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# **ANALYTICAL CHIRAL SEPARATION METHODS**

## **(IUPAC Recommendations 1997)**

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## Analytical chiral separation methods

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*Abstract:* In recent years there has been considerable interest in the synthesis and separation of enantiomers of organic compounds especially because of their importance in the biochemistry and pharmaceutical industry. Frequently the methods used for the separations, for monitoring the progress of an asymmetric synthesis or optical purity of the products are chromatographic with either liquids, gases, or supercritical fluids as the mobile phase. More recently capillary electrophoresis has been added as an analytical chiral separation method.

These applications have led to a number of terms and expressions in addition to those commonly used or recently recommended for the chemistry and physical properties of chiral compounds. This Nomenclature provides the descriptions and definitions for additional terms particularly related to analytical separation methods, and to the formation and enantiomeric purity of chiral products.

### INTRODUCTION

Enantiomers are two chemically identical molecular species which differ from each other as non-superposable mirror images. The most simple and vivid model for enantiomeric structures is the two hands, left and right. Enantiomers, in addition to diastereomers and *cis-trans*-isomers, are thus a special case of stereoisomers.

The chirality (handedness) of enantiomeric molecules is caused by the presence of one or more chirality elements (chirality axis, chirality plane, or chirality centre, e.g., asymmetric carbon atom) in their structure. The chirality sense and optical activity of the enantiomers are determined by their absolute configuration, i.e., the spatial arrangement of the atoms in the molecule. In contrast to their conformation, the configuration of enantiomers cannot be changed without a change in the connectivity of constituent atoms. Designation of the configuration of enantiomers should be made in accordance with the Cahn-Ingold-Prelog *R, S*-system. The Delta-Lambda designations for enantiomers of octahedral complexes and the D, L Fischer-Rosanoff designations for amino acids and sugars are also in use.

Conventional chemical synthesis, in contrast to asymmetric synthesis, deals mostly with the 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 crystallise 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. (Energetic inequivalence of enantiomeric species, which can arise from the violation of parity by the weak interactions [1], is negligibly small - of the order of  $10^{-14}$  J mol<sup>-1</sup>).

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.

Nowadays, direct separation methods are commonly used in which the enantiomers are placed in a chiral environment. As a matter of principle, only chiral selectors or chiral irradiation (e.g., a polarised light beam which consists of two chiral circular-polarised components) can distinguish between two enantiomers. Chiral selectors can be an appropriate chiral molecule or a chiral surface (e.g., a chiral seed crystal). Due to the enantioselectivity (a special case of stereoselectivity) of the interaction with the two enantiomers, the chiral selector either transforms the enantiomers at a different rate into new chemical entities (kinetic enantioselectivity) or forms labile molecular adducts of differing stability with the enantiomers (thermodynamic enantioselectivity). Enzymic selective transformation of L-enantiomers of racemic D, L-amino acids is a typical example of a kinetically enantioselective process (kinetic resolution). Enantioselective (chiral) chromatography does not modify the enantiomeric species to be separated and thus represents an example of a thermodynamically enantioselective process.

Direct enantiomeric resolutions are only feasible in chromatographic systems which contain an appropriate chiral selector. The latter can be incorporated into the stationary phase (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. Combinations of several chiral selectors in the mobile phase [2] as well as mobile and stationary phases [3] 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, non-selective types of solute-sorbent interactions are negligible. 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). However, in the majority of chiral mobile phase systems, the chiral selector 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 [4].

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 [5].

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 realised 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. Numerous review papers and special monographs [6-15] describe the technical details as well as the achievements and potential of these important modern separation techniques.

In the following glossary of definitions and terms related to the chromatographic and capillary-electrophoretic separation of chiral compounds some terms (those marked with asterisks) were defined in the Basic Terminology of Stereochemistry, recently published by the IUPAC Joint Working Party on Stereochemical Terminology [16]. Some of these definitions contain further cross references which are to be found in the original paper.

## TERMS AND DEFINITIONS

### 1. General Terms Related to Chirality

#### 1.1 \*Chirality

The geometric property of a rigid object (or spatial arrangement of points or atoms) of being non-superposable on its mirror image; such an object has no symmetry elements of the second kind (a mirror plane,  $\sigma = S_1$ , a centre of inversion,  $i = S_2$ , a rotation-reflection axis,  $S_{2n}$ ). If the object is superposable on its mirror image the object is described as being achiral. See also handedness

#### 1.2 \*Diastereoisomers (Diastereomers) see diastereoisomerism

#### 1.3 Diastereoisomerism

*Stereoisomerism* other than enantiomerism and *cis-trans* isomerism. Diastereoisomers (or diastereomers) are stereoisomers not related as mirror images. Diastereoisomers are characterised by differences in physical properties, and by differences in chemical behaviour towards achiral as well as chiral reagents.

#### 1.4 \*Enantiomer

One of a pair of molecular entities which are mirror images of each other and non-superposable. See also *enantiomorph*.

#### 1.5 \*Stereoisomers

Isomers that possess identical constitution but which differ in the arrangement of their atoms in space. See *enantiomer*, *diastereomer*, *cis-trans-isomers*.

### 2. Terms Related to the Separation Process

#### 2.1 Chiral additive

The *chiral selector* which has been added as a component of a mobile phase or electrophoretic medium.

#### 2.2 Chiral mobile phase

A mobile phase containing a *chiral selector*.

- 2.3 **Chiral selector**  
The *chiral* component of the separation system capable of interacting *enantioselectively* with the *enantiomers* to be separated.
- 2.4 **Chiral stationary phase**  
A stationary phase which incorporates a *chiral selector*. If not a constituent of the stationary phase as a whole, the 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.
- 2.5 **Enantioselective chromatography (electrophoresis)**  
The separation of *enantiomeric* species due to the *enantioselectivity* of their interaction with the *chiral selector(s)* of a chromatographic (electrophoretic) system. Also called *Chiral chromatography (electrophoresis)*.
- 2.6 **Enantioselective column**  
A chromatographic column containing a *chiral stationary phase*. Also called a *chiral column*.
- 2.7 **Enantioselectivity (in chiral separations)**  
The preferential interaction with the chiral selector of one enantiomer over the other.
- 2.8 **Enantioselectivity of a chromatographic (electrophoretic) system**  
The ratio of the retention factors of two solute enantiomers in a chiral chromatographic (electrophoretic) system.

### 3. Terms Related to the Chiral Purity of the Sample

- 3.1 **\*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. See *stereoselectivity*; *diastereoisomerism*.
- 3.2 **\*Enantiomer excess/Enantiomeric excess**  
For a mixture of (+) and (-) enantiomers, with composition given as the mole or weight fractions  $F_{(+)}$  and  $F_{(-)}$  (where  $F_{(+)} + F_{(-)} = 1$ ) the enantiomeric excess is defined as  $|F_{(+)} - F_{(-)}|$  (and the percent enantiomer excess by  $100|F_{(+)} - F_{(-)}|$ ). Frequently this term is abbreviated as e.e. See *optical purity*.
- 3.3 **\*Enantiomeric purity see Enantiomer excess.**
- 3.4 **\*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*.

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