Chiral Separations

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Key Words

transient diastereomeric complexes, chiral chromatography, chiral capillary electrophoresis, chiral environment

Abstract

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. To researchers who are not familiar with this area, there is currently a bewildering array of commercially available chiral columns, chiral derivatizing reagents, and chiral selectors for approaches that span the range of analytical separation platforms (e.g., high-performance liquid chromatography, gas chromatography, supercritical-fluid chromatography, and capillary electrophoresis). This review begins with a brief discussion of chirality before examining the general strategies and commonalities among all of the chiral separation techniques. Rather than exhaustively listing all the chiral selectors and applications, this review highlights significant issues and differences between chiral and achiral separations, providing salient examples from specific classes of chiral selectors where appropriate.

1. INTRODUCTION

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. Because individual enantiomers of chiral drugs may evince very different bioactivities and/or biotoxicities, the emphasis on chiral separations in this review is on pharmaceutical applications. However, note that for any chiral entities interacting with a chiral environment such as biological organisms or chiral separation media, stereospecific differences can be manifested in a variety of ways. For instance, enantiomers of chiral pesticides degraded through enzymatic pathways may exhibit differences in environmental activity, persistence, or end products (1–3). Also, individual enantiomers of chiral flavoring or fragrance components may elicit different notes (4, 5). Thus, chiral separations are significant to investigators pursuing a broad array of interests.

Over the past 30 years, chiral separations have become an important tool in the analytical separation science arsenal. As a result, researchers unfamiliar with this area should know that there is currently a bewildering array of commercially available chiral columns, chiral derivatizing reagents, and chiral selectors for approaches that include all of the analytical separation platforms [e.g., high-performance liquid chromatography (HPLC), gas chromatography (GC), supercritical-fluid chromatography (SFC), and capillary electrophoresis (CE)]. This review begins with a brief discussion of chirality before examining the general strategies and commonalities among all of the chiral separation techniques. Rather than provide an extensive list of chiral selectors and applications, I refer the reader to References 6–10 for general reviews, References 11–14 for GC, References 15–19 for SFC, References 20–29 for HPLC, and References 30–36 for CE. This review highlights significant issues and differences between chiral and achiral separations, providing salient examples from specific classes of chiral selectors where appropriate. It is hoped that the inclusion of some areas outside more traditional separation applications will also stimulate further research.

A complete discussion of stereochemistry, its terminology, and the controversies surrounding it is beyond the scope of this review; therefore, the reader is directed to other sources (37–41). Briefly, the relationship among various types of isomers, with relevant examples and terms, is given in **Figure 1**. Stereoisomers are compounds that are made up of the same atoms and have the same sequence of bonds. Chiral compounds are a specific type of stereoisomer. They can exist as pairs of compounds that are nonsuperimposable mirror images of each other; these pairs are termed

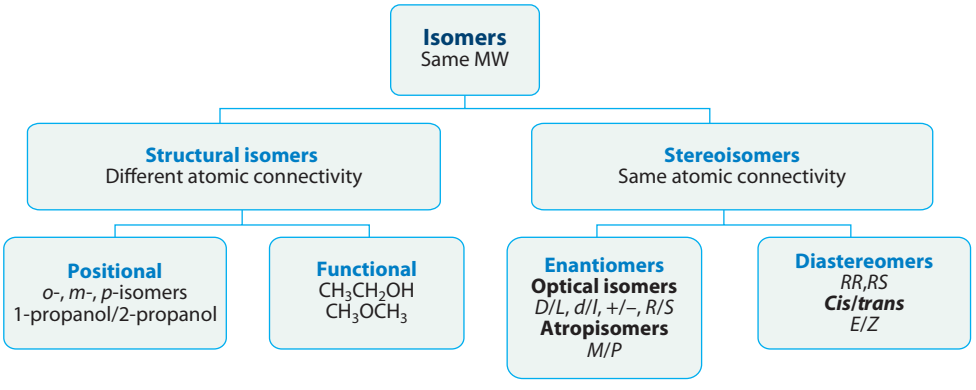

Figure 1

Relationship among isomers. Abbreviation: MW, molecular weight.

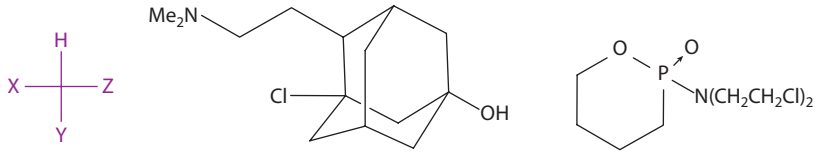

Figure 2

Examples of chiral compounds containing a stereogenic atom.

optical isomers or enantiomers. Separating these types of isomers is the focus of chiral separations and of this review.

The center of asymmetry in chiral molecules is called the stereogenic center. Molecules with this type of stereochemistry can have up to 2^N stereoisomers, where N is the number of chiral centers; however, the number of possible stereoisomers may be reduced if the molecule has an internal plane of symmetry in which one-half of the molecule is a reflection of the other half. The most familiar type of stereogenic center is an atom, such as carbon, with four different substituents (**Figure 2**). However, there also exist chiral compounds with stereogenic heteroatoms [e.g., phosphorus (42), nitrogen (43, 44), and sulfur (45, 46)]. Diastereomers are pairs of stereoisomers that are not enantiomers (39). Thus, stereoisomers include *cis/trans* isomers. Nonplanar chiral compounds that do not have a chiral center have a center of asymmetry that is not assigned to any specific atom (**Figure 3**). These types of enantiomers are sometimes referred to as atropisomers (47–49).

Historically, chiral separations have been considered among the most difficult of all analytical separations, and the wide-scale adoption of chiral separation technology in the pharmaceutical industry was initially greeted with some resistance. Mounting regulatory pressures requiring pharmaceutical companies to provide bioactivity and biotoxicity data on individual enantiomeric drugs as well as on 50:50 or racemic mixtures dampened initial enthusiasm for the development of chiral drugs. There was also some resistance to adopting an incompletely developed technology in a highly regulated environment. Chiral recognition mechanisms were not well understood for many of the most broadly applicable chiral stationary phases (e.g., polysaccharides), and early chiral columns lacked uniformity and robustness. Additionally, some members of the synthetic community thought that chiral separations would become passé as stereosynthetic pathways became available.

However, several factors have contributed to the persistent interest in chiral separations. The technology has advanced significantly as understanding of chiral recognition has grown

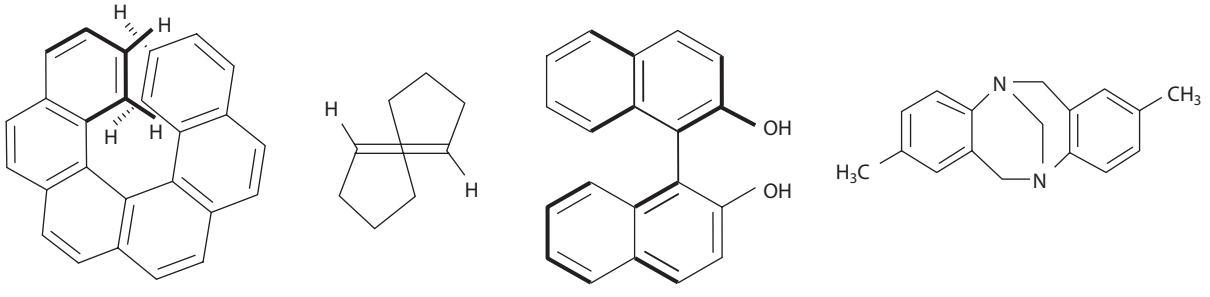

Figure 3

Examples of atropisomers.

(50–61). Racemization during storage, handling (62), or in vivo (63) is always a potential issue that makes it necessary to perform analytical testing of chiral compounds. In some nonstereospecific synthetic pathways, a cost-effective method for recovery of the desired enantiomer, or eutomer, coupled with racemization of the distomer increases overall stereospecific yields (64, 65). In the pharmaceutical industry, the original patent life for racemic drugs has been essentially extended by the issuance of patents for the single enantiomer form of the drug (sometimes referred to as racemic switches) in cases in which therapeutic activity resides in a single enantiomer (66, 67). Prilosec® (omeprazole) and Nexium® (esomeprazole or S-omeprazole), as well as Sensorcaine (bupivacaine) and Chirocaine (*levo*-bupivacine), are examples of drug entities that share this relationship; others will probably follow (68). In some instances, accounting for chirality has also provided much needed insight into bioactivity/biotoxicity mechanisms (69). Indeed, many adverse drug reactions are attributed to drug metabolism; many drug-metabolizing enzymes are known to be polymorphic (70) with varying stereo- and regiospecificity in their activity (71). Thus, chirality will probably play a critical role in the emerging field of pharmacogenomics (72).

2. CHIRAL SEPARATIONS

Chiral separations are concerned with separating stereoisomers, which exist as nonsuperimposable mirror image isomers. Because enantiomers have identical physical and chemical properties in achiral environments, most classical separation methods (e.g., distillation, liquid-liquid extraction, and chromatography) that rely essentially on differences in boiling points or solubilities are ineffective.

In achiral chromatography, separations are essentially driven by relative net differences in analyte interactions with the mobile-phase and stationary-phase environments. In chiral separations, selection or creation of an appropriate chiral environment is key to all chiral separations because enantiomers have the potential to stereospecifically interact with that chiral environment. Chiral recognition requires that two chiral entities be in close proximity to each other. Thus, a crystal may represent the ultimate chiral environment. **Figure 4**, which shows the chirodichroism in the solid state of dicarbonyl–rhodium(I) 3-(trifluoroacetyl)-(1*R*)-camphorate, dramatically illustrates how enantiomers can behave in different chiral environments (12). However, interaction between two chiral species is no guarantee of chiral discrimination. In general, as noted by Woodward et al. in 1963 (73), chiral discrimination requires that the chiral center of the analyte and the chiral center(s) of the chiral environment be in fairly close proximity to each other. Although some chiral

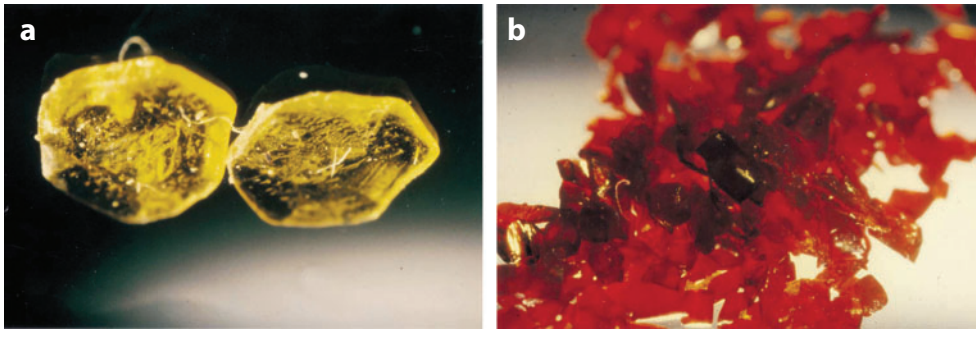

Figure 4

Chirodichroism in the solid state of dicarbonyl–rhodium(I) 3-(trifluoroacetyl)-(1*R*)-camphorate (13). (*a*) Dicarbonyl–rhodium(I) 3-(trifluoroacetyl)-(1*R*)-camphorate. (*b*) Racemate (12).

selectors have demonstrated wide applicability, the existence of a truly universal chiral selector is unlikely. Thus, all successful chiral separation strategies rely on providing a carefully chosen chiral environment for the analytes through the formation of either formal diastereomers or transient diastereomeric complexes.

2.1. Formal Diastereomers: Chiral Derivatization

Chiral environments can sometimes be created covalently through derivatization of a chiral compound with an optically pure chiral derivatizing reagent, thereby creating pairs of diastereomers (74). In general, chiral derivatization requires the presence of readily derivatizable functional groups (e.g., amines, hydroxyls, carboxylic acids, and thiols) in the analyte that is in close proximity to the stereogenic center. Common useful reaction pathways and examples of appropriate derivatizing agents can be found in References 75 through 77. Because diastereomers belong to the same class of compounds and have identical molecular weights, they have very similar but not identical chemical and physical properties; thus, they are easier to separate by classical methods than are enantiomers. Derivatives that can impart some structural rigidity to the diastereomers (e.g., amides versus esters) (78) or multiple interaction modalities (e.g., dipole-dipole and hydrogen bonding) are preferable. For instance, derivatization of chiral amino acids with one enantiomer of Marfey's reagent introduces an additional site for internal hydrogen bonding in one of the enantiomer derivatives that changes the hydrodynamic radius sufficiently to enable an electrophoretic separation of the diastereomers (**Figure 5**) (79). Differential populations of the conformational isomers (**Figure 6**) associated with individual diastereomers can also produce truly remarkable separations of diastereomers on conventional achiral supports through slightly different distributions of bulky substituents and hydrogen-bonding functionality on diastereomeric faces (80–82).

Frequently, more than one type of chiral derivatizing reagent may be available, and often both enantiomers of the chiral derivatizing reagent are available. Thus, there are considerable flexibility (83) and validation opportunities that may not be available with a direct, chiral stationary phase–based approach. However, additional validation is required to ensure that the chiral derivatizing agent is optically pure and that the derivatization does not racemize the chiral analyte.

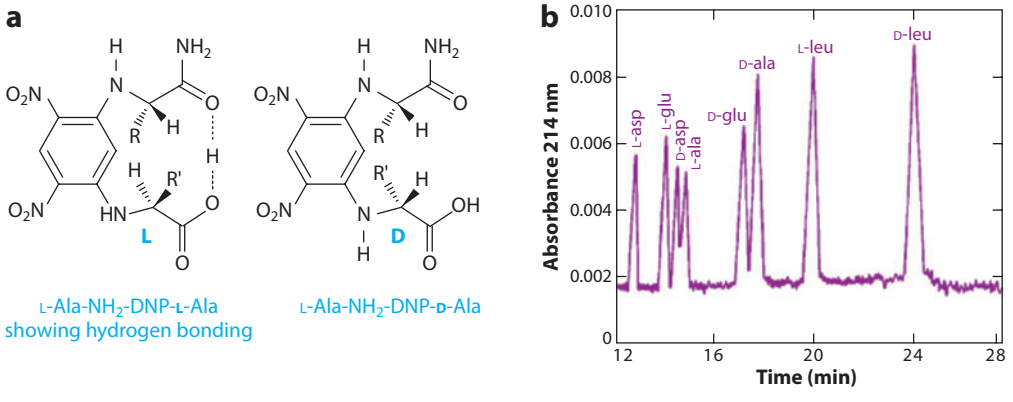

Figure 5

Separation of amino acid diastereomers by means of Marfey's reagent. (*a*) Alanine diastereomer formed via Marfey's reagent. (*b*) Capillary electrophoresis separation of amino acid diastereomers (79).

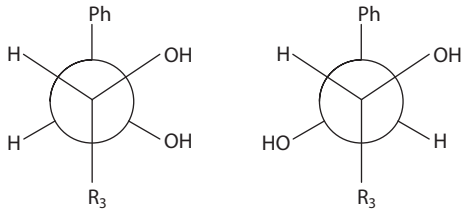

Figure 6

Conformers of a diastereomeric pair.

Like achiral derivatization, chiral derivatization can be used simultaneously not only to alter or improve sample recovery/extractability (84, 85) and chromatographic (86) or electrophoretic behavior of analytes but also to enhance detectability (87, 88). To maximize diastereomeric product yield, excess chiral reagents are generally used, but excess reagent can also introduce additional chromatographic complexity. Multiple products may be produced with chiral analytes incorporating more than one type of functionality (e.g., aminoalcohols), if care is not taken to ensure complete or very selective derivatization (89). Additionally, multifunctionalized chiral derivatizing reagents such as GITC (1,2,3,4-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate) (90, 91) may yield multiple products if partially hydrolyzed. The presence of undesirable side products can confound attempts to enhance sensitivity while further complicating the separation (**Figure 7**). Finally, chiral derivatization may not be an option if one desires to recover the optically pure underivatized analyte because of poor reaction reversibility or potential for racemization.

2.2. Transient Diastereomers: Chiral Chromatography

Transient diastereomeric complexes can be formed with chiral selectors either in free solution (e.g., as a chiral additive) or immobilized on a solid support (e.g., as a chiral stationary phase). In either case, chiral recognition is generally thought to require three unique interactions emanating from the stereogenic centers of both the chiral selector and the chiral analyte (92, 93). Thus, the formation of transient diastereomers, which can be exploited for chiral separations, usually requires that there be a primary docking mechanism between the chiral selector and the chiral analyte, accompanied by a combination of secondary interactions that define the stereospecificity of the interaction. This does not mean that the primary docking mechanism is the only interaction point between the chiral selector and the analyte. Most chiral selectors offer a variety of potential interaction sites. For example, **Figure 8** illustrates the structures and potential interactions between β-chloro-*tert*-butyl carbamoyl quinine and N-(3,5-dinitrobenzoyl)-(S)-leucine (54). This pair of interacting species also provides an excellent example of the interchangeability of chiral selectors and chiral analytes, a feature of chiral separations that Pirkle & Pochapsky (94) described as reciprocity. Simply stated, for chiral species that exhibit mutual stereospecific interactions, either one can be used as chiral media to enantiomerically resolve the other (94). Indeed, both N-(3,5-dinitrobenzoyl)-(S)-leucine (95) and β-chloro-*tert*-butyl carbamoyl quinine (96) have been used as chiral selectors in HPLC.

Chiral analytes may nonstereospecifically interact with the chiral moiety of the chiral selector (56) if the relative orientations of their stereogenic features preclude stereospecific interactions. Chiral analytes can also interact with achiral features of the chiral selection media [e.g., residual silanols (97, 98) or hydrophobic tethers (99) in chromatographic media]. Nonstereospecific

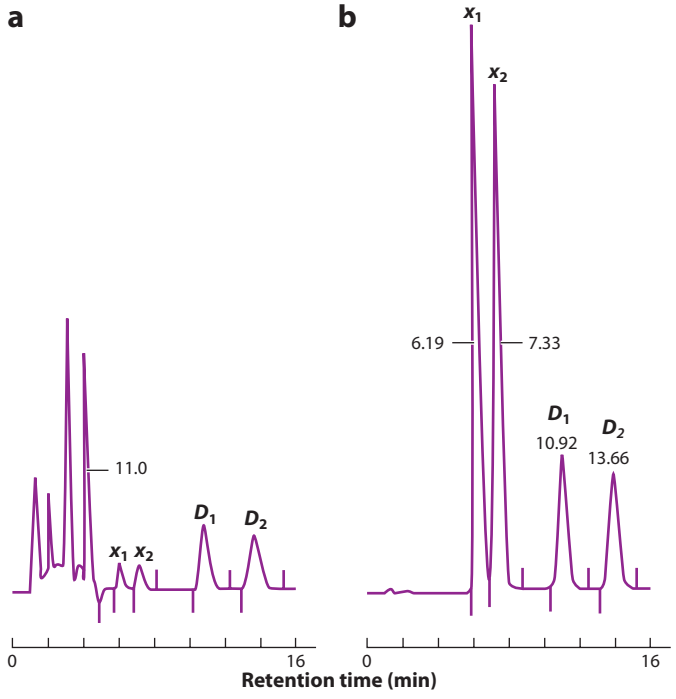

Figure 7

Separation of racemic-propranolol reacted with GITC (1,2,3,4-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate). Chromatographic conditions: Nucleosil 120-3 C_{18}; mobile phase, 55% (v/v) acetonitrile in 50 mM ammonium acetate buffer; pH, 6. (*a*) Ultraviolet detection ($\lambda = 228$ nm). (*b*) Fluorescence detection (excitation at 228 nm, emission at 342 nm) (91).

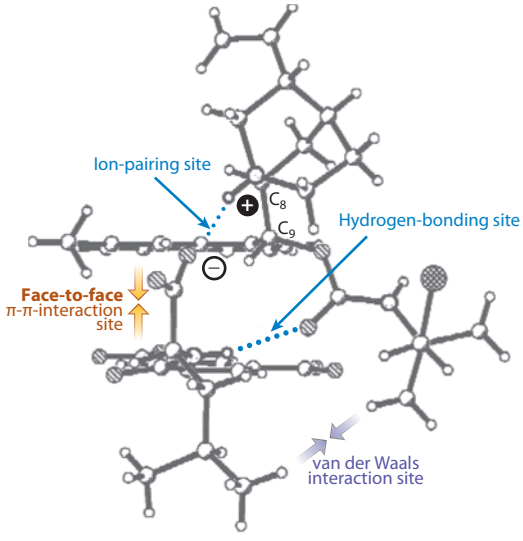

Figure 8

Schematic illustrating various potential interactions between β-chloro-*tert*-butyl carbamoyl quinine and N-(3,5-dinitrobenzoyl)-(S)-leucine (54).

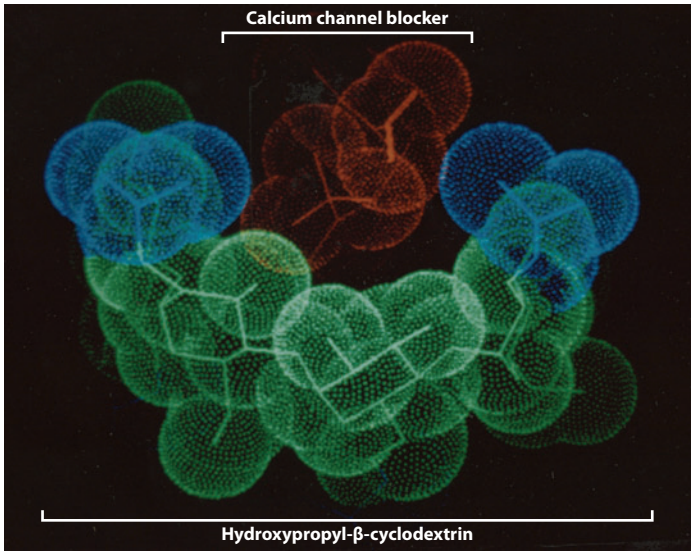

Figure 9

Computer-generated model of the inclusion complex formed between hydroxypropyl-β-cyclodextrin (*green and blue*) and a calcium channel blocker (*red*). The back side of cyclodextrin is not visible in the figure. Adapted from Reference 129.

interactions between the chiral analyte and the chiral selector are generally parasitic to the chiral separation (100, 101).

Making an informed choice of chiral selector requires relying on an inventory of potential interaction sites in the chiral analyte that can form the basis of the primary docking mechanism, their relative proximities to the chiral center, and any directionality constraints. Examples of primary docking mechanisms include electrostatic, π-acid:π-base, and/or inclusion complexation, but such mechanisms are usually based on the strongest interaction mode between the chiral selector and the chiral analyte under the specific experimental conditions. Meanwhile, the secondary interactions are usually weaker than the primary docking mechanism and can include dispersion, steric, hydrogen-bonding, and/or dipole-dipole interactions or combinations thereof. Achiral derivatization may also be used to introduce or enhance specific docking mechanisms such as hydrogen-bonding and dipole-dipole interactions (102) and π-π stacking (103).

Note that the primary docking mechanism can also be altered by changes in experimental conditions (103). For instance, in the case of native cyclodextrin–based chiral selectors (**Figure 9**), inclusion complexation generally dominates in aqueous media (104), but a combination of hydrogen-bonding interactions can provide the primary docking mechanism under nonaqueous liquid (105) or gas chromatographic conditions (52). Changes in pH that alter the ionization of various functionalities within the chiral analyte or chiral selector can affect the primary docking mechanism, thereby modifying chiral recognition. For example, protonation of the aromatic amine in aminoglutethimide redefines the primary docking mechanism with sulfated cyclodextrin from an inclusion-based mechanism to an electrostatic-based interaction located some distance from the chiral center of the analyte (106). As a result, enantioselectivity decreases despite increased retention (**Figure 10**). Thus, experimental conditions that promote the selected primary docking mechanism follow.

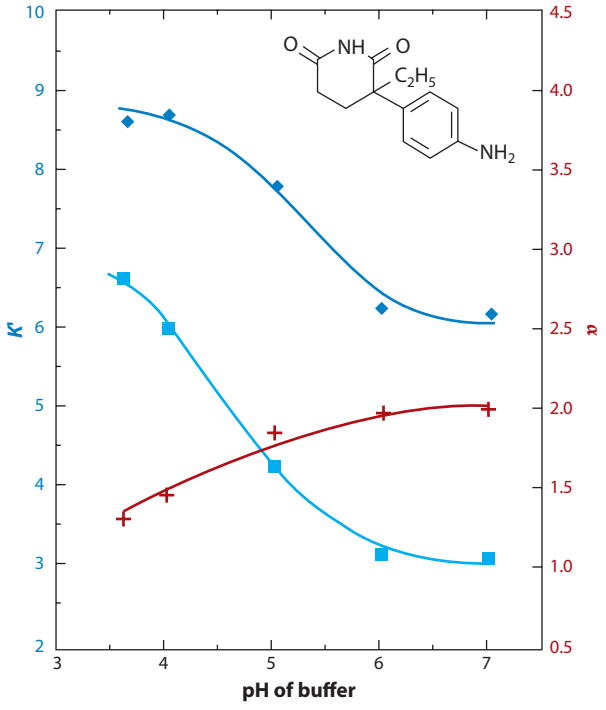

Figure 10

Plot of retention (K') and selectivity (α) versus pH for aminoglutethimide on a sulfated cyclodextrin chiral stationary phase (106).

2.2.1. Transient diastereomers: chiral additives. Chiral additives are chiral selectors that are solubilized in a liquid phase to form transient diastereomeric complexes with chiral analytes. Differences in the formation kinetics or relative stability of the transient diastereomeric complexes, as well as differences in the migration or partitioning behavior of the complex (104), drive the chiral separations. When the chiral additive is much larger than the chiral analyte, the chemical and physical properties of the diastereomeric complexes tend to be dominated by properties of the chiral additive, and only slight or insignificant differences in the properties or lifetimes of the individual transient diastereomeric complexes may be insufficient to effect a chiral separation. In such cases, the concentration of the chiral additives must not exceed the concentration at which both enantiomers are totally complexed. This situation is shown in **Figure 11**, which illustrates the effect of chiral additive concentration on enantiomeric separations through the R_f values in thin-layer chromatography (107) and electrophoretic migration times in CE (108). In CE, this can be expressed as (109)

$$\mu_1 - \mu_2 = \frac{(\mu_c - \mu_f)(K_1 - K_2)(\mathrm{CA})}{[1 + K_1(\mathrm{CA})][1 + K_2(\mathrm{CA})]},$$

where CA stands for concentration of the chiral additive. This equation shows that chiral separation requires there to be a difference in the mobilities of the free and complexed analyte as well as a difference in the binding affinities of the two enantiomers toward the chiral selector. This model also predicts an optimum chiral additive concentration that depends upon the binding constants of the two enantiomers.

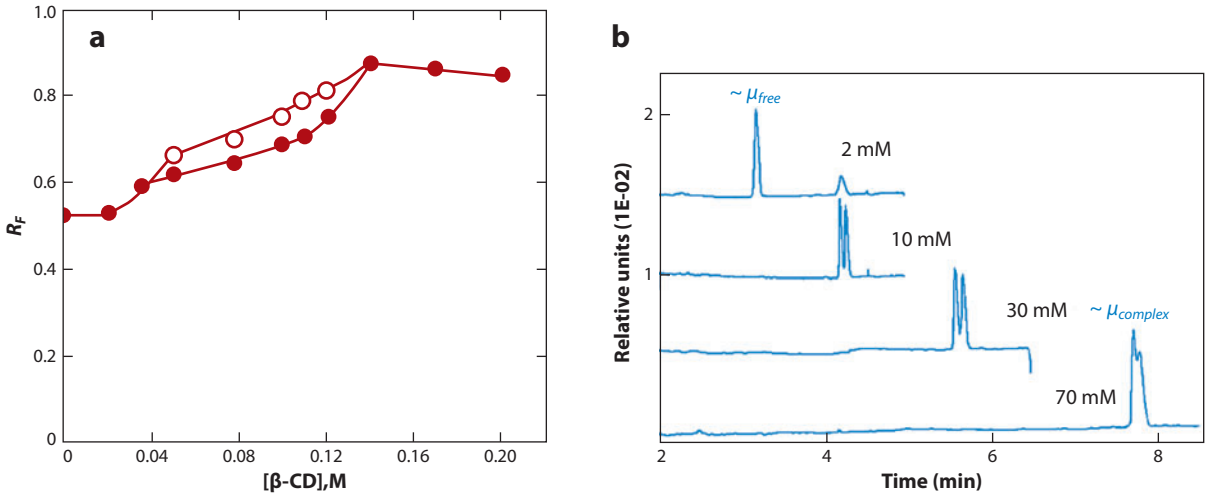

Figure 11

Impact of chiral additive concentration on the migration of enantiomers (*a*) in thin-layer chromatography (107) and (*b*) in capillary electrophoresis (108). Abbreviation: β-CD, β-cyclodextrin.

The presence of chiral additives allows the use of conventional solution-based separation methods, such as CE, although chiral additives can also be used with chiral stationary phases (110) in techniques such as thin-layer chromatography (113, 114), liquid chromatography (50, 115, 116), and centrifugal partition chromatography (117). Chiral additives have been used in liquid chromatography (104, 116) and CE under aqueous (120) and nonaqueous conditions (34, 121). Additionally, there have been several reports of chiral additives in SFC (118, 119).

Chromatography on achiral phases is generally better behaved than chromatography on chiral stationary phases. For instance, the use of chiral additives in thin-layer chromatography allows for the exploitation of secondary equilibria [e.g., micellization (33)], parallel processing, and potential two-dimensional and preparative scale separations, although the presence of chiral additives can complicate analyte recovery. Also, as in the case of chiral derivatization, additional validation is required to insure the optical purity of some chiral additives (122).

Detection may be facilitated (123) or complicated by the presence of a chiral additive. Given their ultraviolet transparency and the preponderance of spectroscopic-based detection in HPLC and CE, it is not surprising that cyclodextrins have generally been used as the dominant chiral additive (36). However, clever strategies have also been developed to isolate the analyte from the chiral additive in CE, facilitating a variety of detection modes including ultraviolet (124, 125), mass spectrometry (126), and electrochemistry (127).

The simplicity of this approach allows for considerable flexibility in the rapid screening of chiral additives, analytes, and conditions. Indeed, several chiral selectors adopted for chiral stationary phases (128–130) were first investigated as chiral additives in thin-layer chromatography (107) and CE (131, 132). Many chiral selectors are very good at separating enantiomers but less capable of separating closely related compounds (133). Thus, mixtures of additives [e.g., chiral and chiral (134) or chiral and achiral (135, 136)] may facilitate the separation of closely related compounds.

Perhaps the most valuable feature of the use of chiral additives is its straightforward ability to access chiral analyte–chiral selector binding information (56, 137). Binding constants for the individual enantiomers in a racemic mixture with chiral additives can be determined by TLC (138), HPLC (139), and CE (137, 140) within the same experimental protocols.

2.2.2. Transient diastereomers: chiral chromatography. Chiral chromatography relies on the differences in the relative stability of the transient diastereomeric complexes formed between the chiral analyte and an immobilized chiral selector. The chiral selector can be coated onto a chromatographic support (113, 141) or capillary wall (12, 52) or dissolved in a nonvolatile media (142), but most chiral selectors are commonly covalently attached to a chromatographic support, particularly in liquid chromatographic applications.

Chiral chromatographic methods have evolved via the same platforms (GC, HPLC, SFC, and CE) and exhibit retention modes (e.g., normal phase, reversed phase, and nonaqueous reversed phase in HPLC; micellar electrokinetic in CE) that are similar to those of achiral analytical separation methods. However, there are some unique and complicating aspects of chiral separations that arise from the very specific interactions between the chiral analyte and the chiral stationary-phase selector.

First, as mentioned above, the primary docking mechanism between the chiral selector and the chiral analyte can be altered by changes in experimental conditions. Thus, it is common for chiral analytes on chiral HPLC stationary phases to exhibit multimodal retention, which manifests as a minimum in retention with modifier concentrations (143). Typically, at low modifier concentrations, increases in modifier concentration decreases retention, but retention increases, again, at high modifier concentrations (**Figure 12**). In some cases, such an increase can be accompanied by a reversal in enantiomeric elution order (**Figure 13**) (51).

In achiral chromatography, increases in temperature generally lead to a decrease in retention. The impact of temperature on retention and enantioselectivity in chiral chromatography depends, to some extent, upon the primary docking mechanism. For instance, elevated temperatures increase mass-transfer efficiency in ion chromatography and micellar chromatography (144) and may play the same role in chiral separations, where the primary docking mechanism is similar. For instance, **Figure 14** illustrates the effect of increasing temperature on a chiral ligand exchange–based separation (145). However, perhaps as a consequence of potential multimodal retention mechanisms often encountered with chiral stationary phases, the impact of changing temperatures on retention for chiral species on chiral stationary phases may differ; retention for one enantiomer may actually increase, whereas the retention for another enantiomer may decrease on the same chiral phase at higher temperatures (146). **Figure 15** illustrates the impact of temperature on the separation of the R- and S-propranolol enantiomers via a protein phase. Protein phases can also exhibit allosteric interactions, in which nonsubstrate binding events occur that induce conformational changes in the protein tertiary structure far from a substrate-binding site on a protein. These allosterically induced conformational changes can induce profound changes in substrate retention on an immobilized protein stationary phase (147, 148).

Another unique aspect of chiral chromatography is its sensitivity to conformational exchange. This sensitivity typically manifests as the appearance of peaks with a plateau between them; these are also known as "Batman" peaks (**Figure 16**). Such peaks can occur with some atropisomers with hindered rotation (135) or with species that can undergo ring opening (133, 149), analogous to the mutarotation of sugars (150). Indeed, analytical chiral separations can be exploited to determine the barriers of inversion or enantiomerization (12, 135, 149) for these types of analytes.

2.2.3. Chiral separation validation. In a regulatory environment, most analytical separation assays are validated for linearity, precision, accuracy, limits of detection, and quantitation (151). Validation of achiral separations usually involves (*a*) comparison of the retention or migration behavior of sample components with the retention or migration behavior of authentic standards, (*b*) comparison of the retention or migration behavior of sample components with the retention or migration behavior of authentic standards via orthogonal separation methods, and (*c*) coupling of

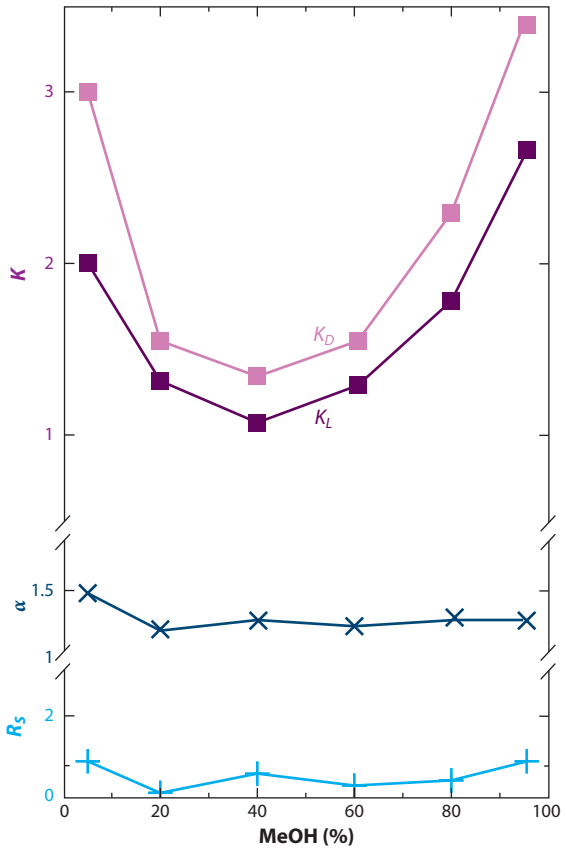

Figure 12

Impact of mobile-phase modifier on retention (K), selectivity (α), and resolution (R_s) on a ristocetin A macrocyclic antibiotic chiral stationary phase (143).

the separation method to highly specific detection methods (e.g., mass spectrometry and infrared spectroscopy). However, there are some additional validation issues relevant to chiral separations. Authentic, enantiomerically pure standards are frequently unavailable, and orthogonal chiral separation methods are frequently lacking. Also, note that the presence of multiple peaks with the use of chiral separation media does not guarantee that the separations obtained are chiral. For instance, cyclodextrin-based selectors have also been used to separate *ortho*-, *meta*-, and *para*-isomers (152, 153).

To circumvent these issues, numerous strategies have developed. Supporting evidence for chiral separation may be provided by incorporating highly specific detection such as mass spectrometry or infrared spectroscopy. Supporting evidence for chiral separations can be provided via chiroptical detection (154). Chiroptical detection relies on either circular dichroism or polarimetry (155) and produces positive and negative peaks for the two enantiomers (**Figure 17**) (156). Quantitation must be performed in tandem with nonchiroptical detection because chiroptical signal suppression through enantiomeric coelution or low concentration cannot be easily distinguished. Further evidence for chiral separations can be obtained through a comparison of detector response under different conditions (e.g., $\lambda = 240$ nm and $\lambda = 254$ nm for ultraviolet detection). The ratio of

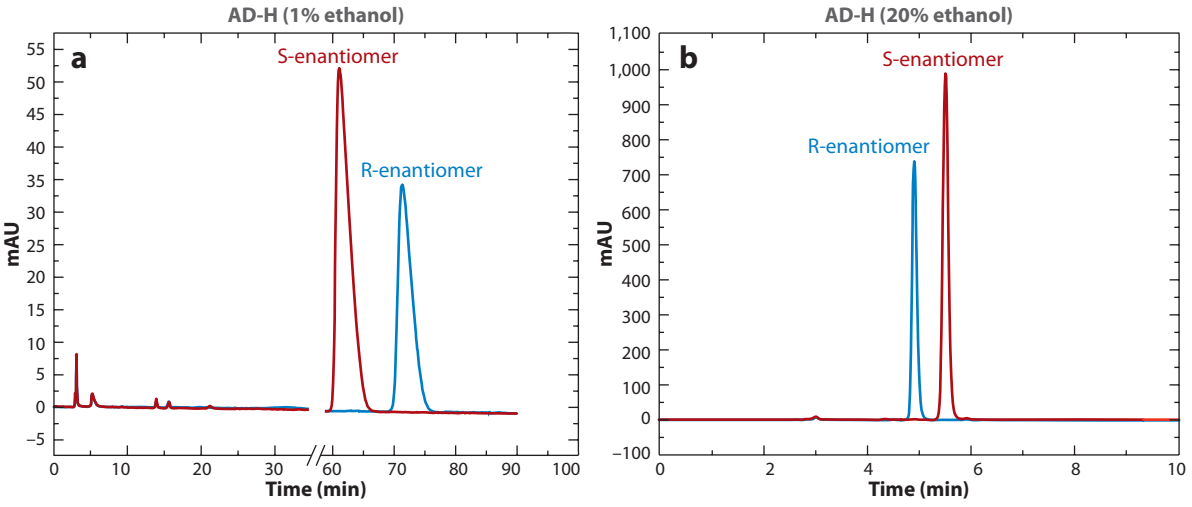

Figure 13

Impact of mobile-phase composition on the elution order from a derivatized amylosic chiral stationary phase (51).

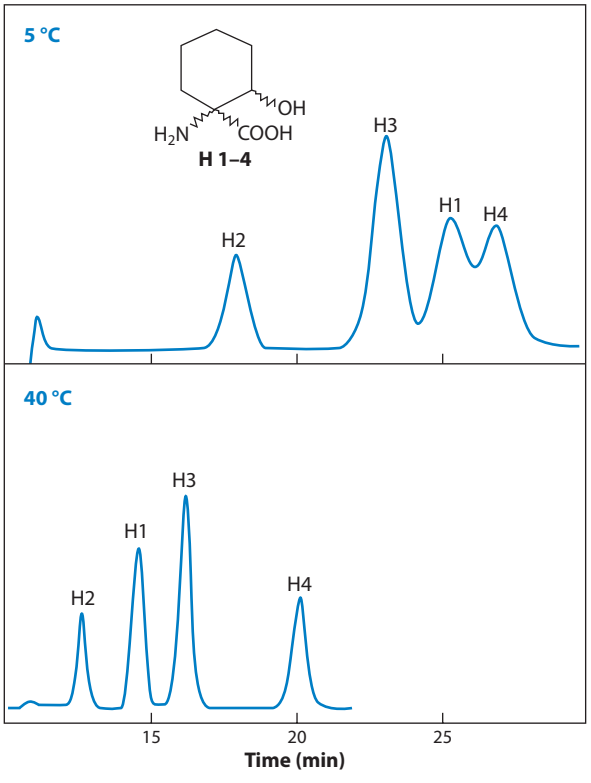

Figure 14

Effect of temperature on chiral ligand exchange separation of the four stereoisomers (H1 through H4) of 1-amino-2-hydroxycyclohexanecarboxylic acid. Adapted from Reference 145.

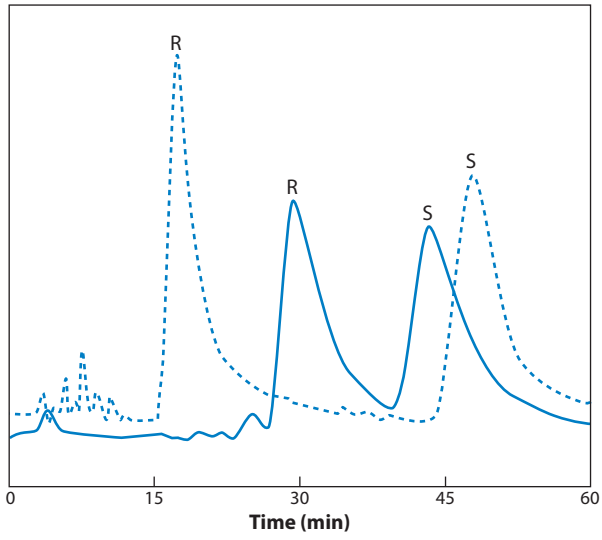

Figure 15

Effect of temperature on the chiral separation obtained on a protein phase (146).

the peak height or area for suspected enantiomers should be the same under different conditions in the absence of chiral additives, which would provide further evidence of chiral separation.

2.3. Transient Diastereomers: Chiral Enzymatic Methods

Chiral enzymatic methods rely on the stereospecificity of enzymatic reactions coupled to achiral separation techniques. In this approach, a racemic mixture with an enzymatically active motif is subjected to enzymatic transformation. In principle, only one enantiomer is preferentially

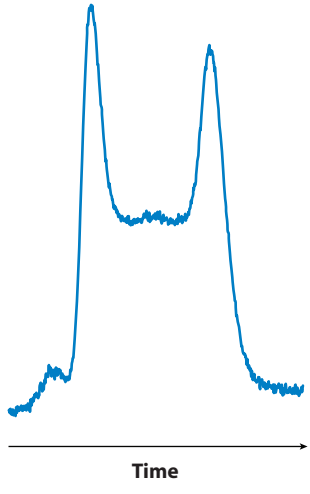

Figure 16

Chromatogram illustrating the impact of interconversion barriers for atropisomers in chiral separations (135).

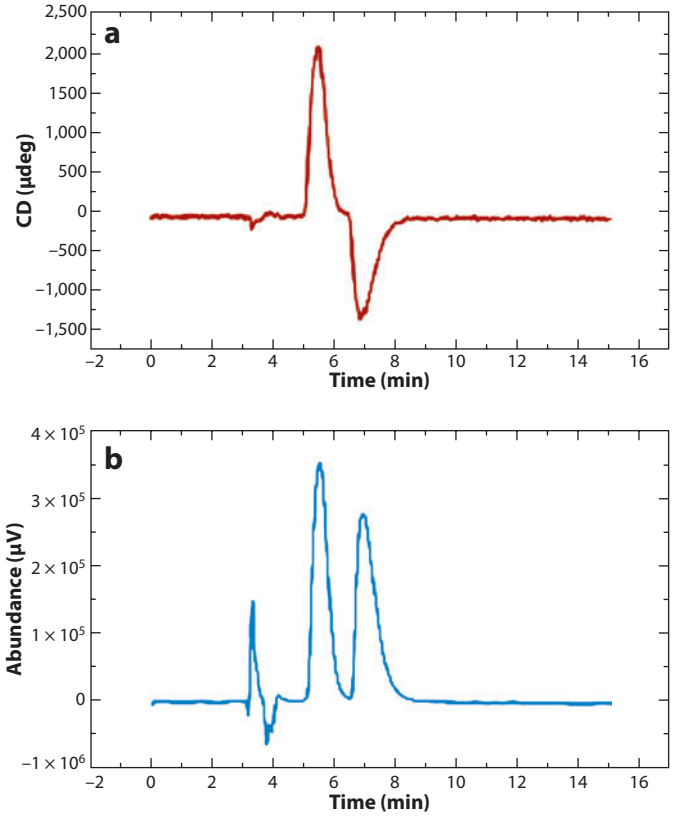

Figure 17

Chromatograms of the separation of bupivacaine enantiomers obtained at 230 nm via (*a*) circular dichroism (CD) detection and (*b*) ultraviolet detection (156).

transformed, but in actuality, enantioresolution is typically kinetic through differences in the enzyme-turnover rates. Lipases are the most widely used enzymes because of their ready availability, adaptability to nonnatural substrates, and amenability to organic solvents (64). A common approach is to transesterify a racemic ester in the presence of an achiral alcohol. The enzyme stereospecifically de-esterifies one enantiomer, and what would have been a difficult chiral separation of chiral esters then becomes a much easier separation of an ester of one enantiomer and an alcohol or carboxylic acid of the other enantiomer. Enzyme immobilization often improves enzyme stability and facilitates coupling to a chromatographic separation, leading to dramatic reductions in sampling handling. Therefore, enzymes such as penicillin G acylase (157) have been immobilized on solid supports and could serve as alternatives to conventional chiral chromatographic stationary phases (158). A further advantage of this approach is that, in many cases, the stereospecific reactive site and structural constraints on substrate structure are well understood.

3. CONCLUSIONS AND FUTURE TRENDS

Since the introduction of commercially available chiral stationary phases for chromatography in the 1970s and 1980s, chiral separations have become a mature science with an incredible array of strategies available to researchers. Selection or creation of an appropriate chiral environment

is key to all chiral separations because enantiomers can stereospecifically interact with a chiral environment. Making an informed choice of chiral selector relies on an inventory of potential interaction sites in the chiral analyte that can form the basis of the primary docking mechanism, their relative proximities to the chiral center, and any directionality constraints. Success in chiral separations also builds upon the lessons learned in achiral separations while recognizing the additional validation issues relevant to chiral separations.

Chiral separations will continue to play a significant role in analytical chemistry. However, major challenges remain in the area of preparative scale separations. Therefore, inherently "green" processes such as SFC and enzyme reactor–coupled chromatography are expected to play increasingly important roles in the area of chiral separations in the future.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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