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CHIRAL CHROMATOGRAPHY AND ITS APPLICATION TO THE PHARMACEUTICAL INDUSTRY: A REVIEW

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ABSTRACT

Chiral chromatographic enantioseparation has been in practice by researchers. There has been a considerable interest in the synthesis and separation of enantiomers of organic compounds especially because of their importance in the biochemical and pharmaceutical industries. Often, these compounds are purified rather than being produced by chiral-specific synthesis. We herein present a general discussion that focuses on the chromatographic enantioseparation, which we hope will be useful to chromatographic and pharmaceutical industries.

***Keywords*:** Chiral chromatography, enantioseparation, pharmaceutical industry.

INTRODUCTION

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a “stationary phase”, which separates the analyte from other compounds in the mixture based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus effecting the separation (Laurence and Christopher, 1989; Pascal *et al.*, 2000).

Chromatography may be *preparative or analytical*. The purpose of preparative chromatography is to separate the components of a mixture for further use and is thus a form of purification technique. Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture (Laurence and Christopher, 1989; Pascal *et al.*, 2000).

Chiral chromatography involves the separation of stereoisomers. In the case of enantiomers, these have no chemical or physical differences apart from being three-dimensional mirror images. Conventional chromatography or other separation processes are incapable of separating them. To enable chiral separations to take place, either the mobile phase or the stationary phase must themselves be made chiral, giving differing affinities between the analytes (Schreier *et al.*, 1995).

The main goal of this review is to provide a brief overview of chiral separations to researchers who are versed in the area of analytical separations but unfamiliar with chiral separations. This review highlights significant issues of the chiral separations and provides salient examples from specific classes of chiral selectors where appropriate.

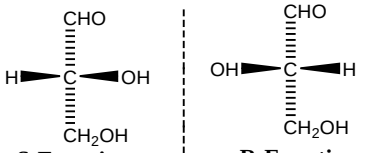
Terms and Definitions

General Terms Related to Chirality

Chirality; The word "chiral" comes from the ancient Greek "cheir" which means "hand". The definition of chirality is something which has an object to mirror image relationship like a pair of hands which cannot be superimposed. These two mirror images are known as "enantiomers". A mixture of these enantiomers in equal proportions is said to be "racemate or racemic mixture" (Eliel, *et al.*, 1994; Moss, 1996).

Stereoisomers: Isomers that possess identical constitution but which differ in the arrangement of their atoms in space, i.e. *enantiomers, diastereomers*, *cis-trans-isomers* (Eliel, *et al.*, 1994; Moss, 1996).

Enantiomer: One of a pair of molecular entities which are mirror images of each other and non-superposable (Eliel, *et al.*, 1994; Moss, 1996). Example, 2,3-dihydroxypropanal.



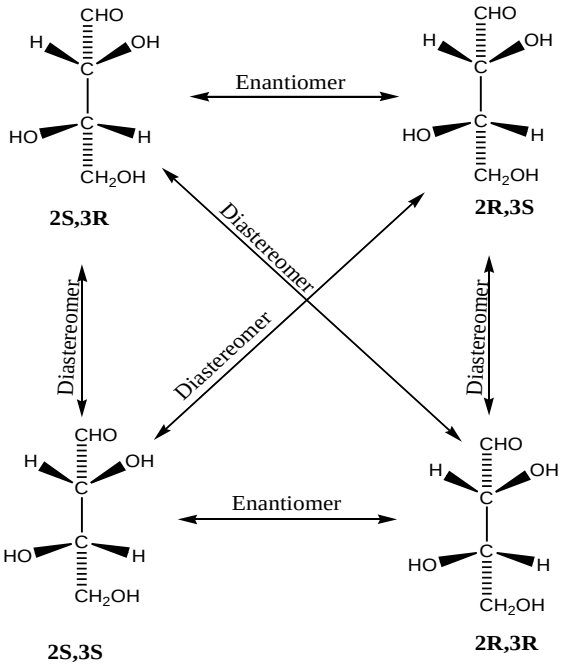
S-Enantiomer **R-Enantiomer**

Diastereoisomers (Diastereomers)*:* Are stereoisomers with a different relative configuration and not related as mirror images. They have different chemical and physical properties (Schreier *et al.*, 1995).

Molecules possessing more than one stereogenic centre also exhibit diastereoisomerism because inverting one or more (but not all) of the centres leads to structures which do not have an object to mirror image relationship (Schreier *et al.*, 1995). Inversion of a single stereogenic centre gives an epimer of the original structure. Inversion of all stereogenic centres gives the enantiomer.

A molecule possessing n stereogenic centres has a maximum of: 2^n stereoisomers, 2^{n-1} pairs of enantiomers and n epimers. Molecular symmetry within the molecule may result in a reduction of the numbers of different isomers due to internal compensation (Eliel, *et al.*, 1994; Moss, 1996).

Example: 2,3,4-Trihydroxybutanal;



Diastereoisomerism; Stereoisomerism other than enantiomerism and *cis-trans* isomerism. Diastereoisomers (or diastereomers) are stereoisomers not related as an object to mirror images. Diastereoisomers are characterised by differences in physical properties, and by differences in chemical behaviour towards achiral as well as chiral reagents (Eliel, *et al.*, 1994; Moss, 1996).

Terms Related to the Separation Process

***Chiral* additive**: This is a *chiral* selector which has been added as a component of a mobile phase or electrophoretic medium that change it to *chiral* mobile phase (Eliel, *et al.*, 1994; Moss, 1996).

***Chiral* selector**: The *chiral* component of the separation system capable of interacting *enantioselectively* with the *enantiomers* to be separated (Eliel, *et al.*, 1994; Moss, 1996).

***Chiral* stationary phase**: A stationary phase which incorporates a *chiral selector*. If not a constituent of the stationary phase as a whole, then chiral selector can be chemically bonded to (*chiral* bonded stationary phase) or immobilised onto the surface of a solid support or column wall (*chiral* coated stationary phase), or simply dissolved in the liquid stationary phase (Eliel, *et al.*, 1994; Moss, 1996).

Enantioselective chromaltography (electrophoresis): The separation of *enantiomeric* species due to the *enantioselectivity* of their interaction with the *chiral* selector(s) of a chromatographic (electrophoretic) system. The term is also sometimes referred to as *Chiral chromatography (electrophoresis)* (Eliel, *et al.*, 1994; Moss, 1996).

Enantioselective column; A chromatographic column containing a *chiral stationary phase is* also called a *chiral column* (Eliel, *et al.*, 1994; Moss, 1996).

***Enantioselectivity (in chiral separations)*:** The preferential interaction with the chiral selector of one enantiomer over the other (Eliel, *et al.*, 1994; Moss, 1996).

***Enantioselectivity of a chromatographic (electrophoretic) system*:** the ratio of the retention factors of two solute enantiomers in a chiral chromatographic (electrophoretic) system (Eliel, *et al.*, 1994; Moss, 1996).

Terms Related to the Chiral Purity of the Sample

Enantiomer excess/Enantiomeric excess; for a mixture of (+) and (-) enantiomers, with composition given as the mole or weight fractions; *F*(+) and *F*(-); Where, *F*(+) + *F*(-) = 1.

Thus, the enantiomeric excess (frequently this term is abbreviated as e.e. (Cox, 2001)) is defined as:

$$\%\, e.e = \frac{|F(+) - F(-)|}{|F(+) + F(-)|} \times 100$$

Enantiomeric purity see Enantiomer excess.

Optical purity: the ratio of the observed optical rotation of a sample consisting of a mixture of enantiomers to the optical rotation of one pure enantiomer: See *enantiomeric excess* (Cox, 2001).

Diastereoisomer excess/Diastereoisomeric excess*;* this is defined by analogy with *enantiomer excess*, as *D*1 - *D*2; and the percent diastereoisomer excess as 100 (*D*1 - *D*2), where the mole fractions of the two diastereoisomers in a mixture or the fractional yields of two diastereoisomers formed in a reaction are *D*1 and *D*2 (*D*1 + *D*2 = 1). The term is not applicable if more than two diastereoisomers are present. Frequently this term is abbreviated to d.e. (Cox, 2001).

Chiral Separations

There are series of technique used in separating racemate below some of these methods listed (Timothy, 2006)**.**

- Capillary Electrophoresis, CE.
- Thin-Layer Chromatography, TLC.
- Supercritical Fluid Chromatography, SFC.
- Gas Chromatography, GC.
- High-Performance Liquid Chromatography, HPLC.
- Capillary Electrochromatography, CEC.

Thin-layer chromatography

Though TLC is used less often than other separation techniques in enantioresolution methods, it remains a reliable separation technique. Chiral separations of ibuprofen and propranolol were reported on commercially available TLC plates with detection by densitometry, and a TLC method for the enantioseparation of ibuprofen was used in physicochemical studies of the oscillatory instability of profens when stored for long periods in aqueous media (Cox, 2001). A method using silica gel plates and (-)-brucine as a chiral selector was reported for the enantioseparation of ibuprofen (Cox, 2001). The TLC enantioseparation of verapamil was also achieved using the macrocyclic antibiotic vancomycin as the chiral selector impregnated on silica gel plates (Cox, 2001*)*.

Applications of Chirality to Pharmaceutical Industries

Every living body contains amino acids, sugars, proteins and nucleic acids. All of these are important to living body and are of chiral molecules. An interesting feature of these chiral biomolecules is that in nature they usually exist in only one of the two possible enantiomeric forms. When a chemist synthesizes a chiral molecule in an achiral environment using achiral starting materials, an equal mixture of the two possible enantiomers (i.e. a racemic mixture) is produced. In order to make just one enantiomer, some enantioenriched starting material, reagent, catalyst, or template must be present in the reaction medium. Oftentimes, only a single enantiomer of a chiral molecule is desired, as is the case when the target molecule is a chiral drug that will be used in living systems. Drug molecules can be likened to tiny keys that fit into locks in the body and elicit a particular biological response. Since the 'locks' in living organisms are chiral, and exist in only one of the two possible enantiomeric forms, only one enantiomer of the 'key' molecule should be used (the mirror image of our car key will not start our car) (Lien *et al.*, 2006).

In general, the use of both enantiomers in a racemic formulation of a chiral drug may be wasteful, and sometimes even introduces extraneous material that may lead to undesired side effects or adverse reactions. The importance of chirality has been appreciated and addressed by the pharmaceutical industry for decades. As technologies for measuring and making enantiopure materials have improved, the production of enantiopure pharmaceuticals has become commonplace, with many of the top selling drugs in the world now being sold in enantiopure form. Consequently, the subject of chirality and the pharmaceutical industry is a topic of considerable recent interest and importance (Lien *et al.*, 2006).

Transformations of achiral compounds

If these reactions result in the formation of a chirality element in the molecule, the reaction product appears to be an equivalent mixture of a pair of enantiomers, a racemate, which is optically inactive. Racemates are also formed through racemisation of chiral compounds. Racemates crystallize in the form of a racemic compound or, less frequently, as a conglomerate. Separation of the enantiomers comprising the racemate, i.e., the resolution of the racemate, is a common problem in stereochemical research as well as in the preparation of biologically active compounds, in particular, drugs. The problem is that in contrast to diastereomers and all other types of isomeric species, enantiomers, in an achiral environment, display identical physical and chemical properties (Cox, 2001; Duff *et al.*, 1993).

One approach to separate enantiomers, sometimes referred to as indirect enantiomeric resolution, involves the coupling of the enantiomers with an auxiliary chiral reagent to convert them into diastereomers. The diastereomers can then be separated by any achiral separation technique (Cox, 2001; Duff *et al.*, 1993).

Direct enantiomeric resolutions are only feasible in chromatographic systems which contain an appropriate chiral selector. The latter can be incorporated into the stationary phase that is, chiral stationary phase or be permanently bonded to or coated onto the surface of the column packing material, *chiral bonded and chiral coated stationary phases*. In all these cases it is appropriate to refer to the chromatographic column as an enantioselective (chiral) column. Enantioselective chromatography can also be performed on achiral chromatographic columns using the required chiral selector as a chiral mobile phase or a chiral mobile phase additive (Timothy, 2006). Combinations of several chiral selectors in the mobile phase as well as mobile and stationary phases are also feasible. In the case of chiral stationary phases, the enantiomer that forms the more stable association with the chiral selector will be the more strongly retained species of the racemate. The enantioselectivity of the chiral chromatographic system is then expressed as the ratio of the retention factors of the two enantiomers. This ratio may approach the value of the thermodynamic enantioselectivity of the association of the chiral selector with the enantiomers.

This situation occurs when the association with the chiral selector governs the retention of the enantiomers in the chromatographic system and other nonselective types of solute-sorbent interactions are negligible (Cox, 2001; Duff *et al.*, 1993). On the other hand, a chiral mobile phase reduces the retention of the solute enantiomer which forms a stronger association with the chiral selector. Here again, the limit for the enantioselectivity of the chiral chromatographic system is set by the enantioselectivity of the selector-solute association in the mobile phase (Timothy, 2006). However, in the majority of chiral mobile phase systems, the chiral selectors as well as its associates with the solute enantiomers are distributed between the mobile and stationary phases. The effective enantioselectivity of the chromatographic system will therefore be proportional to the ratio of the enantioselectivities of the association processes in the stationary and mobile phases. Interaction of the chiral selector of the system with the enantiomers of the solute results in the formation of two labile diastereomers. These differ in their thermodynamic stability, provided that at least three active points of the selector participate in the interaction with corresponding sites of the solute molecule. This three-point interaction rule is generally valid for enantioselective chromatography, with the extension to the rule, stating that one of the required interactions may be mediated by the adsorption of the two components of the interacting pair onto the sorbent surface (ACS Symposium Series, 1991; Cox, 2001; Duff *et al.*, 1993; Welch, *et al.*, 2004) .

Because of the multiplicity and complexity of the interactions of the enantiomers to be separated with the chiral selector, sorbent surface and other components of the chromatographic system, the total enantioselectivity can depend strongly on the composition, pH and temperature of the mobile phase. Therefore, in papers on enantioselective chromatography, it is important to define these parameters. Enantioselective chromatography and capillary electrophoresis are extensively employed in the analysis of the enantiomeric composition (enantiomeric excess, optical purity) of chiral compounds. Liquid and supercritical fluid chromatography are also used for the isolation of chiral compounds from racemic mixtures on a preparative scale. Enantioselective separations have been realized in all possible separation techniques, including gas chromatography, column liquid chromatography, thin-layer chromatography, supercritical fluid chromatography, as well as electromigration methods, counter current liquid chromatography and liquid-liquid extractions (Krstulovic, 1989; Pirkle and Pochapsky 1989; Welch, *et al.*, 2004).

CONCLUSION

Chiral chromatography has become a preferred method for rapid separation of enantiopure compounds in the pharmaceutical industry, largely owing to the speed with which a chromatographic method can be developed and executed as well as the comparatively small labor requirements of the chromatographic approach. The use of chiral chromatography within the field of organic synthesis can be expected to increase as the technique becomes more familiar to synthetic chemists. It is important today to develop fast, cost effective chiral chromatographic methods in research labs and stimulate chemists to take advantage of available chiral stationary phases for enantiomer screening.

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