.

Among the various liquid chromatographic techniques mentioned above, HPLC remains as the best modality, due to its several advantages in comparison to the other options. High speed, sensitivity and reproducible results make HPLC the method of choice in almost all laboratories. About 90 % of the chiral separation of pharmaceuticals has been carried out using the HPLC mode of chromatography. Due to the wide range of applications of HPLC in chiral separation, several chiral selectors are available in the form of HPLC columns. A variety of mobile phases, including normal, reversed, polar organic and polar ionic modes, are used in HPLC. The composition of the mobile phases may be modified by the addition of various aqueous and nonaqueous solvents. The optimization of the chiral separation is carried out using a number of parameters.

The use of a supercritical fluid (SFC) as the mobile phase for chromatographic separation was first reported more than 30 years ago, but most of the growth in SFCs has occurred recently. A supercritical fluid exists when both the temperature and pressure of the system exceed the critical values; that is, a critical temperature T_c and a critical pressure P_c . Critical fluids have physical properties that lie between those of a liquid and a gas.

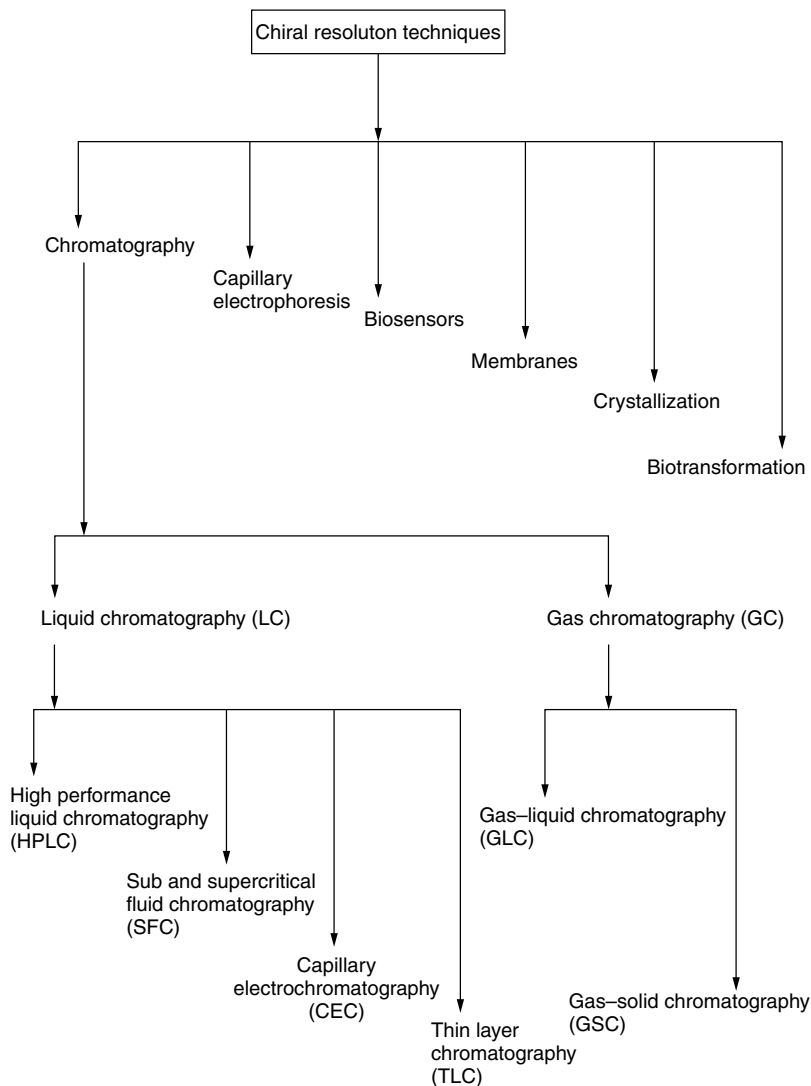


Figure 1.4 The different techniques of chiral resolution.

Like a gas, a supercritical fluid is highly compressible, and the properties of the fluid – including the density and the viscosity – can be maintained by varying the pressure and temperature conditions. In chromatographic systems, the solute diffusion coefficients are often of a higher order of magnitude in supercritical fluids than in traditional liquids. On the other hand, the viscosities are lower than those of liquids [69]. At temperatures

below T_c and pressures above P_c , the fluid becomes a liquid. On the other hand, at temperatures above T_c and pressures below P_c , the fluid behaves as a gas. Therefore, a supercritical fluid can be used as part of a liquid–gas mixture [70]. The commonly used supercritical fluids (SFs) are carbon dioxide, nitrous oxide and trifluoromethane [69–71]. Its compatibility with most detectors, low critical temperature and pressure, low toxicity and environmental burden, and low cost make carbon dioxide the supercritical fluid of choice. The main drawback of supercritical carbon dioxide as a mobile phase is its inability to elute more polar compounds. This can be improved by the addition of organic modifiers to the relatively apolar carbon dioxide. Chiral sub-FC and SFC have been carried out in packed and open tubular columns and capillaries [72]. The first report on chiral separation by SFC was published in 1985, by Mourier *et al.* [73]. Since then, several papers and reviews have appeared on the subject [74–79].

Basically, capillary electrochromatography (CEC) is a hybrid technique that works on the basic principles of capillary electrophoresis and chromatography [80]. This mode of chromatography is used either on packed or tubular capillaries/columns. Packed column ECE was first introduced by Pretorius *et al.* [81] in 1974, while open tubular CEC was presented by Tsuda *et al.* [82] in 1983. In 1984, Terabe *et al.* [83] introduced another modification in liquid chromatography – micellar electrokinetic capillary chromatography (MECC). Of course, this mode too depends on the working principles of capillary electrophoresis and chromatography, but it also involves the formation of micelles. CEC and MECC have been used recently in the chiral separation of racemic compounds and hence some publications have appeared on this issue [84–89]. Their high speed, sensitivity, lower limit of detection and reproducible results make CEC and MECC the methods of choice in chiral separation. However, these methods are not yet in common use, as the techniques are not fully developed and research is still under way.

The development of thin layer chromatography (TLC) has a very long history, but its use in chiral separation goes back about 25 years. Most TLC enantioseparations of pharmaceuticals and other compounds have been carried out in the indirect mode; that is, by preparing diastereoisomers and resolving them using TLC. The derivatization of racemic mixtures and their subsequent separation on silica gel or RP TLC plates represents a method of chiral separation. Only a few reports have appeared on direct enantiomeric separation on chiral TLC plates; that is, using CMPAs or CSPs. Among the direct approaches, the use of CSPs is also very limited. Only ligand exchange based chiral thin layer chromatographic plates are commercially

available for the chiral separation of racemates. It is worth mentioning here that TLC has not been used for chiral separation of environmental pollutants. However, it can easily be used for this purpose.

It is obvious that various modalities of chromatography have been used for the chiral separation of pharmaceuticals and drugs. Therefore, these approaches can also be used for the chiral separation of pollutants. In view of the importance of chromatography in the chiral separation of pollutants, and to familiarize the reader with chromatographic techniques, it is necessary to set out the chromatographic terms and symbols by which chromatographic separations can be explained. Some of the important terms and equations of chromatographic separations are discussed below. Chromatographic separations are characterized by retention (k), separation (α) and resolution (R_s) factors. The values of these parameters can be calculated using the following standard equations [90]:

$$k = (t_r - t_0)/t_0 \quad (1.7)$$

$$\alpha = k_2/k_1 \quad (1.8)$$

$$R_s = 2\Delta t/(w_1 + w_2) \quad (1.9)$$

where t_r and t_0 are the retention time of the chromatogram of the enantiomers and the dead time (solvent front) of the column, respectively, both in minutes. Δt , w_1 and w_2 are the difference between the retention times of the two peaks of the separated pollutant, and the base widths of peaks 1 and 2, respectively. If the individual values of α and R_s are one or greater, the separation is supposed to be complete. If the individual values of these parameters are lower than one, the separation is considered to be partial or incomplete.

The number of theoretical plates (N) characterizes the quality of a column: the larger the value of N , the more complicated is the sample mixture that can be separated using the column. The value of N can be calculated from the following equations:

$$N = 16(t_r/w)^2 \quad (1.10)$$

or

$$N = 5.54[(t_r)/w_{1/2}]^2 \quad (1.11)$$

where t_r , w and $w_{1/2}$ are the retention time (min) of the peak, and the peak widths at base and at half of the height of the peak, respectively. The height equivalent to a theoretical plate (HETP) h is a section of a column in which a solute is in equilibrium with mobile and the stationary phases.

Since a large number of theoretical plates are desired, h should be as small as possible. Naturally, there are no real plates in a column. The concept of a theoretical plate is a variable, the value of which depends on the particle size, the flow velocity, the mobile phase (viscosity) and, especially, on the quality of the packing. h can be calculated using the following equation:

$$h = L/N \quad (1.12)$$

where L is the length of the column used.

1.11.2 The Capillary Electrophoretic Method

At present, capillary electrophoresis (CE), a versatile technique that offers high speed, a high sensitivity and a lower limit of detection, is a major trend in analytical science, and in the field of chiral separation the number of publications has increased exponentially in recent years [91]. Among the electrophoretic methods of chiral separation, various forms of capillary electrophoresis, such as capillary zone electrophoresis (CZE), capillary isotachphoresis (CIF), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), affinity capillary electrophoresis (ACE) and separation on microchips, have been used. In contrast to the others, the CZE model has frequently been used for this purpose [91]. However, it is necessary to mention here that capillary electrophoresis cannot achieve the status of a routine analytical technique in chiral separation, because of some associated drawbacks. The limited application of these methods is due to the lack of development of modern chiral phases.

Again, it is worth explaining some fundamental aspects of capillary electrophoresis, so that the reader can use this technique in the proper way. The separation mechanism in CE is based on the difference in the electrophoretic mobilities of the pollutants. Under the CE conditions, the migration of the pollutants is controlled by the sum of the intrinsic electrophoretic mobility (μ_{ep}) and the electro-osmotic mobility (μ_{eo}), due to the action of electro-osmotic flow (EOF). The observed mobility (μ_{obs}) of the pollutant is related to μ_{eo} and μ_{ep} by the following equation:

$$\mu_{obs} = E(\mu_{eo} + \mu_{ep}) \quad (1.13)$$

where E is the applied voltage (kV).

The simplest way to characterize the separation of two components, the resolution factor (R_s), is to divide the difference in the retention times by the average peak width, as follows:

$$R_s = 2(t_2 - t_1)/(w_1 + w_2) \quad (1.14)$$

where t_1 , t_2 , w_1 and w_2 are the retention times of peaks 1 and 2 and the widths of peaks 1 and 2, respectively.

The value of the separation factor may be correlated with μ_{app} and μ_{ave} by the following equation:

$$R_s = \frac{1}{4}(\Delta\mu_{\text{app}}/\mu_{\text{ave}})N^{1/2} \quad (1.15)$$

where μ_{app} is the apparent mobility of the two enantiomers and μ_{ave} is their average mobility. The utility of Equation (1.9) is that it permits independent assessment of the two factors that affect separation, selectivity and efficiency. The selectivity is reflected in the mobility of the analytes, while the efficiency of the separation process is indicated by N . Another expression for N is derived from the following equation:

$$N = 5.54(L/w_{1/2})^2 \quad (1.16)$$

where L and $w_{1/2}$ are the capillary length and the peak width at half height, respectively.

It is important to point out that it is misleading to discuss theoretical plates in CE: it is simply a carryover from chromatographic theory. In electrophoresis, the separation is governed by the relative mobilities of the analytes in the applied electric field, which are a function of their charge, mass and shape. The theoretical plate in CE is merely a convenient concept to describe the shape of the analyte peaks and to assess the factors that affect separation. The efficiency of the separations on a column is expressed by N , but it is difficult to use this variable to assess the factors that affect efficiency. This is because it refers to the behaviour of a single component during the separation process, and it is not suitable for describing the separation in capillary electrophoresis. However, a more useful parameter is the height equivalent of a theoretical plate (HETP), given as follows:

$$\text{HETP} = L/N = \sigma_{\text{tot}}^2/L \quad (1.17)$$

The HETP may be considered as the function of the capillary occupied by the analyte, and it is more practical to measure separation efficiency compared to N . σ_{tot}^2 is affected not only by diffusion but also by differences in the mobilities, the Joule heating of the capillary and the interaction of the analytes with the capillary wall, and hence σ_{tot}^2 can be represented as follows:

$$\sigma_{\text{tot}}^2 = \sigma_{\text{diff}}^2 + \sigma_{\text{T}}^2 + \sigma_{\text{int}}^2 + \sigma_{\text{wall}}^2 \quad (1.18)$$

1.12 Chiral Selectors in Chromatography and Capillary Electrophoresis

The presence of a chiral phase, called a chiral selector, is essential for the enantiomeric analysis of chiral pollutants by chromatographic and capillary electrophoretic methods. Therefore, several optically active compounds have been used for this purpose. The most important classes of such types of substances are polysaccharides, cyclodextrins, antibiotics, proteins, Pirkle-type CSPs (see below), ligand exchangers, crown ethers and several other types. The basic requirements for a suitable chiral selector are that it should be easily available and inexpensive, that it should have sufficient groups, atoms, grooves, cavities and so on for complexing with chiral pollutants, and that it should be capable of forming diastereomers that are non-UV-absorbing in nature as, generally, the detection in chromatography and capillary electrophoresis is carried out by a UV/visible detector.

Most of the naturally occurring polymers, including the polysaccharides, are chiral and optically active because of their asymmetric structures. These polymers often possess a specific conformation or higher order structure arising from chirality that is essential for the chiral analysis of racemic pollutants [92]. Therefore, the polysaccharides have a potential application in the chiral separation of chiral pollutants by chromatography and capillary electrophoresis [93, 94]. The polysaccharide polymers, such as cellulose, amylose, chitosan, xylan, curdlan, dextran and inulin, have been used for chiral separation in chromatography [95]. However, these derivatives cannot be used as commercial chiral stationary phases (CSPs), because of their poor separation capacity and handling problems [92]. Therefore, derivatives of these polymers have been synthesized in the past two decades [92]. Among the various polymers of polysaccharides, cellulose and amylose are the most readily available naturally occurring forms, and they have been found to be suitable for chiral separations. Therefore, most chiral applications involving chromatography and capillary electrophoresis have been reported as using these two polysaccharides [92, 95].

Cyclodextrins (CDs) are cyclic and nonreducing oligosaccharides, and are obtained from starch. Schardinger [96] identified three different forms of naturally occurring CDs – α -, β - and γ -CDs – and referred to them as Schardinger's sugars. They are also called cyclohexamylose (α -CD), cycloheptamylose (β -CD), cyclooctamaylose (γ -CD), cycloglucans, glucopyranose and Schardinger dextrans. The ability of CDs to form complexes with a wide variety of molecules has been documented [97–102]. The complex formation of CDs and their binding constants have been determined

and are controlled by several different factors – hydrophobic interactions, hydrogen bondings and van der Waals interactions. Therefore, CDs and their derivatives have been widely used in separation science since the early 1980s [103, 104]. The evolution of CDs as chiral selectors in chromatographic and capillary electrophoretic separations of enantiomers has become a subject of interest in the past two decades. The presence of a chiral hollow basket/cavity in these molecules makes them suitable for chiral separation of a wide range of chiral pollutants. At present, the use of CDs as chiral selectors for enantiomeric separation by chromatography and capillary electrophoresis is very common. As chiral selectors, CDs have been used in the form of chiral stationary phases (CSPs) and chiral mobile phase additives (CMPs).

Macrocyclic antibiotics are one of the newest and perhaps the most varied classes of chiral selectors [105]. The concept of utilizing macrocyclic glycopeptide as a chiral stationary phase for HPLC was introduced by Dr D. W. Armstrong in 1994 [106]. Since then, their use for chiral analysis in chromatography and capillary electrophoresis has increased exponentially [67, 107]. The antibiotics have been found to have a very good potential for the chiral separation of a wide range of racemates. This may be due to their specific structures and the possibility of using a wide range of mobile phases. Additionally, due to their relatively small size and the fact that their structures are known, basic studies on chiral recognition can be carried out easily and precisely. They are often complementary in the types of compounds they can separate. For example, rifamycin B, an ansamycin, is enantioselective for many positively charged analytes, whereas vancomycin, a glycopeptide, can resolve a variety of chiral compounds containing free carboxylic acid functional groups. In addition, the separation of enantiomers by antibiotics is not very sensitive and hence is highly robust. The antibiotics most commonly used for chiral separation are vancomycin, teicoplanin, teicoplanin aglycon and Ristocetin A, although vancomycin aglycon, thioestrepton, rifamycin, fradiomycin, streptomycin, kanamycin and avoparcin are also used.

Proteins are natural polymers and are made of amino acids – which are chiral molecules, with the exception of glycine – through amide bonds. However, some glycoproteins also contain sugar moieties. The protein polymer remains in the twisted form because of the different intramolecular bondings. These bondings are also responsible for different types of loops/grooves that are present in the protein molecule. This sort of twisted three-dimensional structure of the protein makes it enantioselective in nature. Enantioselective interactions between small molecules and proteins

in biological systems are well known [108]. Although all of the protein molecules are complex in structure and enantiospecific, they have not been used as successful chiral selectors yet, because the enantiomeric separation varies from one protein to another. The albumin proteins used as chiral selectors in chromatography and capillary electrophoresis are bovine serum albumin (BSA), human serum albumin (HSA), rat serum albumin (RSA) and guinea pig serum albumin (GPSA), but BSA and HSA have been found to be the successful chiral selectors. However, other protein molecules have been explored for their chiral separation capacities; that is, glycoproteins such as α_1 -acid glycoprotein (AGP), ovomucoid (OVM), ovotransferin, avidin and trypsin (CT), and certain enzymes such as chymotrypsin, riboflavin, lysozyme, pepsin, amyloglucosidase and lactoglobulin. Additionally, cellobiohydrazinase-I (CBH-I), a protein obtained from fungi, has also been used as a chiral selector in HPLC [109].

In 1976, Mikeš *et al.* [110] introduced a new concept by attaching a small chiral molecule to silica gel. In this CSP, the organic groups of the chiral molecule remain directed away from the silica gel, appearing in the form of a brush, and hence this is called a brush type phase. Later on, Pirkle and coworkers developed these types of CSP extensively, and nowadays they are known as Pirkle-type CSPs [111–119]. Normally, the chiral molecule attached to the silica gel contains π electron donors or π electron receptors, or both types of group. Therefore, these CSPs are classified into three groups; π -acidic (with π electron acceptor groups), π -basic (with π electron donor groups), and π acidic–basic (with π electron acceptor and donor groups), respectively. The reciprocity concept put forth by Pirkle has allowed the development of several generations of these types of CSP [113, 116]. The main advantage of these types of phases is that one can choose the type of chiral molecule to be attached to the silica gel. A specific and required chiral molecule (to be attached to the silica gel) can be selected by the reciprocity concept and bonded to the silica gel, and hence the chiral separation of a wide variety of racemic compounds can be performed easily and successfully. Recently, some chiral molecules that have specific groups other than π donors or π acceptors, such as polar and polarizable groups, have been grafted on to the silica gel surface. These types of CSPs have been found to have great potential for the chiral separation of different racemic compounds.

Ligand exchange chiral selectors involve the breaking and formation of coordinate bonds among the metal ions of the complex, the ligands and the chiral pollutants. Therefore, ligand exchange chromatography is useful for the chiral separation of pollutants that contain electron-donating

atoms, such as oxygen, nitrogen and sulfur. These types of pollutant contain amino, hydroxy and acid groups. Sometimes, the fast kinetics of the ligand exchange reactions in the metal ion coordination sphere make this technique suitable for the chiral separation of kinetically labile pollutants. Chiral ligand exchange chromatography was developed by Davankov [120, 121] in 1969. Copper(II) has been used as the ligand metal ion in most of the applications of ligand exchange chromatography. However, some other metal ions, such as nickel and zinc, have also been tested [122, 123]. Nowadays, these selectors are also useful for chiral analysis in capillary electrophoresis.

The crown ethers are synthetic macrocyclic polyethers: their name derives from both the crown-like appearance of their molecular structures and their ability to crown selectively with cations. The ether oxygens that are electron donors remain in the inner wall of the crown cavity, and are surrounded by methylene groups in a collar fashion. The IUPAC nomenclature for these ethers is complex, and hence trivial names are commonly used [124]. For example, 2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxa-cyclo-octadeca-2,11-diene is called dibenzo-18-crown-6 ether, where dibenzo, 18 and 6 indicate the substituent groups, the total number of atoms in the ring and the number of oxygen atoms, respectively. If the oxygen atoms of the ether are replaced by nitrogen or sulfur atoms, the crown ether is called an aza or a thia crown ether, respectively. Chirality in crown ethers is developed by introducing chiral moieties and, hence, the developed crown ether is called a chiral crown ether (CCE). The most important chiral groups used for this purpose are binaphthyl [125–128], biphenanthryl [129–131], hericene derivatives [132], tartaric acid derivatives [133], carbohydrate moiety [134], a chiral carbon atom with a bulky group directly incorporated in the crown ring [135, 136], aromatic bicyclo derivatives [3.3.1], nonane derivatives [137, 138] hexahydrochryse or tetrahydroindenoinden [139], and 9,9'-spiro-bifluorene groups [140]. The capability of these CCEs of crowning selectively, and their stereospecific configurations, make them suitable chiral selectors in chromatography and capillary electrophoresis. The application of these chiral selectors is limited, as they can only be used for the analysis of chiral pollutants containing amine and amide groups.

The new strategy for chiral separation in chromatography and capillary electrophoresis is the development of molecularly imprinted polymers. First of all, Wulf *et al.* [141] presented the idea of a molecularly imprinted polymers technique. This involves the incorporation of a target molecule (an imprint molecule) into a polymer and the removal of the print molecule, to leave a substrate selective site or cavities. This may be achieved either by

a print molecule (template) bonded to functionalized monomers in solution, and where after copolymerization with an excess of a crosslinker the print molecule is chemically cleaved off the polymer, or by the imprint molecule being mixed with functional monomers and undergoing electrostatic (ionic and hydrophobic) interactions with the monomer prior to polymerization in solution. After polymerization, hydrogen bonds are formed between the template and the carboxylic groups of the recognition sites (polymeric). Then the imprint molecule is removed by washing the polymer with an acidic organic solvent. The most commonly used monomers are methacrylic acid, 2-vinylpyridine and 4-vinylpyridine. This technique has not been popular up to the present day, but it is promising for the near future, as the required imprinted polymer can be synthesized and chiral analysis can be achieved easily and successfully. However, the synthesis of these chiral selectors suffers from certain drawbacks. To make a successful polymer of a chiral compound, the proper amount of the enantiopure imprint molecule is required. In some cases, a molecularly imprinted polymer can be so selective for a certain molecule that it would become necessary to make an imprinted polymer for each analyte to be tested [142]. Additionally, a small amount of the print molecule can be irreversibly incorporated into the polymer matrix, which would exclude the use of molecularly imprinted polymers for the determination of enantiomeric purity [143].

1.13 Detection in Chromatography and Capillary Electrophoresis

UV detection is used in most chiral analysis by HPLC and other liquid chromatographic modalities. However, some other detectors, such as conductivity, fluorescent and refractive index types, are also used. The choice of detector depends on the properties of the racemic compound to be resolved [41, 144]. Chiroptical detectors, which are based on the principle of polarimetry [145] or circular dichroism [146, 147], are also available. The enantiomer (+)- or (-)-notation is determined by these detectors. Some organochlorine pesticides are not UV-sensitive, and hence they are difficult to detect in liquid chromatography. The detection of these types of pollutant can be achieved by using a mass spectrometry (MS) detector, and therefore LC–MS instruments are now being put on the market for routine use [148, 149].

Due to the presence of halogen atoms in some pesticides, an electron capture detector is considered to be one of the most efficient detection methods

in gas chromatography. However, other detectors, such as conductivity, flame ionization, nitrogen and phosphorus types, may also be used in GC. Most chiral separation of these types of pollutants is carried out using gas chromatography. Also, GC–MS coupling is used to identify the separated enantiomers in real samples.

In TLC, detection is carried out using a number of approaches. The UV-sensitive enantiomers are observed in a UV cabinet, while some other non-UV-absorbing chiral molecules may be detected by developing a colour using a suitable reagent; for example, compounds containing amino acids and amines are located by developing a colour on the TLC plate using ninhydrin [150]. Moreover, iodine vapour is used as a universal detection method on TLC plates. The separated enantiomers adsorb the iodine vapour and become yellow in colour. The detection limits for chiral pollutants depend on a number of factors, such as the properties of the molecules, the mobile phase, the chiral stationary phase, the type of chiral selector and the sensitivity of the detector. The lowest detection limits have been reported at the milligram to nanogram levels.

1.14 Other Methods of Separation of Chiral Pollutants

Besides chromatographic and capillary electrophoretic methods, some other alternatives are also available for the chiral separation of environmental pollutants. These include spectroscopic, sensor, simulating moving bed adsorption and membrane methods [37–40, 67, 68, 151–153]. Optical rotation measurements, nuclear magnetic resonance (NMR) and infrared (IR) spectroscopic methods have been reported to distinguish enantiomers. In IR, differential scanning calorimetry (DSC) only distinguishes racemic mixtures (\pm) and individual enantiomers. In NMR, chiral solvating agents (CSA) can be utilized to promote a change in the chemical shift of the chiral (carbon) atoms. However, while the spectroscopic and DSC methods are sensitive to interference by impurities of a chiral or nonchiral kind in the sample of interest, they can lack sensitivity and accuracy, since the differences between the isomers and/or the isomer mixtures may be very small.

Biosensors have been used for the discrimination of industrial, medicinal and environmental chiral molecules [68]. The molecularly imprinted polymer based biomimetic sensors are useful for this type of chiral analysis [151]. The advantages of molecularly imprinted polymers over other biomolecules are their high stability and their resistance to the strong medium. Gas sensors have also been used for chiral analysis, this technique involving the use of chiral amide (octyl-Chirasil-Val[®]) attached to a

polysiloxane chain and coated on a solid surface such as quartz, glass and so on [154]. In this method, one enantiomer undergoes a greater interaction with the incorporated chiral selector in the polymer coating as compared to the other antipode, and this difference leads to the enantiodifferentiation. Chiral discrimination of the vaporized racemates results in a small change in the thickness of the polymer film after absorption of the pollutants into the coating, which causes a shift in the reflection of monochromatic light through the transparent film on the glass substrate surface.

Chiral discrimination has been achieved by the use of membranes, this technique involving the principle of enrichment of enantiomers in organic solutions on both sides of a membrane, which prevents the two organic phases from mixing together [152]. These membranes are permeable for the enantiomers, with a preferred passage of only one enantiomer in comparison to its antipode, which results in the enrichment of only one enantiomer. Various types of membranes are available, including polymers, solid supported liquid membranes [155], emulsion liquid membranes [156] and bulk liquid membranes [157]. In spite of the good capacity of these membranes, the method is not yet popular, as it is still under development.

The simulated moving bed adsorption technique is based on the movement of the stationary phase. The front and back ends of a series of columns are connected to form a circle, and during rotation of the columns a countercurrent movement of the phase relative to the liquid stream in the system is developed [158]. Injections of the fresh chiral analyte and the solvent are made at various connecting points, and the separated enantiomers are withdrawn simultaneously at certain time intervals. This is a continuous process that provides certain advantages for enantiodiscrimination. The chiral selectors used in this technique are the same as those utilized in liquid chromatography and capillary electrophoresis.

In view of the importance of the enantiomeric separation of chiral pollutants, attempts are made in this book to explain the art of enantiomeric separation of pollutants by chromatography and electrophoresis. Following this introduction, the remaining nine chapters discuss enantioselective toxicities, metabolism and biotransformation, extraction, purification and pre-concentration, and the determination of chiral pollutants by chromatography and capillary electrophoresis. In this book, the art of the chromatographic and electrophoretic analysis of chiral pollutants is described in a well defined, systematic and scientific way, including discussions on optimization of the methods and the separation mechanisms. We hope that this book will be useful to academicians, scientists, workers in other regulatory authorities and students dealing with the analysis of chiral pollutants.

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