A. Seidel-Morgenstern and H. Lorenz

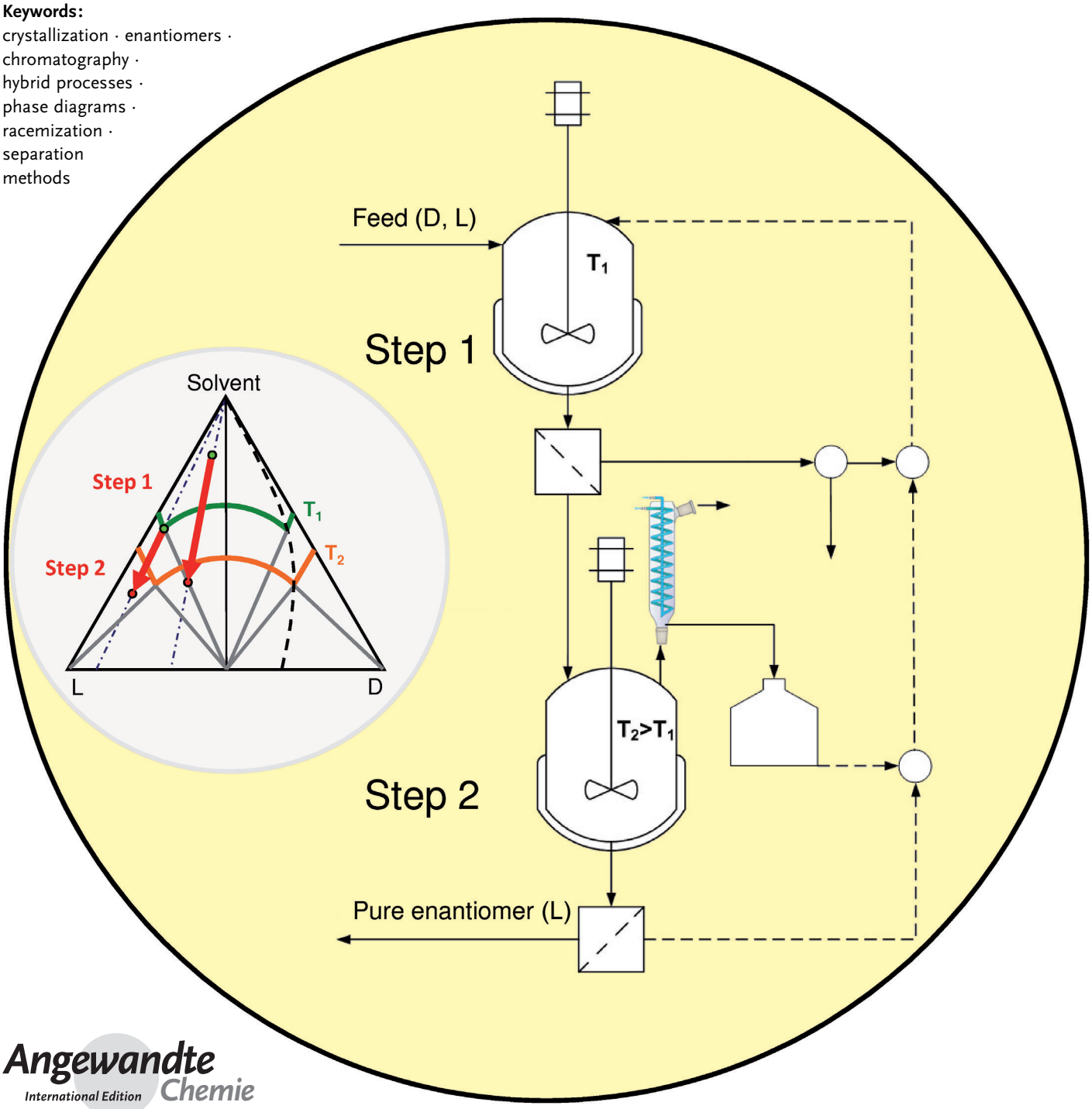
DOI: 10.1002/anie.201302823

Enantiomer Separation

Processes To Separate Enantiomers

*Heike Lorenz and Andreas Seidel-Morgenstern**

Keywords:
crystallization · enantiomers · chromatography · hybrid processes · phase diagrams · racemization · separation methods



Angewandte International Edition Chemie

The provision of pure enantiomers is of increasing importance not only for the pharmaceutical industry but also for agrochemistry and biotechnology. In general, there are two rival approaches to provide pure enantiomers. The "chiral" approach is based on developing an asymmetric synthesis of just one of the enantiomers, while the "racemic" approach is based on separating mixtures of the two enantiomers. In the last few years remarkable progress has been achieved in the latter area. This Review focuses in particular on enantioselective crystallization processes and preparative chromatography, including hybrid processes and the incorporation of racemization steps. Several examples from our research are used for illustration purposes.

From the Contents

1. Introduction

Enantiomers are stereoisomers that are nonsuperimposable mirror images. They are designated by classical notations as D or L, as *R* or *S*, or as (+) or (−).[1] There is still no final answer to the question of why life is essentially constructed using L-amino acids as building blocks. This fascinating and unsolved problem continues to stimulate a large spectrum of research (e.g. Refs. [2–5]). There is tremendous interest in producing pure enantiomers for the food and agrochemical industries, and in particular for the pharmaceutical industry.[6–8] Nowadays, there is clear evidence that often only one enantiomer of a chiral drug provides the desired physiological effect. In many cases, the other enantiomer has no effect or is even harmful. Regulators increasingly demand that chiral drugs are administered in an optically pure form.[9–11] This has intensified efforts in industrial and academic research to develop techniques which are capable of producing pure enantiomers. The approaches applied can be divided into: A) the "chiral approach" based on developing an asymmetric synthesis of just one of the enantiomers and B) the "racemic approach" based on separating mixtures of the two enantiomers.[12–14]

In the last few years remarkable progress has been achieved in the field of asymmetric synthesis (often also called enantioselective synthesis or stereoselective synthesis) by applying essentially the four main concepts indicated in Figure 1 and described shortly below.

Fermentation Methods

Methods based on exploiting the selective natural metabolism of microorganisms are applied very successfully on a very large scale to produce optically pure amino acids, in particular for use as food additives. For example, the production of L-lysine by fermentation is realized by exploiting genetically adapted cell lines of *Corynebacterium glutamicum*. There are many excellent reviews available that describe both the mechanisms of various successful biotransformations and related industrial applications.[15]

Chiral Pool Synthesis

This is the easiest approach, whereby available chiral starting materials are manipulated through successive reac-

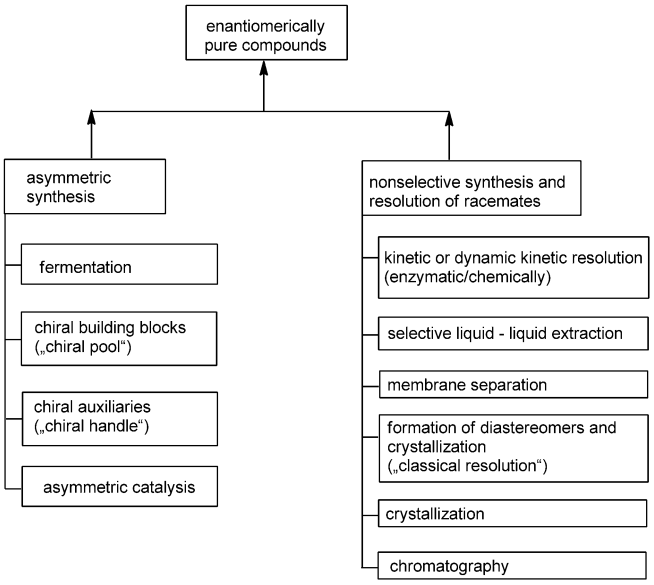


Figure 1. Possible pathways leading to pure enantiomers.

[*] Prof. H. Lorenz, Prof. A. Seidel-Morgenstern
Max-Planck-Institut für Dynamik komplexer technischer Systeme
Sandtorstrasse 1, 39106 Magdeburg (Germany)
Prof. A. Seidel-Morgenstern
Otto-von-Guericke-Universität Magdeburg
Institut für Verfahrenstechnik
Universitätsplatz 2, 39106 Magdeburg (Germany)
E-mail: seidel-morgenstern@mpi-magdeburg.mpg.de

tions using achiral reagents to obtain the desired chiral target molecules. This concept is especially attractive for target molecules with chirality similar to that of a relatively inexpensive naturally occurring building block, such as, for example, a sugar or an amino acid. However, the number of possible reactions the molecules can undergo is restricted, and tortuous synthetic routes may be required. This approach requires stoichiometric amounts of suitable starting materials, which should be sufficiently enantiopure and, thus, could be rather expensive (e.g. Ref. [16]).

Use of Chiral Auxiliaries

A common feature of many strategies in asymmetric synthesis is asymmetric induction to transform enantiomers into diastereomers, which have different reactivities. One asymmetric induction strategy is, for example, the use of a chiral auxiliary to form an adduct with the starting material which leaves only the desired trajectory open and physically blocks the other one. Assuming the chiral auxiliary is enantiopure, the different reaction pathways are not equivalent and lead to different products. The application of the concept requires one reaction step to add and another one to remove the auxiliary, which typically increases costs and decreases yields.[17]

Asymmetric Catalysis

Small amounts of chiral enantiomerically pure (or just optically active) catalysts can promote desired reactions and lead to the formation of larger amounts of enantiomerically pure or enriched products.[18–20] Currently three different kinds of chiral catalysts are mostly employed:

- metal–ligand complexes derived from chiral ligands,
- chiral organocatalysts, and
- biocatalysts.

The first methods were pioneered by William S. Knowles and Ryoji Noyori (Nobel Prize in Chemistry 2001) as well as Henri Kagan. In 1968 Knowles replaced the achiral triphenylphosphine ligands in the Wilkinson catalyst by the chiral phosphine ligands P(Ph)(Me)(propyl) and created a first asymmetric catalyst,[21,22] which was employed in an asymmetric hydrogenation that resulted in an enantiomeric excess of 15%. The method was ultimately used in an asymmetric hydrogenation step in the industrial production of L-DOPA. In the same year, Noyori and co-workers[23] published a chiral ligand for the cyclopropanation of styrene. In common with the observations made by Knowles, the enantiomeric excess for this first generation ligand was with 6% still disappointingly low. A major breakthrough was achieved by Kagan and Dang in 1971/1972 with the development of a very effective chiral bidentate diphosphine ligand.[24]

Examples of successful applications of asymmetric catalysis include:

- applications of the chiral phosphine BINAP in combination with ruthenium or rhodium compounds (these complexes catalyze effectively the hydrogenation of functionalized alkenes on only one face; a corresponding process developed by Noyori for the synthesis of menthol using a chiral BINAP–rhodium complex is commercialized[25]),
- the synthesis of Naproxen with a chiral phosphine ligand in a hydrocyanation reaction,[26]
- reactions of an alkene with osmium tetroxide in the presence of a chiral quinine ligand to form a vicinal diol (Sharpless dihydroxylation and aminohydroxylation).[27]

There are excellent reviews available that summarize the progess achieved in the field of enantioselective organocatalysis.[28] Comprehensive overviews regarding the current status of asymmetric catalysis including also fermentation and biocatalysis can be found in Refs. [29,30]. Various examples of successful industrial application have been described. In some cases, combinations of all three catalytic above-mentioned approaches are used to optimize productivity.

Nonselective Synthesis and Resolution of Racemates

Despite the tremendous progress achieved in the last decades, the number of highly selective reactions that supply pure enantiomers on an industrially relevant scale is still limited. Consequently, there is great interest in developing and using cheap, reliable, and widely applicable enantioselective separation processes.

In this Review we will discuss the current status of the most successful techniques for separating enantiomers. The focus is on techniques that can be applied on a preparative

Heike Lorenz is head of the crystallization research team at the Max Planck Institute for Dynamics of Complex Technical Systems and Adjunct Professor at the Otto von Guericke University in Magdeburg (Germany). She obtained a Diploma in Chemistry from the Freiberg University of Mining and Technology and completed her PhD and Habilitation at Magdeburg University. She received the "Research Award for Applied Research" of the state of Saxony-Anhalt (2008) and is elected chair of the Board "Crystallization" of Dechema/VDI-GVC's ProcessNet (since 2009).

Andreas Seidel-Morgenstern is professor of chemical engineering at the Otto von Guericke University and Director at the Max Planck Institute of Dynamics of Complex Technical Systems in Magdeburg (Germany). He received his PhD in 1987 from the Institute of Physical Chemistry of the Academy of Sciences of Berlin and completed his Habilitation in 1994 at the Technical University in Berlin. He received the Max Buchner Award of Dechema (2000) and Honoris Causa Doctorates of the Lappeenranta University in Finland (2008) and the University of Southern Denmark (2012).

and industrial scale, and thus the large arsenal of techniques developed for analytical purposes is not considered. The latter aspect is extensively discussed in various reviews and textbooks that focus mostly on analytical chromatography and electrophoresis.[31–33]

After a short introduction on important concepts which allow the separation of enantiomers, a particular focus will be on selective crystallization and chromatographic methods. Significant progress has been achieved in regard to these two methods and they are currently seen as the most flexible and productive ones. Various concepts will be illustrated by using examples of separations performed in our laboratory. Finally, promising new process variants based on coupling different separation principles and the inclusion of racemization steps will be discussed, presenting results of joint work carried out recently within the European project "INTENANT".[34]

2. Techniques for Separating Enantiomers

As a consequence of the importance and difficulty in separating enantiomers they have been studied intensively in the last few decades. Comprehensive overviews were given recently[35–39] and excellent descriptions of selected process concepts are available (e.g. Refs. [31,40]).

The provision of an initial asymmetry is usually needed to permit enantioseparation. In the various separation methods developed this is provided by specific chiral partners, which could be for example, reactants, solvents, carriers, or solid surfaces. If available, some of the target enantiomers could also be initially used and recovered later in increased amounts. The methods developed differ essentially in the types of chiral partner used and the phase situations present during the separation process.

Before we concentrate in more detail on the currently most promising enantioseparation methods, namely crystallization and chromatography, we describe below briefly the principle and status of other important resolution methods.

Kinetic and Dynamic Kinetic Resolution

Kinetic resolution exploits the difference in the consumption rates of two enantiomers in a chemical reaction. Through this difference, an excess of the less-reactive enantiomer is created, the concentration of which goes through a maximum before it disappears on full completion of the reaction. Kinetic resolution is a very old concept in organic chemistry and has been used successfully in the synthesis of various organic chiral molecules. It was first observed by Marckwald and McKenzie in 1899 during the esterification of racemic mandelic acid with optically active (−)-menthol to form a pair of diastereomeric esters.[41] In this reaction the *R* enantiomer of mandelic acid has the higher reaction rate and, with incomplete conversion, the reaction mixture becomes enriched in (*S*)-mandelic acid. Full hydrolysis of the incomplete esterification mixture gives an excess of (*R*)-mandelic acid. Taking the reaction to 100% completion will again produce equal amounts of both esters.

An important extension of kinetic resolution is dynamic kinetic resolution (DKR). It tackles the drawbacks of the above-described system, namely that the maximum conversion in the reaction is only 50% and the product has to be separated from the reactants. In DKR, it is possible to convert the achiral reactant with 100% completion because both enantiomers are connected in a chemical equilibrium. In this way, the faster-reacting enantiomer is replenished during the course of the reaction at the expense of the slower-reacting enantiomer. One of the earliest demonstrations of this method is an adaptation of the Noyori asymmetric hydrogenation.[42,43]

The principle of DKR has been used successfully in both enzymatic and chemical reactions. Several examples of enzymatic DKR processes are given in Ref. [44]. An overview on nonenzymatic kinetic resolution is provided in Ref. [45].

The combination of kinetic resolution in ionic liquids (ILs) and selective extraction with supercritical carbon dioxide (scCO2) provides a new approach for the separation of enantiomers, as exemplified by the lipase-catalyzed esterification of chiral secondary alcohols.[46]

The need to identify suitable reaction partners and the typically high requirements in terms of product purity still restrict wider application of the attractive kinetic resolution approach.

Enantioselective Liquid–Liquid Extraction

Liquid–liquid extraction is a mature separation method that can be operated in a productive countercurrent mode to fractionate racemates continuously into their enantiomers.[47] Enantioselective liquid–liquid extraction (ELLE) is closely affiliated to the large field of host–guest chemistry.[48] It combines the concepts of enantiomeric recognition and solvent extraction in a single technique. The possibility of operating on different reaction scales makes the use of liquid–liquid extraction interesting for enantioseparation.

There are many reports concerning successful enantioselective liquid–liquid extractions on a laboratory scale. Most of these studies deal with the identification and characterization of extraction systems. The most frequently used carriers are cyclodextrin derivatives (e.g. Ref. [49]), tartaric acid derivatives (e.g. Refs. [50,51]), and crown ethers (e.g. Ref. [52]). Metal complexes and metalloids have also been applied successfully as reactive extractants.[53,54] The resolution of racemic *N*-benzyl-α-amino acids by using a chiral cobalt(III)-salen complex was studied in Ref. [55]. Recently, successful attempts were reported that combine the effects of two carriers.[56] A review regarding technological aspects was given in Ref. [57].

The development of efficient processes requires multistage systems with back-extraction sections to recover the host. Many types of extraction columns are available with a broad range of internal constructions to enhance mass transport and facilitate separation. Numerous types of centrifugal extractors have been developed. The first device that was especially designed for ELLE purposes was the chiral resolution machine developed by Cram and co-work-

ers.[58] A novel more-advanced centrifugal contactor separator was introduced recently.[59] The application of a related concept designated as continuous countercurrent chromatography is described in Ref. [60]. Overviews regarding enantioselective liquid–liquid extraction were given recently in Refs. [61,62]. A broader commercialization of ELLE has not been achieved to date. Currently, the selectivities achieved are typically still below 1.2, whereas for successful applications a minimum selectivity of 1.5 is desired to limit the number of extraction stages required. To promote industrial applications, easily accessible hosts with a high selectivity towards a wider substrate spectrum are required to reduce the time for developing the separation processes.

Application of Membranes

Another strategy to separate enantiomers is based on the use of membrane-based approaches. Examples and overviews that introduce different possibilities are given in Refs. [63–65]. Membrane separations offer, in general, attractive options for clean, energy efficient, ease of performance, and continuous operation.

Two basic types of membrane processes can be distinguished for the separation of racemic mixtures: 1) direct separation using enantioselective chiral membranes and 2) separation in which a non-enantioselective membrane assists in an enantioselective process.

Chiral membranes can be liquids or dense polymers. In the former case the membrane liquid can be chiral or may contain a chiral additive (carrier). Often the membrane liquid is not used as a bulk phase but instead is immobilized in a porous matrix (supported liquid membrane) through capillary and interfacial tension forces.[66,67] An interesting concept is based on a combination of countercurrent fractionation and liquid membrane technology and makes use of two liquids, which are designed to have the opposite chirality by addition of the *R* or *S* enantiomer of a chiral selector and separated by another nonmiscible liquid immobilized in a porous membrane.[68] A main disadvantage of liquid membrane systems is their instability over long periods of time.[69]

Enantioselective solid polymer membranes typically consist of a nonselective porous support coated with a thin layer of an enantioselective selector. Various examples using different selectors and supports have been suggested (e.g. Refs. [70–73]).[70–73] Alternatively, molecularly imprinted polymers, that is, polymers having enantiospecific cavities in their bulk phase, have been suggested as a basis for chiral membranes. One of the enantiomers is used as a template during the synthesis of the polymer.[74] A detailed characterization of enantioselective membranes obtained by applying various L-proline derivates as chiral carriers is given in Ref. [75].

Significant progress has been made in the application of cheap and widely accessible ultrafiltration membranes as nonselective achiral membranes. The selectivity is achieved by preferably creating micelles or enzyme complexes in the feed phase with only one of the enantiomers. The separation is then simply based on retaining the larger complexes and allowing permeation of the unbound enantiomer.[76–78]

Membrane processes typically have to be carried out in several stages to reach high purities, because of the limited separation factors.[79] General drawbacks of membrane technology, which currently limit the application potential, are the relatively low transport rates through the membranes and the risk of membrane fouling.

Other Resolution Techniques

An overview regarding other, less-frequently studied and not so far developed chiral resolution concepts is given in Refs. [35,36]. Enantioselective foam flotation, preparative gel electrophoresis, and distillation are, for example, introduced. Enantioselective distillation is discussed, for example, in Ref. [80]. However, all the techniques mentioned and other alternatives have not yet acquired larger industrial relevance.

In the next two main sections we will concentrate in more detail on enantioselective crystallization and chromatography. Mainly examples of separations carried out in our laboratory will be used for illustration.

3. Crystallization-Based Methods To Separate Enantiomers

Since Pasteur's famous experiments on separating the enantiomers of sodium ammonium tartrate by direct crystallization,[81] extensive research has been devoted to study and apply crystallization processes for enantioseparation purposes. Substantial progress has been achieved both in understanding the thermodynamic and kinetic fundamentals behind enantioselective crystallization and also in exploiting this knowledge for the development of suitable crystallization methods. In general, crystallization techniques have the advantage of being widely applicable, simple, and cost-efficient. Only standard equipment which is readily available in the pharmaceutical and fine chemical industries is required to carry out crystallization. Crystallization techniques are not restricted to the resolution of racemates, but can also be applied for further purification of nonracemic mixtures of enantiomers resulting from other techniques, such as partially selective synthesis, chromatography, or membrane separation. Compared to asymmetric synthesis and chromatographic separations, crystallization processes often bear a "low-tech-image" or are sometimes considered as "out-of-date technology". However, when reviewing manufacturing methods that are used to provide pure enantiomer drugs, it becomes apparent that the majority of drugs are produced by classical resolution, that is, crystallization.[39] An overview on crystallization methods for the resolution of racemates is given in the textbook by Jacques et al.[82] A review on current patents in the field of optical resolution by crystallization methods with a focus on amino acids has been published recently.[83]

In principle, Pasteur's technique of hand-sorting crystals of two enantiomers is still a feasible technique to separate

racemic conglomerates that show enantiomorphism.[82] However, provided that crystals with well-defined morphological characteristics can be produced, a broader, in particular industrial, application would require reliable methods for automatic shape recognition and sorting. The challenging task of developing such methods might be the subject of future activities.

Classical Resolution

In classical resolution the racemate to be resolved is converted with a suitable enantiomerically pure resolving agent to provide two diastereomeric salts which have different solubilities and, thus, are separable by crystallization. This technique has the advantage of being robust and simple to operate. However, without additional efforts to incorporate racemization the achievable yields are limited to 50%. Classical resolution is still the most frequently applied technology for enantioseparation on an industrial scale and increasingly also the method of choice in the manufacture of active pharmaceutical ingredients (APIs).[39] For example, thousands of tons of (*S*)-naproxen, D-phenylglycine, and D-4-hydroxyphenylglycine are produced per year by the formation and separation of diastereomeric salts (see, for example, a Highlight in *Angew. Chem. Int. Ed.* in 1998).[84] More recently launched drugs produced by classical resolution are Frovatriptan (2002), Duloxetine (2004), and Eszopiclone (2004).[39]

When racemization of the unwanted enantiomer is feasible, the low yield inherent in a diastereomeric resolution can be overcome, thus offering significant increases in product amount and, thus, process performance. As an exemple, it is reported that the use of chemical racemization in the production of (*S*)-naproxen can enhance the overall yield to $\geq 95\%$.[85] A more recent example of a so-called crystallization-induced diastereomeric transformation refers to the API Sertraline, where a semicontinuous resolution-racemization process is performed.[86] The most frequently used acidic and basic resolving agents are natural L-tartaric acid, its derivatives such as L-/D-dibenzoyl- and L-/D-ditolouyl-tartaric acid, (*R*)-/(*S*)-mandelic acid, and (+)-/(−)-α-methylbenzylamine.[39] The sometimes elaborate search for an optimal resolving agent that forms easy to separate diastereomeric salts with the compound to be resolved has clearly been improved by the introduction of a combinatorial approach.[87] In this case, a family of resolving agents is simultaneously added to a solution of a racemate, thereby causing a rapid precipitation of a crystalline diastereomeric salt with high purity and yield. Since the mechanism behind this so-called "Dutch resolution" is not yet fully understood, studies are directed to study its thermodynamic and kinetic origins.[88] A compilation of new developments in crystallization-induced resolution focusing on Dutch resolution and examples of crystallization-induced asymmetric transformation (i.e. combination of classical resolution with in situ racemization) was published recently.[89] Novel aspects that should be mentioned here refer to the addition of achiral compounds with similar chemical structures (achiral additives) to the resolving agents which act as "catalysts" and thus affect the rate as well as the efficiency of the resolution process.[90]

Broader reviews on classical resolution are given in Refs. [82, 91, 92]. Suggestions for the conceptual design of enantioselective crystallization processes involving classical resolution and other alternatives are provided in Ref. [93].

Preferential Crystallization of Conglomerates

The direct crystallization of an enantiomer from a racemic solution is only feasible when the enantiomers in their mixtures form separate but pure crystals and, thus, the corresponding racemates are just conglomerates, that is, mixtures of crystals of both enantiomers. However, only 5 to 10% of the chiral substances belong to this type.[94] Preferential crystallization is an attractive approach for racemates of such substances that allows for direct crystallization of the desired enantiomer without the need for any chiral auxiliary. Generally, two techniques of direct crystallization can be distinguished: a) the entrainment process and b) simultaneous crystallization; both are applicable to solution and melt phases. In the first case, the enantiomers crystallize consecutively from a supersaturated racemic solution and the process is not allowed to reach equilibrium. In fact, it is a kinetically driven separation that relies on different crystallization rates of the enantiomers in the presence of homochiral seeds. The entrainment process is usually applied on smaller scales in a batch mode. Examples of industrial use concern, in particular, the production of broad-spectrum antibiotics such as chloramphenicol, thiamphenicol, and β-lactames.[94] The second approach, that is, simultaneous crystallization, is an option for larger scales. In this case, the enantiomers crystallize simultaneously but locally separated from a solution, which always remains close to the racemic composition. The process can be performed both in batch and continuous modes and has been industrially realized, for example, for the production of (−)-menthol (via the benzyl ester, Haarmann & Reimer, $1400\ t\,a^{-1}$) and an L-α-methyldopa intermediate (Merck, $>100\ t\,a^{-1}$). Ajinomoto produced L-glutamic acid at about $13\,000\ t\,a^{-1}$ over a period of ten years by simultaneous crystallization.[82] The design and features of continuous resolution processes using different configurations of stirred tank and fluidized bed systems were described in a monography published in 2009.[95] In this publication, the application of fluidized bed crystallizers and the implementation of ultrasonic cleavage of seed particles to maintain the process regime are emphasized in particular. The advantages of preferential crystallization were demonstrated by resolving racemic calcium pantothenate (with the *R* enantiomer an important commercial precursor of vitamin B_5) and an industrially required resolving agent, both on a laboratory and technical (3000 L and 35 L) scale with high yields.[96,97]

Detailed information on preferential crystallization can be found in several overview articles (e.g. Refs. [94, 98]) and book contributions.[82,99] Capabilities and limitations ("pitfalls

and rewards") have been highlighted in an article by Levilain and Coquerel.[100]

Understanding the phenomenon of conglomerate formation, also referred to as spontaneous resolution of racemates upon crystallization, is reported to be "one of the great challenges in stereochemistry",[101] and several review articles are devoted to this topic (e.g. Refs. [101,102]). Efforts to systematically derive possible conglomerates among a group of derivatives of a chiral substance of interest were described by the research group of Bredikhin (e.g. Refs. [103,104]). Recently, the second harmonic generation effect has been proven to be an alternative and efficient prescreening technique for the detection of conglomerates.[105] Studies on the application of so-called "tailor-made" additives to selectively inhibit the crystallization of the undesired enantiomer and, thus, to make the separation more robust have been performed in particular by the research group of Lahav and Leiserowitz (e.g. Refs. [106–108]). In a recent study[109] the authors describe a cyclic separation process in the presence of a tailor-made enantiomerically pure polymer that causes differences in the growth and dissolution of the enantiomers and thus assists in resolution.

In the last few years different innovative process modifications have been suggested to enhance the performance of the classical isothermal process. These include, for example, polythermal crystallization procedures, autoseeding strategies, and innovative reactor concepts.[99,110–117] Selected applications of preferential crystallization will be presented and discussed in Section 3.3. Advanced process concepts such as an autoseeded polythermal process mode, first introduced by Coquerel et al.,[118] innovative crystallizer configurations, and the application of preferential crystallization to racemic compounds in integrated process schemes will be addressed. With regard to the latter, the ability of tailor-made ("molecularly" designed) additives to selectively inhibit the crystallization of the racemic compound and, accordingly, to support enantioselective crystallization in such systems was studied and reported.[119,120] Further noteworthy studies concern enhancing the yields of preferential crystallization by increasing the solubility of the racemic mixture through addition of appropriate modifiers.[121]

The combination of preferential crystallization with racemization permits transformation of the unwanted enantiomer and thus, an increase in the overall yield, which for preferential crystallization alone (as for classical resolution) is inherently limited to 50%. Examples of implementing razemization procedures are given, for example, in Refs. [96,122].

Attrition-Enhanced Deracemization (Viedma Ripening).

Attrition-enhanced deracemization is a new technique based on ground-breaking studies by Viedma,[123] who discovered that a 1:1 mixture of enantiomorphous $NaClO_3$ crystals in contact with the saturated solution "deracemizes" on grinding the crystals in the suspension. This technique was shown to be applicable in cases where the racemic substance to be resolved is 1) a conglomerate and 2) quickly racemizes in liquid state.[124] The driving mechanism behind attrition-enhanced deracemization,[125,126] novel application-related aspects,[127,128] and a first proof of its practical feasibility for a drug (Clopidogrel) intermediate have recently been reported.[129] Furthermore, it could be shown that the implementation of grinding and in situ racemization in a preferential crystallization facilitates an improved process performance with respect to yield and productivity.[130]

The more general exploitation of Viedma ripening as well as the extension of the applicability of preferential crystallization to racemic compounds are expected to increase the attractiveness of both techniques for the separation of enantiomers on an industrial scale.

Optically Active Solvents

A further method for the resolution of racemates is the application of optically active solvents, which might also be achiral solvents containing definite amounts of a pure enantiomer as the solute. In principle, specific diastereomeric interactions can occur when enantiomers are dissolved in an optically active solvent. As a result, these diastereomeric complexes should possess different physicochemical properties that might lead to differences in the solubility of the two enantiomers of the chiral substance to be resolved and, thus, should introduce a certain asymmetry in the appropriate solubility phase diagram. However, despite the fact that this concept has been considered as a relevant tool for enantioseparation since the beginning of the 20th century, only a few investigations have been reported[82] and there is a lack of systematic studies that allow for generalization of the results.

On the other hand, a couple of successful chiral resolutions have been described that make use of kinetic effects for enantioselective crystallization with the help of optically active solvents. For example, the resolution of racemic glutamic acid by using a chiral solvent made from lysine and water was demonstrated.[131] Small amounts of L- or D-lysine were added to retard the crystallization rate of the corresponding glutamic acid enantiomer, thereby leading to a transient optical resolution during crystallization. In addition, successful chiral resolutions of some racemic conglomerates using the chiral solvents D-isopropyl tartrate and (−)-α-pinene were reported.[132,133] This direct crystallization was feasible due to different rates of nucleation and/or growth of the two enantiomers. The resolution of a racemic compound in (−)-α-pinene was not successful.[133] Further studies have been carried out by applying tailor-made additives in kinetic resolutions, as already mentioned above (e.g. Ref. [106] or more recently Ref. [134]). All the examples given only apply to conglomerate systems. Comprehensive studies performed in our research group were devoted to the evaluation of both thermodynamic and kinetic effects of chiral solvents on enantioselective crystallization. Specifically chosen solvents were also applied to verify the feasibility of resolving racemic compounds.[135–138] Selected results will be depicted in more detail in Section 3.3.2.

3.1. Solid–Liquid Equilibria and Related Possibilities for Enantioseparation

The application of crystallization for the separation or purification of enantiomers requires a comprehensive knowledge of the fundamental solid–liquid equilibria (SLE), which form the thermodynamic basis of all crystallization processes. SLE data are graphically represented in phase diagrams, which depict the equilibria between solid and liquid phases for a specific system over a wide range of temperatures and compositions. These phase diagrams specify the equilibrium conditions and the corresponding phases present in this state. Thus, they provide information about the identity of the solid phases involved, such as polymorphs, solvates, or solid solutions. The relevant phase diagrams for crystallization-based enantioseparation are 1) the binary melt phase diagram of the two enantiomers that describes the melting behavior in the binary system and 2) the ternary solubility phase diagram of the two enantiomers in a specific solvent that depicts the solubility behavior of the enantiomers and their mixtures in the presence of this solvent. Overviews covering the identified types of phase diagrams and their description have been published elsewhere.[82,94,139,140] Examples of systematic studies of binary and ternary phase diagrams are given in articles from the research groups of Grant (e.g. Ref. [141]) and Klussmann.[142,143] To our knowledge, there is no monograph available that focuses mainly on the phase equilibria of organic compounds. A comprehensive overview on the structure and characterization of molecular crystals and the formation of solid solutions in general can be found in the excellent books of Kitaigorodski.[144,145]

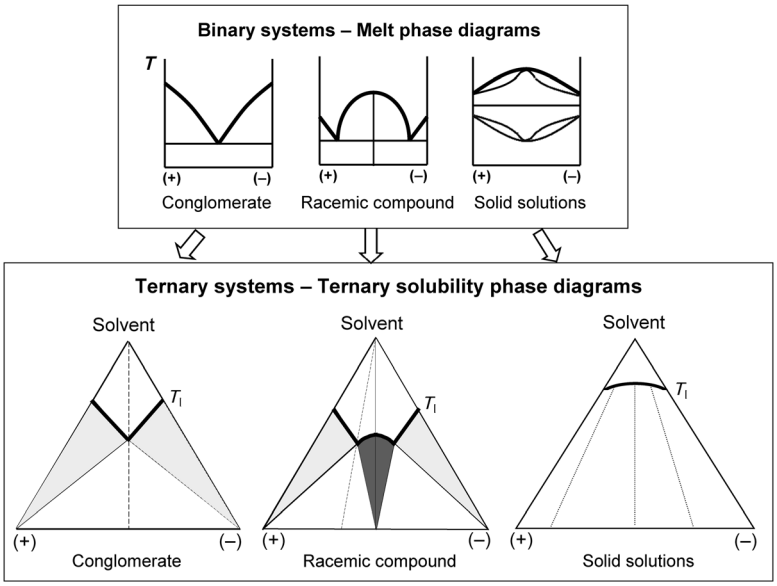


Figure 2. Fundamental types of binary melt phase diagrams and ternary solubility phase diagrams for chiral systems. The solubility phase diagrams are represented as isothermal slices depicting the SLE at a certain temperature T_l. Thick black lines mark related liquidus (saturation) lines. In the ternary phase diagram the eutectic composition in the chiral system is indicated by a dashed line connecting the solvent corner with the binary chiral system ("eutectic line"). In the case of miscibility in the solid state (exemplified by the right phase diagram), dotted tie-lines characterize the composition of the liquid and solid phases coexisting in equilibrium. (Adapted from Ref. [146].)

The fundamental types of phase diagrams that occur in chiral systems are shown schematically in Figure 2.[146] In general, since the enantiomers exhibit identical physical properties, such as melting points, melting enthalpies, and solubilities, both the binary melt phase diagrams and the ternary solubility phase diagrams (upper and lower parts of Figure 2, respectively) are symmetrical with respect to the racemic composition. This simplifies the determination of SLE for such systems, since just one half of the phase diagram has to be measured.

Basically, one can distinguish between three main types of phase diagrams, which arise from the particular characteristics of the crystalline racemate in the system and which were first described in a pioneering study by Roozeboom.[140] In the first case, a simple eutectic is formed between the enantiomers at the racemic composition. Here, mixtures of the two enantiomers are just mechanical mixtures of crystals formed by homochiral molecules (conglomerates). Thus, in the racemic mixture the enantiomers form separate solid phases that may crystallize separately, which is the main requirement for preferential crystallization. However, as already mentioned, only 5 to 10% of the chiral substances belong to this group.[94]

In the second case, that is, when the enantiomers form an intermediate stoichiometric 1:1 compound, at the racemic composition there is only one solid phase present, the racemic compound. Most chiral systems (90–95%) form racemic compounds. From a purely thermodynamic point of view, the generation of pure enantiomers from the racemates is not feasible here by direct crystallization. An important property of such systems is the composition of the two eutectics that occur between the enantiomers and the racemic compound (mirror-shaped around the racemic composition). It will be shown later that this composition essentially determines the crystallization techniques that can be applied to provide the desired pure enantiomer from enriched mixtures.

In the third case, complete miscibility in the solid state occurs, that is, the enantiomers form mixed crystals (solid solutions), at all compositions. The liquidus curve in the phase diagram can exhibit a melting point maximum, a melting point minimum, or a constant melting temperature. The same applies to solubilities in the ternary phase diagram. It is clear from the tie-lines shown that no pure enantiomer can be crystallized from an enriched solution within one step. Therefore, this type of system is the most unfavorable one for separation or purification purposes. Fractional crystallization might be used to further purify an already enriched mixture. Fortunately, this type of phase diagram is very rare in the case of molecular crystals in general and enantiomers in particular. Not considered in Figure 2 is the fact that partial miscibility in the solid state can occur for both conglomerates and racemic compound forming systems. Related phase diagrams are rare.

Examples referring to partial solid solutions on the enantiomer side are given in Refs. [147,148], cases depicting limited miscibility in the solid state for the racemic compound in Ref. [149], and examples with partial solid solutions for both enantiomers and the racemate in Ref. [150].

The ternary solubility phase diagrams shown in Figure 2 are isothermal cuts of the three-dimensional representation of the ternary system of the two enantiomers and a solvent in an equilateral prism with temperature as the vertical axis perpendicular to the prism base. Light gray areas represent the existence regions of the enantiomers where in equilibrium these enantiomers can be crystallized as solid phases. The dark gray area shows the region where the racemic compound exists, and is the solid phase that crystallizes from solution compositions in this region. In the three-phase region embedded by the two-phase regions a pure enantiomer can only be crystallized under kinetically driven conditions. The "eutectic line" indicated must not necessarily be linear, it can also be curved. Examples will be shown in Section 3.3.2. In contrast to the binary chiral systems, solvates can occur as additional solid phases in the solubility phase diagrams. For this reason and also since polymorphism might be solution-mediated (thus, providing polymorphic varieties not revealed in the binary system), solid-phase analysis should always accompany solubility measurements to avoid wrong allocation of solubility values to solid phases.

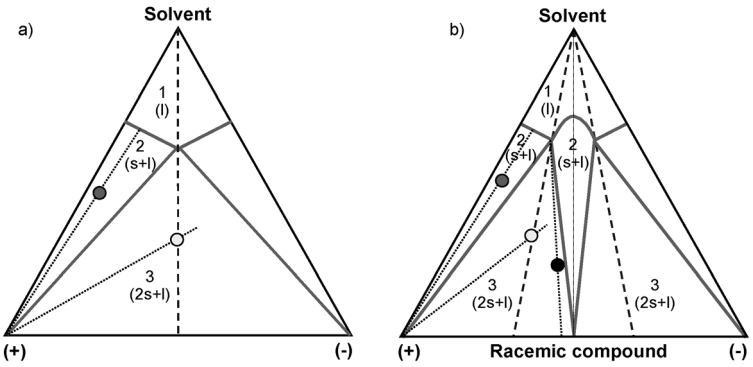


Figure 3. General possibilities of crystallization-based enantioseparation without a chiral auxiliary illustrated for a) conglomerates and b) systems with racemic compounds. The numbers and states of aggregation of the phases present in equilibrium are depicted in the particular regions (s: solid; l: liquid).

Often the phase diagrams required are not known, in particular for newly synthesized substances. Frequently, one faces a lack of consistent solubility data for the substance of interest. Experimental determination is a tedious and time-consuming undertaking and requires sufficient amount of substance, which is often not available at an early stage of development. In addition, a combination of different analytical techniques is usually necessary to obtain solubility values and to identify the solid phases in equilibrium with the saturated solution. Since ternary solubility diagrams are inherently closely related to the corresponding melt phase diagrams, preliminary determination of melt equilibria of the chiral system prior to the determination of solubility equilibria rationalizes the experimental efforts by specifying the type of crystalline racemate and the location of the eutectic composition in the chiral system. Moreover, ideal solubilities can be calculated from the melting data (temperature and heat of fusion), thereby providing a first rough estimate of the solubility curve by using classical thermodynamic equations (simplified Schröder-van Laar equation).[82,146]

The possibilities for crystallization-based enantioseparation that arise from the ternary solubility phase diagrams are illustrated in Figure 3 for a conglomerate and a racemic compound forming system.

If enantiomerically enriched mixtures of the enantiomers are already available (characterized by a composition in the existence region of the pure enantiomer, for example, dark gray points in Figure 3), the target enantiomer can be directly crystallized. This so-called "classical enantioselective crystallization" is feasible for both conglomerates and racemic compound forming systems.

Alternatively, one can work in the three-phase region of the phase diagram, where both enantiomers of conglomerates, and one enantiomer and the racemic compound of racemic compound forming systems are present as solid phases in equilibrium. There, two options arise: 1) If the initial solution contains the enantiomers close to the eutectic composition (e.g. light gray point in Figure 3a), preferential crystallization can be applied. By adding seeds of just one enantiomer to the supersaturated solution selective crystallization of this enantiomer is induced. However, since both enantiomers are stable solid phases at equilibrium, this is a non-equilibrium kinetically driven process, where nucleation of the counter-enantiomer has to be avoided. As mentioned before, preferential crystallization for the resolution of racemates is in principle only applicable to conglomerates. However, below it will be demonstrated that it can also be used for racemic compounds starting from solutions having a composition close to that of the eutectic (e.g. light grey point in Figure 3b).[111,113] 2) As indicated in Figure 3b by the black point, an initial step might be applied to exploit enrichment in the liquid phase. Hereby, only a slight initial enantiomeric enrichment is sufficient to provide a liquid phase, which under certain conditions allows for the subsequent selective crystallization of a pure enantiomer. Attractive process options arise if the eutectic compositions in the chiral system change with temperature and/or solvent composition.[151,152]

In summary, except for preferential crystallizations of conglomerates, crystallization-based racemate resolutions usually require a certain enantiomeric enrichment, which might be provided, for example, in an integrated process by a partially selective synthesis or a prior alternative separation step.

3.2. Illustration of Selected Melt and Solution Equilibria

In the last few years more than 30 chiral substances originating from the fields of fine chemicals (chemical intermediates, amino acids) and pharmaceuticals have been

Table 1: Chiral systems studied regarding their melt and solution phase equilibria.

Chiral substance	Type of phase diagram/ racemate	x_{eu} binary melt system	x_{eu} ternary solution system	Additional characteristics	References
amino acids[a]					
threonine	C	–	0.5 (w,w/EtOH)	–	[153, 154]
asparagine	C	–	0.5 (w)	hydrate	[155]
methionine	RC	–	0.94-0.85 (1-60 °C) (w)	variable x_{eu}	[156]
serine	RC	–	0.988-0.998 (MeOH/ w) 0.987-0.995 (EtOH/w) (20-80 °C)	hydrate formation at selected conditions, variable x_{eu}	[157]
chemical intermediates					
N-methylephedrine	C	0.5	0.5 (8 solvents)	polymorphy	e.g. [137, 158, 159]
mandelic acid	RC	0.69	0.69 (11 solvents)	polymorphy of RC	[137, 138, 153, 160–162]
3-chloromandelic acid	RC	0.89 (0.85 metastable)	0.9–0.84 (5-50 °C) (w) 0.9 (toluene)	polymorphy of enantiomer & RC, variable x_{eu}	[163]
ethanolamine 3-chloromandelate	C	0.5	0.5	ss ($<0.25, >0.75$)	[164]
malic acid	RC	0.967	0.985 (w)	polymorphy of RC, ss of RC (≈ 0.7)	[149]
Tröger's base	RC	0.85	0.92–0.885 (25–50 °C) (EtOH)	variable x_{eu}	[165]
pharmaceutical intermediates and active pharmaceutical ingredients (APIs)					
compound A	C	0.5	0.5 (acetonitrile)	–	[153]
propranolol hydrochloride	RC	0.55	0.55 (w, MeOH)	polymorphy of RC, ss of enantiomer (0.982)	[147, 166]
bicalutamide	RC	0.9	0.977-0.95 (0-60 °C) (MeOH/w)	variable x_{eu}	[167, 168]
2,6-pipecoloxylidide	RC	0.67	0.7 (dibutylether)	solvates, polymorphy of RC	[169]

[a] All amino acids decompose during or before melting, thus no melt phase diagram can be determined. C: conglomerate; RC: racemic compound; x_{eu}: eutectic composition in the chiral system (only given for $x \geq 0.5$); EtOH: ethanol; MeOH: methanol; w: water; ss: solid solutions.

characterized in our research group through their binary and/ or ternary phase diagrams, including diastereomeric salts as well. A selection of 14 chiral substances is compiled in Table 1. The type of crystalline racemate and the eutectic composition in the chiral system determined from the melt phase and ternary solubility phase diagrams are specified. In some cases, different solvents have been studied, for example, solubilities of *N*-methylephedrine as a conglomerate and mandelic acid as a racemic compound forming system in eight and eleven, respectively, different achiral and chiral solvents were measured. Table 1 further contains thermodynamic information (not acquired in our laboratory) for Tröger's base which is discussed below as a model compound.[153–169]

The systems studied show a wide variety of characteristics, such as polymorphy of the enantiomers or/and racemate, solvates, solid solutions, and a variation of the eutectic composition as a function of temperature or solvent. Five chiral systems form conglomerates and nine racemic compounds. Only threonine and the pharmaceutical intermediate A belonged to the "simplest" type of phase diagrams, that is, being a conglomerate and showing no polymorphs, solvates, or solid solutions under the range of conditions investigated. Hard to track cases were 3- and 2-chloromandelic acid (Tables 1 and 2), which are both racemic compound forming systems. In the case of 3-chloromandelic acid, monotropic modifications exist for both the enantiomers and the racemic compound.[163] A metastable conglomerate was found for 2-chloromandelic acid (Table 2).[170] An additional monotropic polymorph of the racemic compound as well as partial miscibility of the enantiomer and the racemic compound were observed for the relatively simple compound malic acid. The rather rare occurrence of systems showing no additional solid phases or solid solutions corroborates the importance of solid-phase analysis when measuring SLE data, an issue not considered in many investigations.

Table 2 summarizes the different eutectic compositions determined in the melt phase diagrams for a selection of mandelic acid derivatives. All belong to the racemic compound forming type of substances. The 2-substituted mandelic acid derivatives show eutectic compositions close to the racemic one. Increased x_{eu} values are obtained for 3- and 4-substituted derivatives with medium values for the 4-substi-

Table 2: Eutectic compositions x_{eu} in the melt phase diagrams of different mandelic acid derivatives.[171]

Substance	x_{eu}
2-chloromandelic acid	0.57
3-chloromandelic acid	0.89
4-chloromandelic acid	0.83
2-bromomandelic acid	0.54
3-bromomandelic acid	0.87
4-bromomandelic acid	0.76
2-chloro-4-fluoromandelic acid	0.56
4-chloro-2-fluoromandelic acid	0.73
3-methylmandelic acid	0.63

tuted compounds. The same holds true for the di-substituted 2-chloro-4-fluoro- and 4-chloro-2-fluoromandelic acid.[171]

In the following, the application of different concepts of crystallization-based separation of enantiomers will be demonstrated with results from selected case studies.

3.3. Concepts of Crystallization-Based Enantioseparation

Classical and innovative crystallization processes capable of separating enantiomers will be illustrated below by using four of the compounds listed in Table 1 that were characterized by different but typical thermodynamic properties and diverse kinetic crystallization behavior. The compounds considered are threonine, mandelic acid, methionine, and Tröger's base. All the results given below have been obtained in our laboratory over the last decade.

3.3.1. Conglomerates

Of the four compounds considered, threonine is the only one that belongs to the group of conglomerates. Threonine, an essential amino acid, occurs in nature as the 2*S*,3*R* form, designated as L-threonine. Since it contains two chiral centers, four stereoisomers are possible, thus giving two pairs of enantiomers, L- and D-threonine (L-Thr, D-Thr), as well as L-*allo*-threonine and D-*allo*-threonine. The corresponding structures are shown in Figure 4.

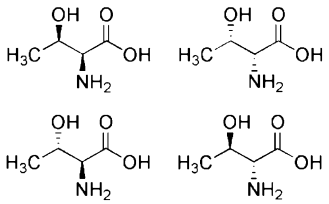


Figure 4. L-(2*S*,3*R*)-(−)-threonine, D-(2*R*,3*S*)-(+)-threonine, L-(2*S*,3*S*)-(+)-*allo*-threonine, and D-(2*R*,3*R*)-(−)-*allo*-threonine.

Separations will be demonstrated for the example of the L- and D-threonine pair. The ternary solubility phase diagram with water as the solvent is presented in Figure 5, and shows four solubility isotherms between 10 and 40 °C. As expected, symmetrical behavior around the racemic composition is obtained and the solubility isotherms exhibit the typical shape of a simple eutectic (conglomerate) system. Solubilities increase with temperature, as frequently observed. Furthermore, the solubility isotherms run almost parallel to the opposite triangle sides, that is, the solubility of one enantiomer is not affected by the presence of the counter-enantiomer, which indicates close to ideal solution behavior.

Figure 6 illustrates the principle of preferential crystallization for conglomerates. Starting from a racemic composition in the three-phase

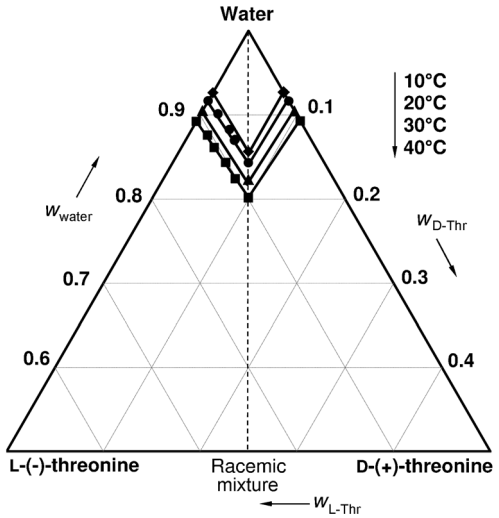


Figure 5. Ternary solubility phase diagram of L- and D-Thr in water. Since the solubilities of the threonine species in aqueous solution are comparatively small, only the upper half of the solubility phase diagram is shown (axes in weight fractions, *w*; isotherm lines are guides to the eyes).

region of the phase diagram (black point in Figure 6a), in the presence of homochiral seeds (here the (−) enantiomer) it is possible to crystallize only the seeded enantiomer for a certain period of time. The crystallization trajectory is indicated by an arrow. When the metastable solubility line of the crystallizing enantiomer is finally reached, the separation process has to be interrupted to avoid nucleation of the counter-enantiomer and, thus, contamination of the target product. What makes the process attractive for industrial application is the fact that it can be performed in a cyclic mode, thereby providing both enantiomers in a periodic manner (Figure 6b). There, trajectories A→B and C→D represent the selective crystallization pathways of the (−) and (+) enantiomers initiated by addition of the respective seeds. In between (B→C, D→A), the crystallized products are filtered off and new racemic feed

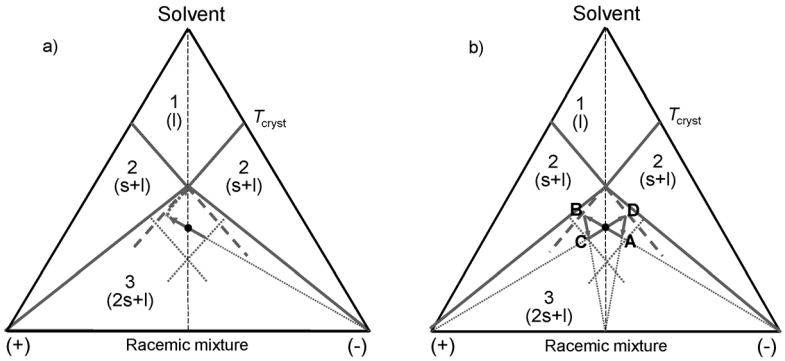


Figure 6. Illustration of the principle of preferential crystallization in the ternary solubility phase diagram. Shown is a) a trajectory for preferentially crystallizing a single enantiomer and b) a cyclic process producing periodically both enantiomers in an isothermal process at a crystallization temperature T_{cryst}. The black points indicate the starting points and gray dashed lines the metastable solubility lines of the enantiomers and, thus, the crystallization limits for successful preferential crystallization.

is added. Continuous monitoring of the separation progress (measuring the solution composition through a combination of polarimetric and density detection) has proven to facilitate a reliable separation process.[172,173] Additional application of inline particle measurement techniques (such as focused beam reflectance measurement (FBRM) or videomicroscopy) allows the evolution of the solid phase with regard to particle size, particle size distribution, particle shape, etc to be monitored.[174–176]

Results of this classically isothermal process are presented, for example, in Ref. [110]. More-sophisticated process modes, namely polythermal crystallization procedures, autoseeding strategies, and alternative crystallizer concepts, have been suggested to enhance the process performance with respect to robustness, productivity, and product purity. For example, the potential of a promising autoseeded polythermal mode of preferential crystallization[118] will be briefly illustrated by using the threonine case study. The process comprises a cooling crystallization that allows for enhanced yields and improved product purities as well as a particular autoseeding for supplying well-conditioned seeds for reliable separation. Figure 7 illustrates a typical temperature profile used for a complete separation cycle. Figure 8 shows the resulting profiles of the optical rotation angle as a function of time for five consecutive separation cycles of DL-threonine using aqueous solutions.

Unlike the isothermal process described before, each new half cycle now starts after addition of a racemic feed with adjustment of the temperature somewhat below the saturation temperature of the mixture (suspension temperature, T_{susp}, Figure 7) to facilitate selective dissolution of just one of the enantiomers from the racemate. For example, at point C* in Figure 7 (i.e. after filtering off the obtained L-Thr crystals and addition of new racemic feed to the mother liquor), the L-Thr crystals in the racemate are completely dissolved. After subjecting the remaining D-Thr crystals to a conditioning phase (e.g. C*→C, Figure 7), they serve as seed crystals for the subsequent cooling crystallization, thereby providing D-Thr as the product (C→D, Figures 7, 8). In the cycles shown, the purity of L- and D-Thr gained was on average 99.1 %.[175] With a connected mean yield of 4.1 % per half cycle,[175] the separation performed appears to be economically attractive according to estimations made by Collet.[94]

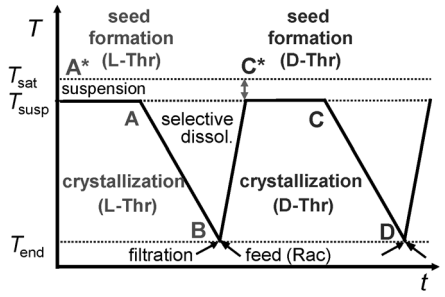


Figure 7. Schematic temperature profile applied in cyclic autoseeded polythermal preferential crystallization. T_{sat}, T_{susp}, and T_{end}: saturation, suspension, and end temperature of the cooling crystallization process, respectively; t: time. The periods A→B and C→D characterize the "production periods" of the separation cycle providing periodically L-Thr and D-Thr, compare with Figure 8.[175]

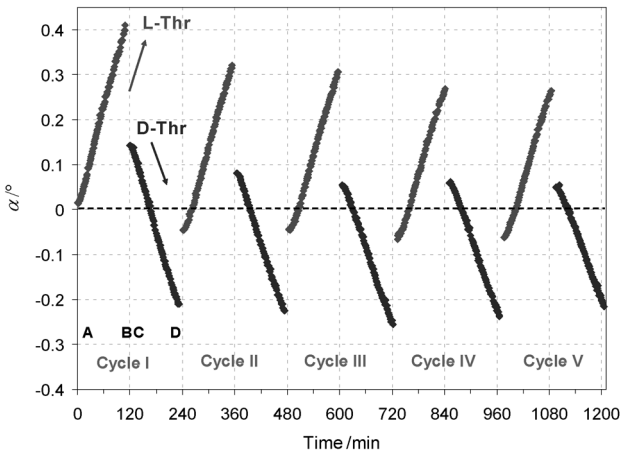


Figure 8. Profiles of the optical rotation angle α for five consecutive separation cycles in the autoseeded polythermal preferential crystallization of threonine. Only the crystallization periods A→B and C→D are shown. Crystallization conditions: 1000 g solution; 20 Kh^{-1} cooling rate; T_{sat}, T_{susp}, T_{end}: 53 °C, 39 °C, 4 °C, respectively.[175]

As for many processes that are based on recycling, the accumulation of impurities is also an issue of importance for cyclic preferential crystallization processes. The influence of L- and D-*allo*-threonine accumulating as impurities with increasing cycle numbers in the preferential crystallization of D- and L-threonine was discussed and evaluated in Ref. [174]. In this study, the application of a modified autoseeding strategy is also presented. It is shown that, within certain limits and depending on the seed characteristics, the properties of the produced crystals (shape, size) and the productivity of the separation can be adjusted.[112]

A further opportunity to improve the performance of preferential crystallization is to apply tailor-made additives that selectively inhibit the crystallization of one of the enantiomers and, thus, allow for increased yields of the desired enantiomer.[106,107] In the case of the resolution of racemic threonine, L-glutamic acid is known to selectively suppress nucleation and the crystallization of L-Thr. Experiments have shown that both the process robustness and the yield in the crystallization of D-Thr as the target product is enhanced in the presence of L-glutamic acid.[112] Furthermore, the application of chiral polymers based on poly(*N*-acryl)-amino acids as chiral additives has been reported to induce enantioselective crystallization of amino acids in solution. For example, high chiral discrimination at the early crystallization stages has been observed for DL-Thr in the presence of 1 $mg\,mL^{-1}$ of poly-L-leucine.[177]

Different innovative concepts of alternative crystallizer configurations are presented in Ref. [110]. One concept is based on simultaneous preferential crystallization in two crystallizers coupled through the liquid phase. The enhanced performance provided by this concept is illustrated in Figure 9, where the measured concentration profiles of the threonine enantiomers in the coupled crystallizer mode are compared to results for classical preferential crystallization performed in a simple batch mode.[116]

As a consequence of the exchange of mother liquors, the concentration of the undesired enantiomer is decreased and

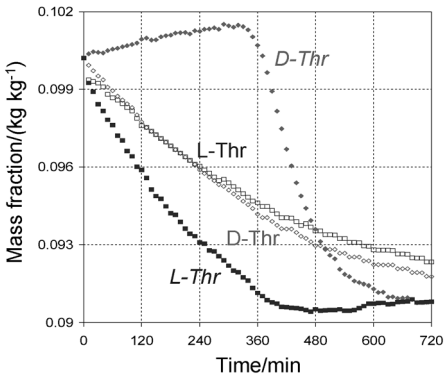


Figure 9. Concentration profiles of the enantiomers in the liquid phase for a simple batch and coupled crystallization mode.[116] Filled symbols and italic letters represent the simple batch mode, open symbols and regular letters represent the mode using two crystallizers coupled through their liquid phases.

the concentration of the crystallizing target enantiomer (i.e. also the driving force for growth) is increased in the two crystallizers. The mother liquor composition is always close to the racemic one in both vessels (i.e. almost identical concentrations of L- and D-Thr, Figure 9), which prevents the corresponding counter-enantiomer from nucleation. As a result, improved process robustness and increased productivity could be achieved. More details and a process option that also considers the selective dissolution is given in Ref. [178].

3.3.2. *Racemic Compound Forming Systems*

3.3.2.1. *Mandelic Acid*

Mandelic acid (MA) and its derivatives have several pharmaceutical applications, such as the treatment of urinary tract infections because of their bacteriostatic properties[179] and as a pharmaceutical constituent through its analgesic, antirheumatic, and spasmolytic effects.[180] Furthermore, pure (*R*)-mandelic acid is used as a precursor for the synthesis of cephalosporin and penicillin.[181] Both enantiomers, (*S*)- and (*R*)-mandelic acid (Figure 10), belong to the most frequently applied acidic resolving agents utilized in classical resolution.[39] For example, mandelic acid is used at an intermediate step in the synthesis of Duloxetine ((*S*)-MA, 2004) and in the manufacture of Sertraline ((*R*-)-MA, 1990).[39] The latter one is one of the top ten drugs and, thus, one of the nine chiral blockbusters.[182] The commercial process to Sertraline was recently modified, starting the synthesis with a continuous chromatographic resolution of the racemic starting material.[39,183] Nevertheless, advanced variants of the diastereomeric resolution with (*R*)-MA to implement racemization of the undesired enantiomer and which provide both good economics and high-quality products are under development.[86]

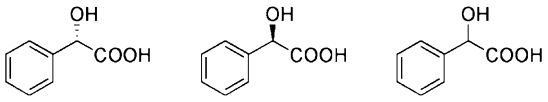


Figure 10. (*S*)-(+)-Mandelic acid, (*R*)-(−)-mandelic acid, and (*RS*)-(±)-mandelic acid.

Here, we use mandelic acid as an example of a racemic compound forming system to be resolved itself rather than utilizing it as a resolving agent for classical resolution. Figure 11 shows the ternary solubility phase diagrams of the mandelic acid enantiomers in a) water and b) (2*R*,3*R*)-diethyl tartrate (DET) as an example of a chiral solvent. Our results confirmed the already known ability of mandelic acid to form a racemic compound.[82] The eutectic composition measured for the binary melt system at a weight fraction (*w*) of 0.7 and 0.3, respectively,[160] remains unchanged in the presence of either solvent. Even when the chiral solvent DET is used (and also other optically active solvents, for example, lactates),[137] no recognizable asymmetry is introduced in the MA phase diagram, which could be applied for separation purposes. The solubility of the mandelic acid species in both solvents increases with temperature and is higher for the racemic compound than for the enantiomers.

Although no quantifiable impact on solution thermodynamics was observed in terms of different solubilities of the mandelic acid enantiomers in the chiral solvents,[135] in the case of kinetics, pronounced selective inhibition effects were obtained in metastable zone width (MZW) studies in (2*R*,3*R*)-diethyl tartrate and also in (*S*)-ethyl lactate.[136] For example, the MZW with respect to primary nucleation was found to be smaller for (*R*)-mandelic acid than for (*S*)- and racemic mandelic acid in (2*R*,3*R*)-diethyl tartrate, thereby facilitating preferential nucleation of the *R* enantiomer and,

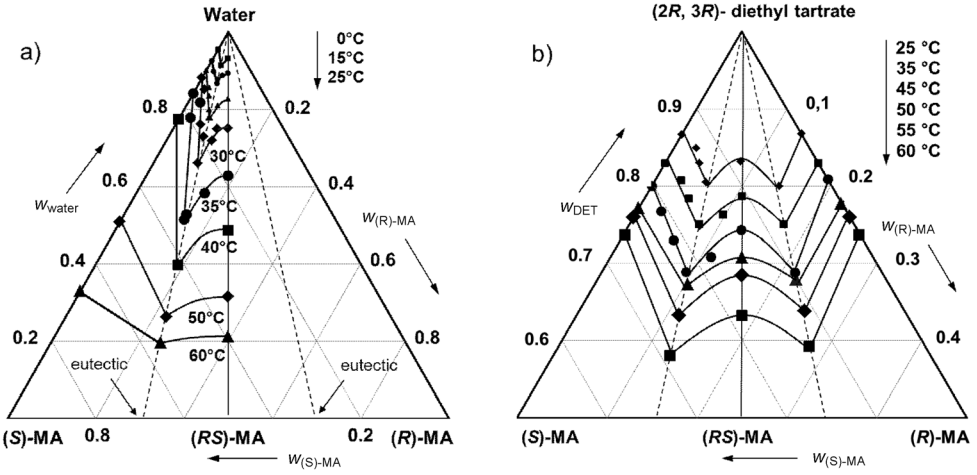


Figure 11. Ternary solubility phase diagrams of the mandelic acid enantiomers in a) water[153] and b) (2*R*,3*R*)-diethyl tartrate.[162] The isotherm lines are guides to the eyes.

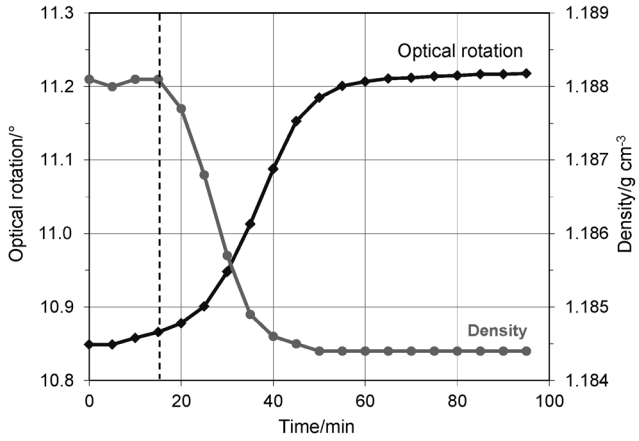


Figure 12. Profiles of the optical rotation of the mother liquor and the solution density during a preferential nucleation experiment starting with racemic mandelic acid in (2*R*,3*R*)-diethyl tartrate at $T_{sat} = 55\,°C$. The initial optical rotation of about 10.85° characterizes the optical rotation of the starting solution influenced by the chiral solvent.[136]

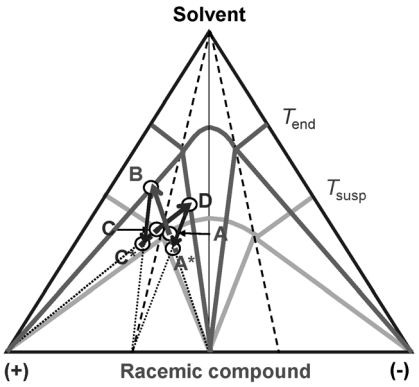


Figure 13. Illustration of a cyclic autoseeded polythermal preferential crystallization process providing periodically the racemic compound and the (+) enantiomer of a racemic compound forming system.[113] The notation refers to the temperature profile given in Figure 7.

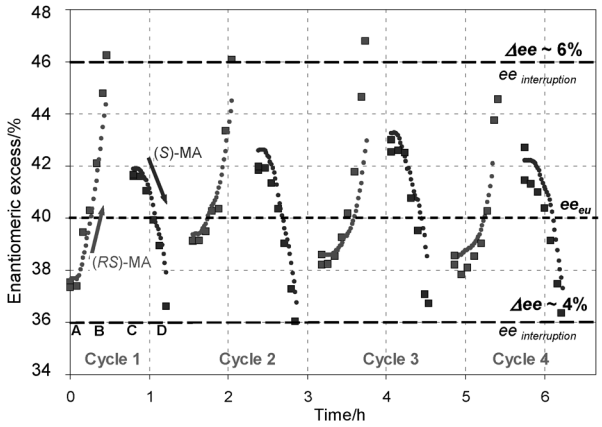


Figure 14. Profiles of enantiomeric excess for four consecutive separation cycles in autoseeded polythermal preferential crystallization of mandelic acid.[113] Only the cooling crystallization periods A→B and C→D are shown. Selected crystallization conditions: 300 g solution; 15 Kh^{-1} cooling rate.

thus, its selective crystallization. Figure 12a presents typical results of a preferential nucleation experiment for racemic mandelic acid in (2*R*,3*R*)-diethyl tartrate. As expected from MZW data, a decrease in the solution density and an increase in the optical rotation of the solution was observed with occurrence of nucleation, thereby indicating crystallization of (*R*)-(−)-mandelic acid. Hence, enantioselective crystallization was feasible directly from the racemic solution without "investing" in a prior enantiomeric enrichment step.

Recently, the application of certain mandelic acid esters as chiral "task-specific" solvents provided evidence for chiral recognition in the solution phase, as indicated by clear solubility differences of the mandelic acid enantiomers.[138]

As shown in the example of threonine, preferential crystallization can be used to resolve racemic mixtures of conglomerates. It should be stated that this method is also applicable to racemic compound forming systems when starting from solutions enriched with the target enantiomer.[110,111,113,114,117,166,184] In principal, since both the enantiomer and the racemic compound can be crystallized separately, it should be feasible to preferentially crystallize them in the three-phase region of the ternary solubility phase diagram in a periodic manner, similar to the two enantiomers of conglomerates.

The characteristic trajectories of a cyclic autoseeded polythermal preferential crystallization for a racemic compound forming system are illustrated in Figure 13. The applicability of this technique for enantioseparation in the mandelic acid system is demonstrated in Figure 14. The measured trajectories principally look similar to the threonine case (Figure 8), but differ in the fact that the process is no longer symmetrical around the eutectic line. The maximum difference in the enantiomeric excess (Δee) that is attainable by preferential crystallization can be unequal for the racemic compound and the enantiomer (e.g. $\Delta ee = 6\,\%$ versus 4%, Figure 14), since it depends on the particular eutectic composition of the chiral system. For mandelic acid, the racemate and enantiomer can be obtained in a ratio of 3:2 according to the eutectic at an *ee* value of 40% (ee_{eut}, Figure 14). In an integrated process scheme, where first a certain enantiomeric enrichment is achieved that is used subsequently for preferential crystallization, the racemic compound can be recycled as a by-product of the cyclic process through the enrichment step. Moreover, as already demonstrated for threonine, simultaneous preferential crystallization in the coupled crystallizer mode also offers further improvement of process performance for mandelic acid in terms of yield and robustness.[115,117] Just recently, the application of hydroquinine-4-methyl-2-quinolyl ether as a chiral additive in the preferential crystallization of mandelic acid was described.[185] As a result of its particular effect on nucleation kinetics, this additive also allows for significant enhancements in yield.

3.3.2.2. ***Methionine and Tröger's Base***

The amino acid methionine (Figure 15) is an essential sulfur-containing amino acid that (like all proteinogenic

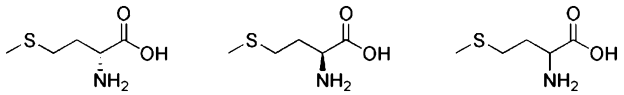


Figure 15. D-(*R*)-(+)-Methionine, L-(*S*)-(−)-methionine, and DL-(*RS*)-(±)-methionine.

amino acids) is found in nature as the L enantiomer. It is of particular relevance as an animal feed additive. For this application, it is not necessary to resolve racemic methionine, since it can be transformed into the desired L enantiomer by the animal organisms. Methionine is used in pharmaceutical applications as a liver protection agent and in infusion solutions.[186] Methionine was used in our study as a model system for the discussion of separation strategies applicable for substances with similar phase diagram characteristics.

Figure 16 shows the ternary solubility phase diagram of the methionine enantiomers in water, which is clearly of a racemic compound forming type. The solubilities are found to be comparatively low, particularly for the racemic compound. The eutectic composition in the chiral system is close to the pure enantiomer side and varies with temperature; it decreases from $x_{eu} = 0.94$ at 1 °C to 0.85 at 60 °C. Such an alteration of the eutectic composition has been reported for several compounds, such as serine,[157] 3-chloromandelic acid,[163] the API Bicalutamide,[167] a further pharmaceutically relevant substance investigated by Wang et al.,[187] and Tröger's base.[165]

The phase diagrams of methionine and Tröger's base[165] exhibit very similar characteristics. The solubilities in both systems (methionine/water, Tröger's base/ethanol) are comparatively low and both form at racemic composition an intermediate compound, which at the same temperature has significantly lower solubility than the enantiomers. As a result, the eutectic compositions left and right of the racemic compound occur close to the enantiomer sides in the phase diagram. Furthermore, as a function of temperature, the eutectic lines show convex profiles, that is, a high eutectic composition at low temperature shifts to lower values at elevated temperatures. For the Tröger's base, this shift in the eutectic composition is, with $w = 0.92$ at 25 °C to 0.885 at 50 °C,[165] similar to the methionine data.

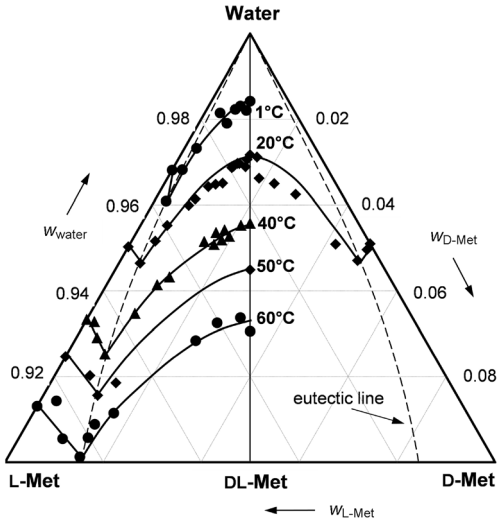


Figure 16. Ternary solubility phase diagram of the methionine (Met) enantiomers in water. Only the upper 10% of the phase diagram is shown due to the low solubilities in the system. The isotherm lines are guides to the eyes.[156]

Tröger's base ((±)-2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine) is a chiral heterocyclic amine whose chirality is solely due to the presence of two stereogenic nitrogen atoms (Figure 17). It is frequently used as a model system to study enantioseparation, for example, by chromatography (see Section 4.4). Furthermore, its derivatives are applied as substrates in organic and biochemistry.[188,189] Worlitschek et al.[165] measured the binary melt and ternary solubility phase diagrams in ethanol and described the SLE using the classical Schröder–van Laar and Prigogine–Defay Equations[82] and the Non Random Two Liquid (NRTL) model to account for non-ideality in solution. Crystallization-based resolution of racemic Tröger's base has been achieved by diastereomer-mediated resolution with a strongly acidic resolving agent. The resolution was found to be accompanied by a crystallization-induced asymmetric transformation of the salt containing the (+) enantiomer of Tröger's base, thereby providing high yield.[190] A hybrid chromatography-crystallization process for the resolution of Tröger's base has been studied by Amanullah and Mazzotti.[191]

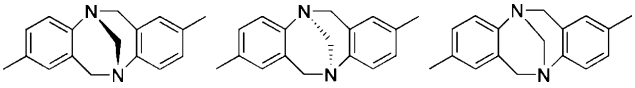


Figure 17. Structures of (−)-Tröger's base, (+)-Tröger's base, and (±)-Tröger's base.

By exploiting the shift of the eutectic composition in the chiral system with temperature, an alternative two-step process for enantioseparation can be proposed (see Figure 18 for methionine).[151,152] Starting at a low temperature, T_1, from a (just slightly) enantiomerically enriched solution (point 1), evaporation of solvent provides a solution with a composition inside the three-phase region of the phase diagram (e.g. point 2). Equilibration results in a solid phase with a composition (point 3) depleted in the target enantiomer and a saturated solution with a eutectic composition, $x_{eu,1}$ (point 4) enriched in the target enantiomer. After filtering off the crystalline phase, further removal of solvent shifts the solution composition at a higher temperature, T_2, within the two-phase existence region of the target enantiomer (point 5), thereby supplying it in an enantiopure form (point 6). The maximum yield is obtained when the solution composition meets the phase boundary between the two- and adjacent three-phase region (point 5′).

The proposed process can be performed in a cyclic manner (Figure 19). The crystalline phase of the first step, the solvent, and the mother liquor of the second step can be fully or partially recycled, thereby resulting in an efficient

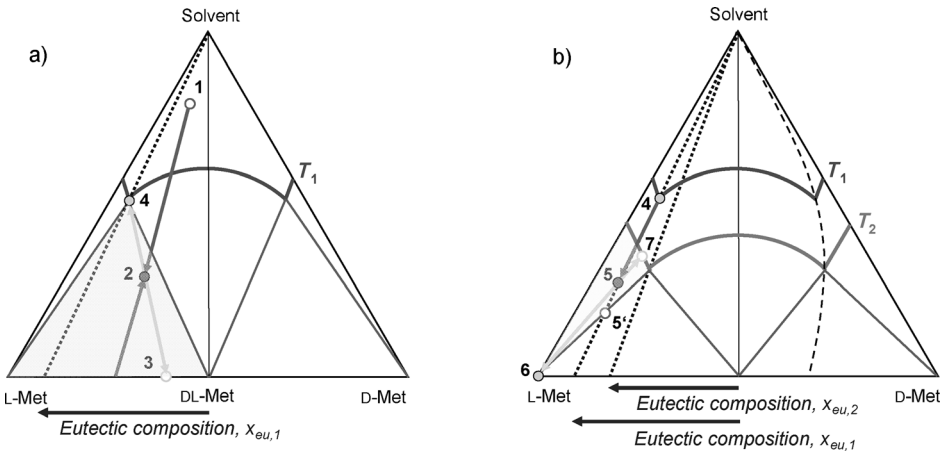


Figure 18. Two-step process for enantioseparation in the methionine system exploiting the shift in the eutectic composition with temperature. a) Step 1: enrichment in the liquid phase at a low temperature, T_1. b) Step 2: evaporation of the solvent and selective crystallization of L-Met at a higher temperature, T_2.[151]

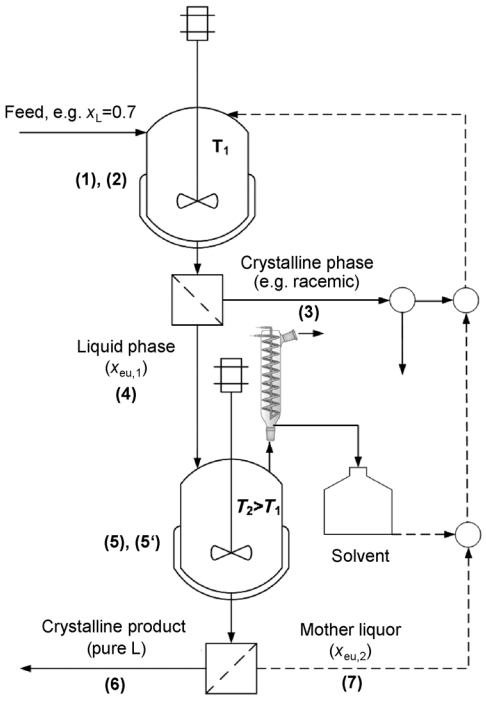


Figure 19. Flow scheme of the two-step separation process for methionine considering partial or full recycling of the solid separated in the first step, the solvent, and the mother liquor of the second step (target enantiomer: L-Met).[151] The numbers correspond to points in Figure 18.

utilization of the process starting materials. The potential of this process was successfully demonstrated.[151]

The next section introduces the principle and process concepts of preparative chromatography as a very versatile and frequently used method to separate enantiomers.

4. Chromatographic Separation of Enantiomers

The development of enantioselective chromatographic methods capable of resolving racemates is described in several excellent books and reviews.[31,36,38,192–202]

As in the area of crystallization, extensive efforts have also been devoted in chromatography to the application of suitable chiral derivatizing reagents to generate diastereomers which can be separated with classical chromatographic techniques used for the separation of achiral molecules. Since this so-called indirect method has a couple of drawbacks, such as the different rates of transforming the two enantiomers and the danger of racemization,[200] the direct method, which circumvents laborious derivatizations, is seen as the more successful one for preparative purposes.

Column chromatography on solid stationary phases is one of the most powerful enantioselective separation techniques. This is mainly due to the wide spectrum of possibilities offered by adjusting both the fluid mobile phase and the stationary phase to the requirements of the separation problem.

Gases, liquids, and supercritical fluids can be applied as the mobile phase, depending on the properties of the feed mixture. Pioneers in enantioselective gas chromatography (GC) are the research groups of Gil-Av,[203] Schurig,[204] König,[205] and Frank and Bayer.[206] However, this technique has still almost no importance for preparative applications. The wide spectrum of liquid chromatographic techniques provides the basis for numerous enantioselective HPLC methods for nonvolatile and/or thermally labile compounds. The current state of the art was recently reviewed in Ref. [207]. A renewed interest in supercritical fluid chromatography (SFC) has led in the last few years to the development of successful enantioselective separations with supercritical CO_2 used as the main constituent of the mobile phase.[208,209]

In the case of conventional high-performance liquid chromatography (HPLC) often several alternative methods can be applied to solve a separation problem, that is, different combinations of mobile and stationary phases.[207,210] This advanced state has not yet been reached for the separation of enantiomers. Suitable chromatographic methods are still often developed empirically to solve such complicated separation problems. However, considerable progress has been achieved in the last few years in understanding important principles of chiral recognition.[211] The development of specific and highly productive process concepts and the provision of the corresponding design tools nowadays

allow virtually any mixture of enantiomers to be separated with chromatographic techniques.

A fundamental question when carrying out the chromatographic separation is which of the two phases should be chiral. Efforts have been made to develop methods that make use of chiral mobile phases or use achiral mobile phases to which specific chiral additives have been added. An example is the separation of enantiomers of acids by forming ion pairs with chiral bases such as quinine derivatives.[212] Ligand-exchange methods have been reviewed in Ref. [213]. However, the use of chiral mobile phases appears not to be well suited for preparative LC. Often the chiral agents can not be easily recovered and recycled. In addition, the capacities of the chromatographic systems are typically much lower than systems based on chiral stationary phases (CSPs).

The application of a CSP is generally the most straightforward and convenient means for chromatographic enantioseparation and typically the method of choice, in particular for preparative applications.[200] It is based on creating and exploiting selective interactions at a solid–liquid interface. The solid support has a large influence on the mechanism of complexation, which causes the selectivity to be different from the selectivity in a liquid–liquid environment. Currently, there is a large and permanently growing number of commercially available selective CSPs, which allows the separation of a broad spectrum of racemates. Important CSPs will be introduced in Section 4.1.

Chromatographic techniques are widely used in industry to purify smaller amounts of target enantiomers at early development stages. However, these techniques are increasingly becoming a tool for the production of larger quantities.[38,199] Since large-scale (industrial) chromatographic processes are expensive, they require careful design and optimization. In the last few years the theoretical understanding of preparative chromatography carried out under overloaded conditions has improved significantly.[214–216] The theoretical analysis of band propagation processes occuring in packed columns revealed that the nonlinearities in the underlying distribution equilibria have to be taken into account in the design of preparative chromatographic processes.[216] Compared to conventional analytical chromatography, isocratic batch elution concepts are frequently not adequate and various alternative concepts have been developed to increase the productivities and yields and to decrease solvent consumptions, for example, by using several recycling techniques.[215,216] A breakthrough in increasing the productivity for chromatographic enantioseparations was achieved by applying the multicolumn simulated moving bed (SMB) technology[217] developed in the petrochemical industry and based on continuous countercurrent principles.[218] The process concept will be explained in Section 4.2.

4.1. Chiral Stationary Phases

The production, characterization, and application of CSPs and their corresponding enantioselective columns has been described in excellent book chapters and reviews.[37,200,219,220]

The chiral selectors of CSPs are preferentially covalently linked or alternatively strongly physically adsorbed (e.g. by coating with a polymeric selector) to a chromatographic support (usually porous silica particles). These CSPs are applied with achiral mobile phases. During migration of the sample through the column, diastereomeric complexes are formed, and the stationary phase retains the individual enantiomers in a specific manner.

The principle of chiral recognition was frequently rationalized earlier by using the classical "three point interaction model" suggested by Dalglish.[221] The numerous more detailed mechanisms that were proposed were reviewed on a regular basis.[211,222] Currently, there are intensive attempts to use density functional theory and molecular dynamic simulations to understand and predict the distribution of the enantiomers at the interface between the mobile phases and the CSP (e.g. Refs. [8,223,224]).

An essential aspect for a successful preparative separation is to find a combination of a CSP and a mobile phase that allows for high solubility of the enantiomers and for "reasonable" retention times in the columns. Both too long and too short retentions are not desirable. Despite the considerable improvement in understanding the separation mechanisms, because of the large number of degrees of freedom, the standard technique to identify a suitable CSP for a specific enantioseparation problem is still based on screening a smaller or larger set of available stationary phases and various mobile phases. Solvent mixtures are typically used as the mobile phase to allow the retention times to be adjusted. This screening is frequently supported by the manufacturers of the CSPs.

A broad variety of chiral selectors, of both natural and synthetic origin, have been used in the CSPs. More than a hundred CSPs are nowadays commercially available, of which around 20 are used most frequently.

There are various classifications for CSPs.[200,220,225] In the following, we give a short overview of the grouping of the most important selectors for CSPs, as applied in Ref. [200].

Macromolecular Selectors of Semisynthetic Origin (Polysaccharide-Based CSPs)

Polysaccharide selectors have a long tradition. Hesse and Hagel introduced in 1973 microcrystalline cellulose triacetate (Figure 20) as a polymeric selector material (without sup-

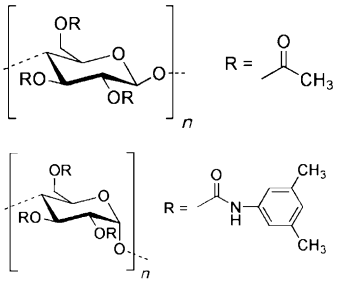


Figure 20. Polymer backbone of polysaccharide-based CSPs; top: cellulose triacetate (CTA), bottom: ChiralpakAD.

port).[226] By coating different cellulose derivatives (e.g. amylose tris(3,5-dimethylphenylcarbamate, Figure 20) on macroporous silica beads, Okamoto et al. made highly enantioselective materials with higher pressure stability and chromatographic efficiency, which were successfully commercialized by Daicel Chemical Industries.[227]

Currently there are phases available with improved selector immobilization which can be used for a wide spectrum of solvents. This allows mobile phases to be chosen that offer maximum solubility, which, together with the typically high enantioselectivities, makes this group of CSPs very attractive for preparative applications.[228,229]

Macromolecular Selectors of Synthetic Origin (Synthetic Polymer CSPs)

Several chiral synthetic polymers have been developed and are applied as potential chiral selectors to mimic the enantioselectivity provided by the semisynthetic polysaccharides. A review on polyacrylamide/silica composites was written by Kinkel.[230] These materials often do not achieve the same enantiorecognition as the polysaccharide phases, probably because of the much less ordered structures of the polymer chains.

Macromolecular Selectors of Natural Origin (Protein Phases)

After the pioneering work of Anderson and Allenmark[231] and others, who investigated the stereoselective binding of chiral components to proteins, a variety of protein-based CSPs are nowadays well-documented[232] and commercially available.[200] A drawback of protein phases is their limited sample loadability, which makes them less attractive for preparative chiral chromatography.

Macrocyclic Oligomeric or Intermediate-Sized Selectors (Cyclodextrins, Macrocyclic Antibiotics, Chiral Crown Ethers)

This type of selectors provides the basis for a large and important group of CSPs.

Cyclodextrin-bonded CSPs are based on α-, β, or γ-cyclodextrins, that is, on macrocyclic structures that are assembled from 6, 7, or 8 glucose units, respectively.[233,234] The cyclodextrins are usually bonded to silica gel, either through an ether linkage or a carbamate linkage.

Inspired by the stereoselective inclusion capabilities of the macrocyclic cyclodextrins, Armstrong et al. developed a new very successful class of CSPs: the macrocyclic antibiotic CSPs. The first type of this class was vancomycin-modified silica.[235] The company ASTEC (now Sigma Aldrich, USA) has commercialized several other CSPs based on glycopeptide antibiotics.[235] The structure of Teicoplanin, which is the selector of the commercially available CSP Chirobiotic T, is given in Figure 21 as an example. High loadabilities were achieved for Teicoplanin aglycone (Chirobiotic Tag).[235c]

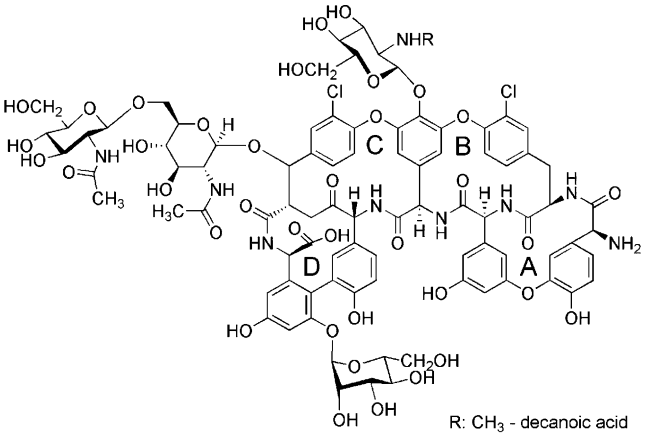


Figure 21. Structure of the glycopeptide antibiotic teicoplanin, which is the selector in the CSP Chirobiotic T (with 4 inclusion cavities A, B, C, and D).

Other interesting representatives of this group of CSPs are chiral crown ether phases developed initially by Cram and co-workers.[236]

Synthetic Neutral Entitities of Low Molecular Weight (Donor–Acceptor Phases/Pirkle Phases)

The first commercialized CSP with entirely synthetic selectors was developed by Pirkle and co-workers.[200] A DNB-phenylglycine derivative immobilized through ionic interactions onto silica was used as the selector. Later a larger collection of donor–acceptor types of CSPs was developed,[237] which frequently exploit π-π stacking interactions between electron-rich and electron-deficient aromatic systems as the primary attractions. Particularly successful is currently the so-called Whelk-O1 phase, which has preorganized clefts for solute insertion and allows for simultaneous face-to-face and face-to-edge π-π interactions. An interesting advantage of these CSPs is the fact that, due to the availability of the selectors in both enantiomeric forms, the elution order can be selected.[238]

Synthetic Ionic Molecules of Low Molecular Weight with Ion-Exchange Properties

Several ion exchangers have been developed for the separation of ionizable chiral compounds: for example, chiral anion exchangers based on cinchona alkaloid derivatives to resolve chiral acids[211,239] or chiral cation exchangers based on chiral amino sulfonic acids and caboxyxlic acids for the separation of chiral bases.[240] Best described are the anion exchangers using the cinchona alkaloids quinine and quinidine as the backbone of the selectors. The corresponding columns appear to be very promising, in particular because of their remarkable sample loadability.[200]

Chelating Selectors for Chiral Ligand-Exchange Chromatography

The first chiral ligand-exchange CSPs were developed in the late 1960s. The method is based on the reversible coordination of immobilized selectors and analytes within the metal-ion coordination sphere that forms a mixed ternary metal-ion/selector/analyte complex.[200] Davankov immobilized, for example, proline onto a polystyrene support and used this enantioselective matrix in combination with mobile phases containing Cu^{II} ions for the enantiomeric separation of amino acids.[241] An overview on the method is available in Ref. [242].

A broad spectrum of separation problems and examples is described in the above-mentioned references. The important problem of the loadabilities and saturation capacities of the CSP is addressed in Refs. [219,220]. The identification of the most suitable chromatographic system to solve a specific separation problem through systematic column screening was described recently,[243] as well as attempts at miniaturization to allow for high-throughput methods.[244]

The intensive research activities in the area of developing improved CSPs will without doubt contribute in the future to the provision of further improved solid chiral stationary phases. However, besides finding the right CSP for tackling an enantioseparation problem, it is still an important task to properly design a separation process for preparative purposes and to select the most suitable operating mode.

4.2. Preparative Chromatography

In analytical applications of chromatographic separation relatively small sample sizes are injected into a single column. This causes more or less Gaussian shapes of the eluting peaks. Together with the efficiencies of the columns (i.e. the plate numbers), the retention times and the selectivities between the components to be separated are the main parameters that must be adjusted and optimized.

The objectives are different in preparative chromatography. Here the columns have to be heavily overloaded to achieve high productivities. This is done by injecting larger concentrations, often close to the solubility limits, and/or larger sample volumes. As a consequence, nonlinear regions of the distribution equilibria control the separation and lead typically to highly asymmetric and distorted peak shapes. Besides the selectivities, the loadabilities of the stationary phases now become very important. To increase the productivities and recoveries as well as to reduce the solvent consumptions, several alternatives to batch chromatography have been developed and are used in industry. Examples are given, for example, in Refs. [245,246]. One class of processes that improves conventional batch chromatography exploits the principle of recycling insufficiently resolved fractions, such as, for example, the so-called steady-state recycling.[247] The most important chromatographic techniques used for enantioseparation are reviewed in Refs. [38,199,248]. Useful hints and rules of thumb for the development of a preparative chromatographic enantioseparation are given in Ref. [249].

As a consequence of the fact that a quantitative understanding and design of preparative chromatography requires incorporating the nonlinear adsorption isotherms, the mathematical framework required for simulating the development of concentration profiles in the columns is more sophisticated than in analytical chromatography. However, sufficiently accurate model equations and the tools required to solve them are nowadays available.[214–216] Thus, important features of nonlinear overloaded chromatography, such as, for example, displacement and tag-along effects and the mentioned peak distortions, are well understood. As a result of the lack of sufficiently accurate prediction methods, an important problem is still the fact that the required adsorption equilibrium isotherms must be determined experimentally. A broad spectrum of methods has been developed to perform this task.[250] Provided the thermodynamic functions and the column efficiencies are known, various types of operating modes can be predicted well and optimized. The theoretical framework available can be used to scale-up laboratory results to industrial-scale columns.[251] A powerful modeling approach capable of simulating steady-state recycling chromatography was developed recently.[252]

As a consequence of the availability of both high-capacity and very selective CSPs as well as mathematical tools that allow the design of various modes of operation, the separation of racemic mixtures by preparative liquid chromatography is currently in many cases the most rapid route to produce small quantities of pure enantiomers. In the last few years a specific process involving several connected columns improved the potential of chromatographic enantioseparation and led to several large-scale applications. This process will be introduced in the next section.

4.3. Simulated Moving Bed Chromatography

The basic concept of simulated moving bed (SMB) chromatography was patented in 1961.[218] It was initially applied in the petroleum and sugar industries to separate, for example, the xylene isomers and fructose from glucose.[253,254]

To explain the SMB process it is expedient to firstly explain a hypothetical true moving bed (TMB) process. As illustrated in Figure 22, the process is based on a counter-

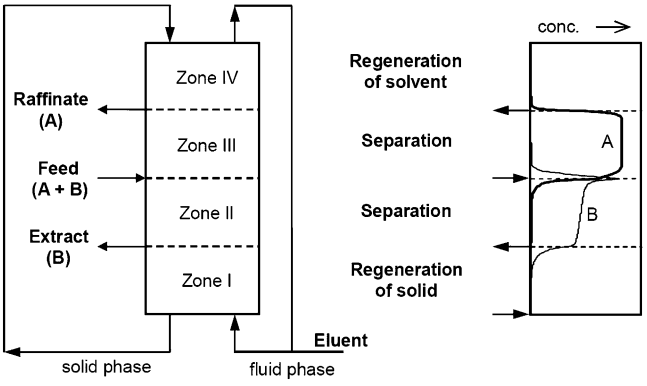


Figure 22. Principle of true moving bed (TMB) chromatography and typical internal concentration profiles for a successful separation of a binary mixture of components A and B.

current of the two phases involved, namely the fluid mobile phase and the solid phase (in this case not stationary). The TMB process is characterized by two streams entering the unit—that is, the feed stream containing the mixture to be separated and the desorbent or eluent stream—and by two product outlet streams. The inlets and outlets divide the unit into four zones I, II, III, and IV. The process acts as a binary fractionator and can separate mixtures consisting of two components *A* and *B*, for example, two enantiomers. Under successful conditions one of the outgoing streams is enriched with the less adsorbable component *A* (the raffinate stream) and one is enriched with the more adsorbable component *B* (the extract stream). Each of the four zones has to fulfill distinct tasks. The separation of the two feed components should happen in the two central zones II and III. Here, the net flow rates need to be set in such a way that component *A* is carried in the direction of the raffinate outlet and component *B* in the direction of the extract outlet. The desorbent is fed to zone I to desorb component *B* and thus to regenerate the solid phase. Component *A* is adsorbed on the solid phase in zone IV to regenerate the eluent. The process reaches a steady state in which the positions of the internal concentration profiles do not change. Provided the flow rates have been adjusted correctly, a complete separation of the feed mixture can be achieved, as illustrated in Figure 22.

The drawback of the TMB process described is the fact that a continuous transport of fragile solid particles without unwanted back-mixing is hardly possible. This led to the suggestion of the simulated moving bed process (Figure 23).

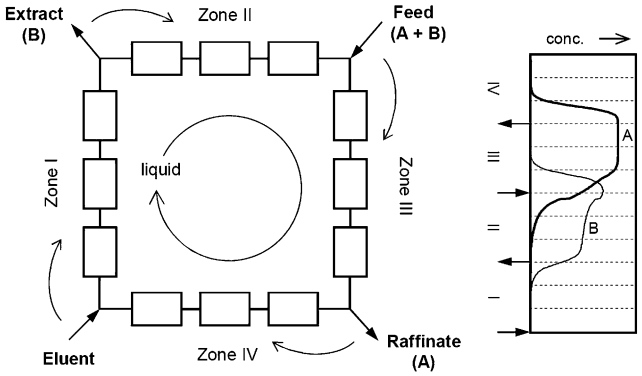


Figure 23. Principle of simulated moving bed (SMB) chromatography and typical internal concentration profiles at the end of a shift period for a successful binary separation.

The countercurrent movements of the liquid and solid phases are simulated by periodically shifting the positions of either the inlet and outlet ports or the columns. The residence time of a column in a zone is called the cycle or switching time, which is related to the solid-phase volumetric flow rate of the corresponding TMB process. Thus, the SMB process mimics the TMB process, and maintains its attractive features. As a result of the discrete switching events, the SMB process does not reach a steady state, which would be characterized, for example, by constant composition of the outlet streams. Instead, it reaches a "cyclic steady state", which is characterized by a periodic repetition of concentration patterns in the zones and at the outlet ports.

The recognition and acceptance of the SMB process in the pharmaceutical industry was retarded due to the difficulty of the separation, the higher product purity requirements, and a lack of reliable design concepts. After the first successful enantioseparation using the SMB process in 1992,[217] many more applications of this highly productive technique have been reported (e.g. Refs. [255–257]). Several comprehensive reviews describe the success story.[258–263] Typical productivities that are achievable with the technology are in the range 1–10 $kg_{enantiomer}\,kg_{CSP}^{-1}\,day^{-1}$. The breakthrough for SMB chromatography as a powerful tool for enantioseparation is strongly related to the development of easy to apply design rules[264–266] together with the improved provision of the hardware required.

In addition to the conventional four-zone closed-loop SMB process explained above, several improved modifications have been developed in the last years (e.g. Refs. [267–270]), including the application of supercritical fluids as the mobile phase.[271,272] The availability of these variants broadened the spectrum of application[273] and led to the first successful continuous chromatographic purifications of biomolecules.[274]

4.4. Illustration of Preparative Chromatographic Enantioseparations

In this section various features of enantioselective preparative chromatographic separation will be illustrated for the chiral molecules considered in the previous section to describe options for enantioselective crystallization.

The large choice of available chiral stationary phases (Section 4.1) provides in most cases several possibilities for performing a chromatographic enantioseparation. Unfortunately, there is still no reliable theoretical framework available to identify quickly the best combination of chiral stationary and mobile phases for a given separation problem. Consequently, this selection is typically based on screening studies involving the injection of analytical (small) sample sizes into small laboratory-scale columns filled with potential CSP candidates. For this, sufficient solubility in the analyzed solvents or solvent mixtures is necessary. The outcome of this empirical search is that selectivities between the two enantiomers under diluted conditions can be evaluated on the basis of the ratios of the rentention times. Typically, more systematic work is carried out in a second empirical stage for the most promising chromatographic system(s). Hereby the adsorption isotherms, which are the most essential information required for predicting chromatograms, have to be determined over a wider concentration range. These thermodynamic functions quantify the column capacities, allow optimization of the operating conditions and discrimination between rivaling operating regimes and, thus, form the essential basis for the quantitative design of the chosen chromatographic separation process.

a) Threonine

An example of the successful chromatographic separation of a racemic mixture under analytical conditions is given in Figure 24 for the amino acid DL-threonine. A Chirobiotic T

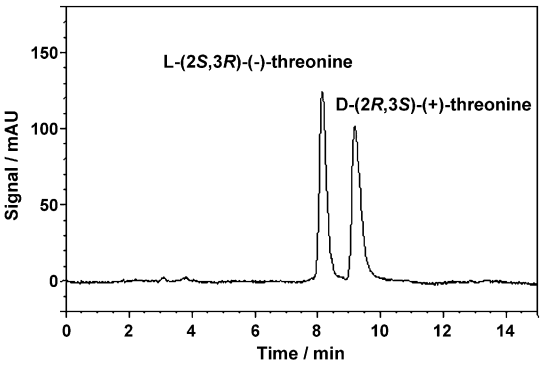


Figure 24. Chromatographic separation of DL-threonine. Chiral stationary phase: Chirobiotic T (5 μm, L = 250, *d* = 4.6 mm, ASTEC/Sigma Aldrich, USA), mobile phase: ethanol/water (60:40, v/v). Injection volume: 5 μL, injection concentration: 1% of DL-threonine in water, flow rate: 0.5 mL min^{-1}, *T* = 24 °C, detection: UV at 200 nm.[173]

stationary phase (see Figure 24) was used with a 60:40 ethanol/water mixture as the mobile phase. There is clearly a considerable selectivity that can be exploited for preparative purposes by injecting periodically larger samples of DL-threonine. However, the development and application of a large-scale chromatographic method appears not to be useful for the separation of these threonine enantiomers, because this amino acid belongs to the relative small group of conglomerates for which preferential crystallization (Section 3) could be applied most efficiently, thus offering a process far more attractive than the more sophisticated chromatographic resolution. Thus, no more efforts were undertaken in scaling up the chromatographic separation of DL-threonine.

b) Mandelic Acid

Figure 25 shows two chromatographic separations of a racemic mixture of the enantiomers of mandelic acid dissolved in water. Two types of chiral stationary phases were used in conventional HPLC columns (CSP I: the macrocyclic glycopeptide Chirobiotic T with triethylammonium acetate/methanol as the mobile phase, CSP II: a cyclodextrin-based CSP with an aqueous buffer solution and acetonitrile as the mobile phase; Table 3). Details regarding the conditions for the separation are given in the Figure caption. Clearly, CSP I provides the higher selectivity for this separation.

Besides the selectivity, the loadabilities of the stationary phase are also of huge importance. The Langmuir adsorption isotherm model can frequently be applied to describe the typically nonlinear distribution equilibria [Eq. (1)].[216]

$$q = q_{sat}\frac{bc}{1+bc} \quad (1)$$

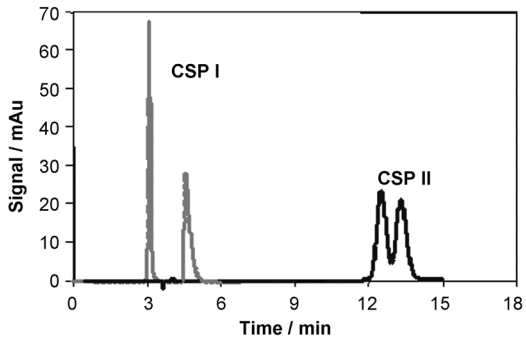


Figure 25. Chromatographic separations of racemic mandelic acid using two different chiral stationary phases. First peaks: (*S*)-(+)-mandelic acid, second peaks: (*R*)-(−)-mandelic acid. Injection volume: 1 μL, injection concentration: 1% of (*RS*)-(±)-mandelic acid in water, flow rate: 0.5 mL min^{-1}, temperature: 20 °C, detection: UV at 254 nm. CSP I:Chirobiotic T (ASTEC/Sigma Aldrich, USA, 5 μm, 150 × 4.6 mm), mobile phase A: 0.3 M methanol/TEAA (20:80, v/v), pH 4, *T* = 40 °C. CSP II: Nucleodex β-OH (Macherey–Nagel, Germany, 5 μm, 200 × 4 mm), mobile phase: 0.05 M NH_4Ac/acetonitrile (95:5, v/v), pH 3, *T* = 20 °C.[173]

Table 3: CSP and mobile phase compositions (v/v) used for the separation of the mandelic acid enantiomers.[275,276]

CSP	Mobile phase[a]
I	A: MeOH/0.3 M TEAAc (20:80), pH 4.02
I	B: EtOH/0.36 M TEAAc (1:1), pH 6.56
I	C: MeOH/MeCN/TEAAc/HOAc (54.5:45:0.25:0.25)
II	MeCN/HOAc/0.05 M AAc (4.5:9.1:86.4), pH 3.0

[a] MeOH: methanol, TEAAc: triethylammonium acetate, EtOH: ethanol, MeCN: acetonitrile, HOAc: acetic acid, AAc: ammonium acetate.

In this Equation, *c* and *q* represent the concentrations in the fluid and adsorbed phases, respectively, q_{sat} is the saturation capacity, and *b* is a parameter describing the isotherm nonlinearity. The parameters of Equation (1), as determined by frontal analysis, are given in Table 4 for the two CSPs that are applicable for separating the mandelic acid enantiomers.[250,275–277] In addition, two more mobile phases (Table 3) that allow for higher solubilities were considered. The isotherm parameters reveal that the different chromatographic systems are characterized by very different initial isotherm slopes, saturation capacities, and separation factors.

Table 4: Adsorption isotherm parameters and rough estimates of solubilities of the mandelic acid enantiomers for different chromatographic systems.[275–277]

System	Enantiomer	*T* [°C]	q_{sat} [g L^{-1}]	*b* [L g^{-1}]	$\alpha = (q_{sat}\,b)_R/(q_{sat}\,b)_S$	c_{max} [g L^{-1}]
I.A	*R*-(−)	40	77.8	0.0207	1.40	15
	S-(+)	40	261.2	0.0044		15
I.B	*R*-(−)	40	181.8	0.0064	1.14	55
	S-(+)	40	178.3	0.0057		55
I.C	*R*-(−)	40	155.1	0.0086	1.24	38
	S-(+)	40	276.4	0.0039		38
II	*R*-(−)	20	70.6	0.1222	1.07	5
	S-(+)	20	72.5	0.1111		5

Table 4 further gives the maximum concentrations c_{max}, which mark the range of the applicability of the adsorption isotherm model, and roughly the limits of solubility as an important constraint in preparative chromatography.

An optimization study was performed for the different chromatographic systems by using this thermodynamic information as well as the numbers of theoretical plates provided by the specific columns in a standard dynamic model capable of predicting the performance of a conventional chromatographic column operated in the batch mode.[216,275,276] Figure 26 shows the results in terms of the achievable productivity. For all systems it is advantageous to operate the column close to the solubility limit. The highest performance is available with CSP I and the mobile phase A. This is essentially due to the highest separation factor provided by this system ($\alpha = 1.40$). Although mobile phase I.B provides a much higher solubility and similar saturation capacities as system I.A, the lower separation factor can not be compensated. Clearly, CSP II can not be considered as an alternative for solving this separation problem.

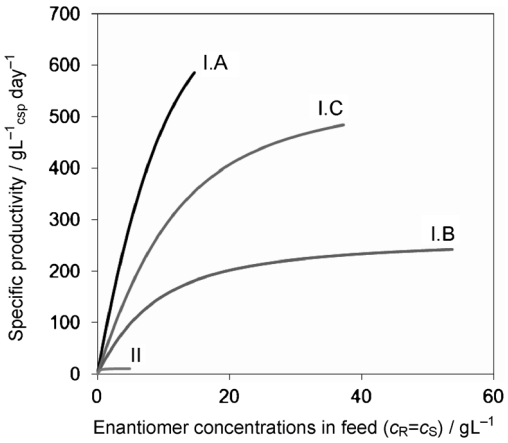


Figure 26. Productivities of performing a chromatographic separation of racemic mandelic acid as a function of the injection concentration for the four chromatographic systems summarized in Tables 3 and 4.[276]

c) Tröger's Base

The separation of racemic Tröger's base is a classical test case in the field of chromatographic enantioseparation. The first successful separation using microcrystalline cellulose triacetate as the CSP was reported in 1973 by Hesse and Hagel.[278] Results of further systematic studies with this chiral compound were published in Ref. [279]. The successful application of cellulose triacetate in a continuous eight-column SMB process was described in Ref. [280]. Many other CSPs were later found to be capable of performing the separation. Results for the screening of polysaccharides and solvents as well as an optimization of loadability and productivity were described.[281] Successful chromatographic enantioseparations of functionalized derivatives of the Tröger's base by using various CSPs were reported in Refs. [282–284].

The first reports about unusual shapes of chromatographic peaks of the enantiomers of Tröger's base were published in Refs. [285,286]. These strange peak forms were confirmed in own studies.[287] Figure 27 shows single-component elution profiles of the two pure enantiomers of Tröger's base with microcrystalline cellulose triacetate (CTA) used as the stationary phase and pure ethanol as the mobile phase. Whereas the development of asymmetric peaks with an increasing tailing could be observed in a series of experiments involving overloading the column with the (−) enantiomers, the peak shapes of the (+) enantiomers follow another trend. The elution profiles of (+)-Tröger's base are characterized by an initial orientation of the peak maximum to longer retention times in the course of successive column overloadings.

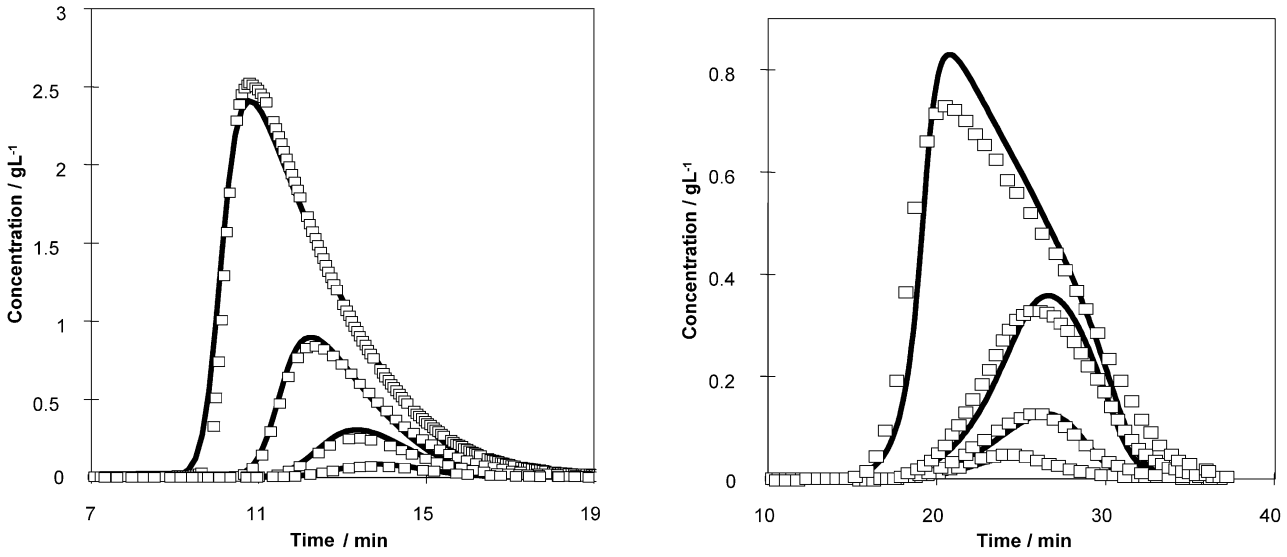


Figure 27. Series of overloading a microcrystalline cellulose triacetate column with injections of the pure enantiomers of Tröger's base. Mobile phase: ethanol, flow rate: 0.5 mL min^{-1}, temperature: 20 °C, injection concentration: 15 g L^{-1}, injection volumes: 10, 30, 90, 250 µl. Left: (−)-Tröger's base. Right: (+)-Tröger's base. Symbols: experiment. Lines: theory.[293]

Further overloading changes this trend and the maximum moves to shorter retention times, as is typical for a Langmuirian system following Equation (1).[216]

Systematic studies devoted to measuring the adsorption isotherms of the two enantiomers using frontal analysis[250] revealed the more complex isotherm shape for the (+) enantiomer (Figure 28). This isotherm is characterized by an inflection point located at a liquid phase concentration of about 0.5 $g\,L^{-1}$.

The isotherm measured for the (+) enantiomer can only be described by using more complex isotherm equations. The quadratic equation [Eq. (2)] arising from statistical thermodynamics can represent the behavior observed.[288]

$$q = q_{sat}\frac{c(b_1 + 2b_2c)}{1 + b_1c + b_2c^2} \qquad (2)$$

The elution profiles of Tröger's base could be rather well represented for the single enantiomers (Figure 27) and racemic mixtures (Figure 29) by applying a) the parameters derived in Refs. [289,291], b) the ideal adsorbed solution theory,[292] which is capable of describing multicomponent adsorption equilibria for arbitrary shapes of the single-component isotherms, and c) the equilibrium dispersive model of a chromatographic column.[216]

Further studies concerning the thermodynamic characterization of this challenging chromatographic system and the first attempts devoted to explaining the behavior on the basis of molecular simulations are given in Refs. [294,295].

Reports on the successful continuous chromatographic separation of the Tröger's base enantiomers by using the SMB process and ChiralPak AD as the CSP were published recently.[296,297]

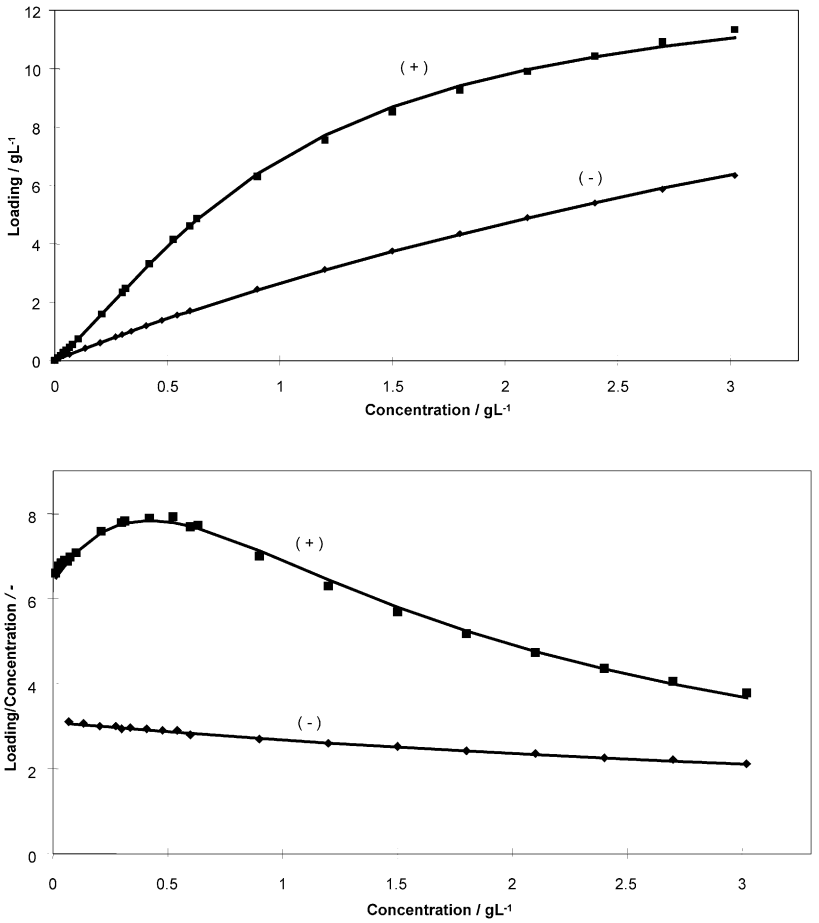


Figure 28. Adsorption isotherms (top) at 20 °C and corresponding profiles q/c versus c (bottom) for the adsorption of the enantiomers of Tröger's base in ethanol on microcrystalline cellulose triacetate.[293]

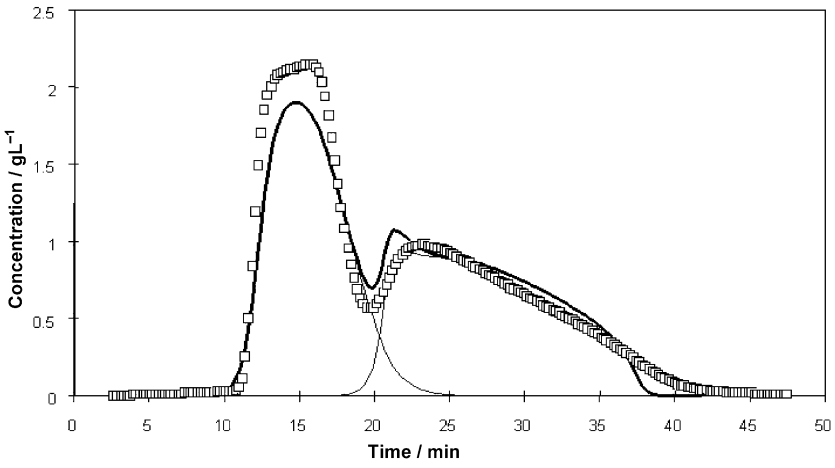


Figure 29. Measured and simulated chromatogram for the separation of the enantiomers of Tröger's base under strongly overloaded conditions. Symbols: experiment, lines: equilibrium dispersive model using the ideal adsorption solution theory [combined with Eq. (2)]. Flow rate: 0.5 $mL\,min^{-1}$, temperature: 20 °C, injection volumes: 1 µL, injection concentrations: 1.5 $g\,L^{-1}$.[293]

*d) **Methionine***

In a comparative study, two CSPs based on macrocyclic glycopeptides were applied for the separation of the enantiomers of methionine, namely teicoplanin (i.e. Chirobiotic T, ASTEC, USA) and eremomycin (i.e. Chirasel E, BioChemMack, Russia).[298] Eremomycin ($M = 1540\,g\,mol^{-1}$)[299] contains 22 chiral centers, three cavities, three sugar moieties, five aromatic rings, one carboxylic group, nine hydroxy groups, seven amido groups, and three amino groups.

Figure 30 shows the adsorption isotherms of the two methionine enantiomers at 25 °C for these CSPs and the corresponding mobile phases compositions indicated in the

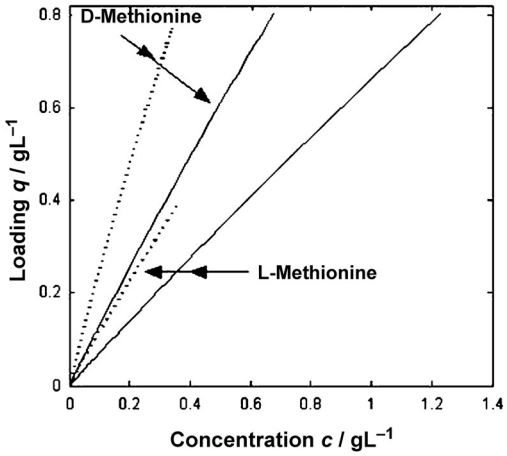


Figure 30. Adsorption isotherms at 25 °C of L- and D-methionine for Chirobiotic T (dotted) with $EtOH/H_2O = 40:60$ (v/v) and Chirasel E (line) with $MeOH/H_2O$ (0.1 M NaH_2PO_4) = 20:80 (v/v).[298]

caption. The selectivities are significant and do not differ much for the two CSPs.

Elution profiles were predicted using the isotherms shown in Figure 30 and the already mentioned equilibrium dispersive model. A typical result, shown in Figure 31, demonstrates the applicability of both the adsorption isotherm and column models to describe the shape of the elution profiles and to evaluate performance parameters.

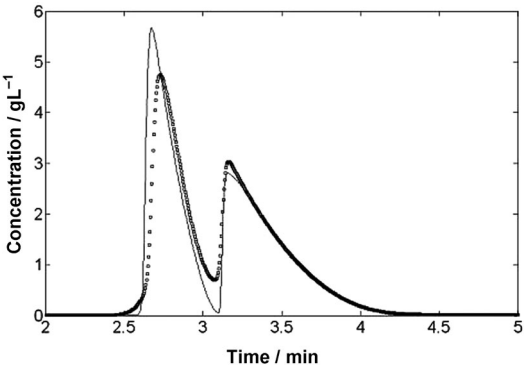


Figure 31. Experimental (symbols) and calculated (dotted) elution profiles of DL-methionine for the Chirasel E column, injection concentrations for both enantiomers: 6.1 g L^{-1}, the first peak corresponds to L-methionine.[298]

Figure 32 summarizes the results of intensive numerical simulations performed with the validated model. The influence of the amount injected on the product of productivity (*PR*) and recovery yield (*RY*) was evaluated for the applied laboratory batch column. The better performance of the Chirasel E CSP is clearly seen. The results provide information about the most beneficial injection quantities. It can be also seen that the performance of the chromatographic process is higher if the first eluting L-methionine is the target component. Based on this information, classical scale-up rules can be applied to predict optimal operating parameters for larger columns.[216]

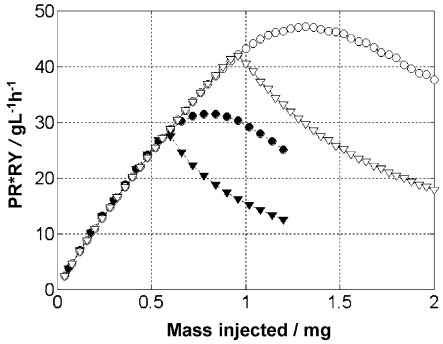


Figure 32. Simulated dependence of productivity (*PR*) multiplied by recovery yield (*RY*) of L- (circles) and D-methionine (triangles) on mass injected for two CSPs (Chirobiotic T, $EtOH/H_2O = 40:60$ (v/v), solid symbols; and Chirasel E, $MeOH/H_2O$ (0.1 M NaH_2PO_4) = 20:80 (v/v), open symbols). Specified purity requirements: 99 %.[300]

Since Chirasel E performed better and also appeared to be more stable than Chirobiotic T, it was used in an experimental SMB study, where eight connected conventional HPLC columns were used (each $L = 10$ cm, $d = 0.46$ cm). Feed concentrations of up to 10 g L^{-1} were applied, which lie in the nonlinear range of the adsorption isotherms. Successful continuous separation of the two methionine enantiomers was achieved after carefully designing the SMB process. The overall process productivity was evaluated to be approximately 0.75 kg $kg^{-1}_{CSP} day^{-1}$.[300]

5. Coupling of Chromatography and Crystallization

After introducing enantioselective crystallization and chromatography individually, a straightforward and very promising concept that is based on combining these two techniques will be described in this section. The basic idea behind this coupling is the well-known fact that any separation process loses performance if the requirements regarding product purity are raised. Considering, for example, SMB chromatography, it is clear that the process must be designed and operated much more careful and conservatively if the purity of the streams leaving the corresponding unit must be very high, compared to a situation in which lower purities are acceptable. In addition, crystallization is a cheaper and in general simpler process than preparative chromatography. However, as discussed above, successful application of standard enantioselective crystallization techniques to racemic compound forming systems requires the provision of a certain initial enrichment. Thus, a reasonable approach is to use the expensive chromatographic step to achieve, prior to crystallization, the required starting composition.[301–303] Examples for such hybrid processes were recently investigated in a systematic manner within the European FP7-project INTENANT (INTegrated synthesis and purification of single ENANTiomers). This project was successful in exploring several possibilities for combining chemical and physical approaches capable of delivering pure enantiomers.[34]

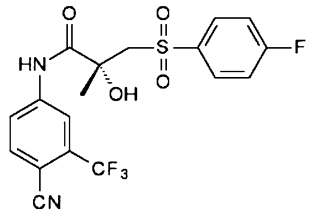


Figure 33. Structure of (*R*)-bicalutamide.[168]

Within the INTENANT project, a comprehensive case study was carried out on the separation of a racemic mixture of bicalutamide, a drug substance used in the treatment of prostate cancer, by simulated moving bed chromatography, with the objective of maximizing throughput at a reduced outlet purity.[168] Figure 33 shows the structure of the target *R* enantiomer. Currently, bicalutamide is sold as a racemate. The isolation of the pure *R* enantiomer from the pre-enriched mixture of bicalutamide enantiomers was successfully scaled up from laboratory experiments processing only a few grams of material to a scale of 600 g of racemate. The throughput of the first SMB chromatography step using Chiralpak AD as the chiral stationary phase could be significantly increased by setting a lower outlet purity. The fraction leaving the SMB extract port possessed a projected enrichment of 92:8 and was subjected to two subsequent crystallization steps, as illustrated in Figure 34. More details regarding the realized exploitation of the shift of the eutectic composition in the bicalutamide phase diagram with temperature and also the addition of water as an antisolvent are given in Ref. [168]. The two simple crystallization steps increased the enantiomeric purity progressively, and the final product was 99.99% pure. The experimentally determined yield was 45%.

Reliable short-cut methods were developed recently to analyze and evaluate the potential of such hybrid chromatography/crystallization processes more systematically and efficiently.[275,304,305] A comparison of competing concepts is shown for the bicalutamide case in Figure 35. The results show that the coupled process based on a "SMB transition purity" of 92% is capable of more than doubling the productivity of isolating the target *R* enantiomer from a racemic solution compared to applying SMB chromatography alone.[168]

A more general analysis revealed that the described type of hybrid process is in particular likely to be beneficial for

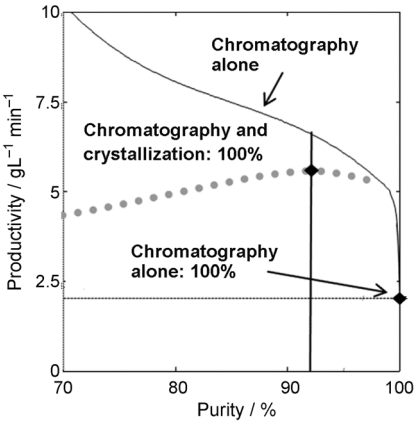


Figure 35. Optimized productivity of (*R*)-bicalutamide in the stand-alone SMB chromatographic process (the right diamond marks productivity for 100% purity, the upper solid line marks the productivity for reduced purity). The gain in productivity for the coupled process compared to SMB alone is reflected by the dotted line. (Adapted from Ref. [168].)

systems possessing a eutectic composition close to the pure enantiomer in the related phase diagram.[168]

Similar results as for bicalutamide were obtained earlier by analyzing the separation of the enantiomers of mandelic acid using a Chirobiotic T column in combination with a crystallization process assumed to reach equilibrium conditions.[304] The reference case was again the exclusive application of SMB (100% purity). An optimal transition purity between chromatography and crystallization of approximately 93% was determined for this particular example, thus providing a performance increase of approximately 45% compared to SMB chromatography alone.

A genetic algorithm was used in Ref. [191] to optimize the combined process for the separation of the enantiomers of Tröger's base. Multi-objective optimization of the productivity and the evaporation costs in terms of essential operating parameters (column length and SMB feed concentration) shows for this coupled process an optimum SMB purity value as a trade-off between SMB performance and recycling of the mother liquor.

Further examples devoted to study quantitatively combinations of chromatography and crystallization were reported in Refs. [306–308]. All the results summarized here clearly

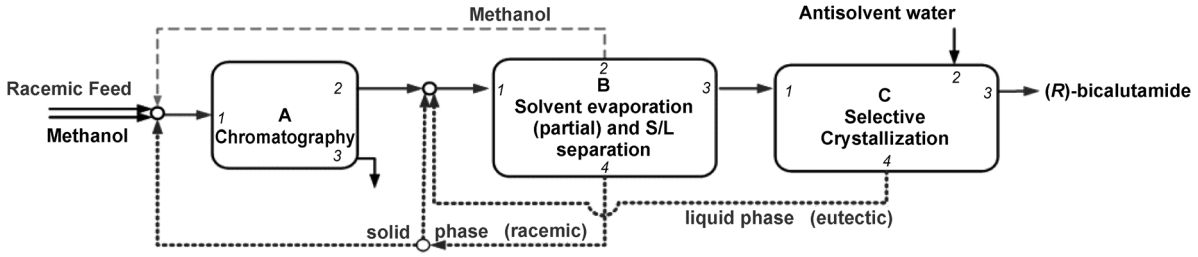


Figure 34. Flow sheet of the coupled process to isolate (*R*)-bicalutamide (target enantiomer). The racemic feed mixture is first enriched by SMB chromatography (A) and further purified in two subsequent crystallization steps (B and C). Recycling of part of the solid phase from (B) and the entire mother liquor from (C) increases the yield. (Adapted from Ref. [168].)

indicate that the combined process can achieve better performance than the SMB process alone.

As an alternative, a differentl enrichment step prior to crystallization was suggested recently in Ref. [309]. It is based on using an enantioselective membrane within a pertraction process. Clearly, other combinations of various separation processes with subsequent crystallization are imaginable.

A powerful and attractive alternative capable of providing initial enrichment for final crystallization is clearly the option of realizing a partially selective synthesis reaction. This approach was applied, for example, in Refs. [171,310].

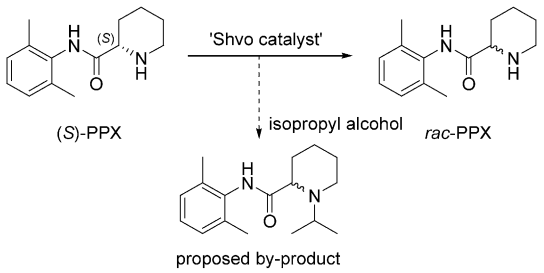


Figure 36. Structure of (*S*)-pipecoloxylidide ((*S*)-PPX) and the reaction scheme for its racemization.[169]

6. Process Concepts with Consideration of Racemization

All amino acids have the L configuration in living tissue, but after the death of an organism a very slow racemization proceeds, which can be used for dating studies.[311]

In separation processes intended to provide pure enantiomers, in addition to the desired product fraction, a second fraction is typically produced, which is enriched in the counter-enantiomer. For economic reasons it is highly desirable to also exploit this side fraction. This can be done by incorporating sufficiently fast racemization reactions. If these reactions are successful, the formed racemic mixtures can be reintroduced into the feed stream of a separation process or at another suitable position within the process sequence, thus allowing an increase in the yield with respect to the target enantiomer. In the optimal case where no counter-enantiomer leaves the process, a theoretical yield of 100 % is possible.

Numerous efforts have been devoted to the development of selective and sufficiently active racemization catalysts for reuse of the "counter-enantiomers". Both chemical and enzymatic approaches are adopted. Concepts and recent examples of racemization using metal catalysis are described in Ref. [312].

The general approach is discussed and illustrated, for example, in Ref. [313] for the combination of chromatographic separations with biotransformations. An overview providing recent examples of the combination of enantioselective HPLC, including SMB chromatography and racemization, is given in Ref. [273].

Referring to an example already discussed extensively above, interesting progress regarding the development of an efficient mandelate racemase is reported in Ref. [314]. In Ref. [315] a recombinant amino acid racemase which offers a broad substrate specificity was prepared from *Pseudomonas putida* cloned into *Escherichia coli*. Enzyme lyophilizates of different purity were applied in the racemization of methionine and asparagine in Ref. [155]. The racemase was further successfully used for in situ racemization during the preferential crystallization of the enantiomers of asparagine. Crystallization experiments accompanied by enzymatic racemization led to a significant increase of crystallized L-asparagine.[122,155]

An integrated process for the separation of the enantiomers of the industrially relevant substance 2′,6′-pipecoloxylidide (PPX, Figure 36) was investigated within the INTENANT project.[169] (*S*)-PPX is an intermediate in the manufacture of a number of local anesthetics. The integrated chiral resolution process developed combines chromatography, crystallization, and racemization. The flowsheet of the entire process is illustrated in Figure 37. Preparative chromatography was used to obtain an enantioenriched solution from the initial racemic mixture of PPX. Despite the fact that the chromatographic separation employed provides unsatisfactory resolution when assessed in isolation, relaxed purity requirements lead to improved productivity, reduced eluent volumes, and an output stream sufficiently enriched for enantioselective crystallization. Crystallization in the two-phase region could then be used to isolate the pure (*S*)-PPX with a purity of $\geq 99.5\,\%$. The (*R*)-PPX-rich chromatography stream was racemized using a homogeneous ruthenium catalyst (Shvo catalyst),[169,312e] which provided efficiently a racemic mixture of PPX (Figure 36). This mixture could be recycled into the chromatography step together with fresh feed. The presence of alcohols led to an undesired side reaction, which necessitated removing the ethanol from the chromatography effluent prior to racemization by distillation

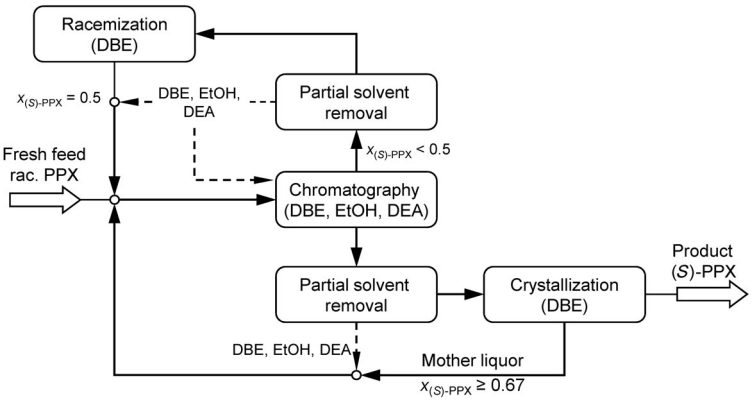


Figure 37. Integrated process scheme to separate racemic PPX. First the racemic feed is enriched by chromatography. The resulting (*S*)-PPX-rich stream is, after creating supersaturation, subjected to crystallization. The mother liquor is returned to the chromatographic enrichment. The (*R*)-PPX-rich stream is racemized and then also returned to the chromatographic step. Intermediate distillations are used to adjust the solvent compositions.[169]

(Figure 37). The rationally designed and successfully applied integrated process uses dibutyl ether (DBE) as a common solvent and allows a full recycling of all the solvents and the undesired (*R*)-PPX. It could be experimentally validated on a laboratory and pilot scale (gram and several 100 g, respectiviely) and resulted in both high productivity and yield.[169]

Considering the large potential of the general approach, reports on industrial processes exploiting racemization are still limited. This is probably partly also because of the fact that problems might occur with the formation of unwanted degradation products. The above-mentioned and other promising results have currently triggered intensive efforts devoted to incorporate more systematically racemization steps in various process chains devoted to the production of pure enantiomers.

7. Summary

Several possible concepts capable of separating enantiomers in a preparative manner have been described in this process-oriented Review and demonstrate that the racemic approach provides a powerful alternative to enantioselective synthesis.

Crystallization-based methods and preparative chromatography have been presented in more detail by using various case studies. These methods currently appear to be the most advanced.

The selection, design, and optimization of crystallization techniques require knowledge regarding the underlying solid–liquid equilibria which has to be gathered experimentally. Productive modes of operation can be identified from the specific type of phase diagram.

As a consequence of the availability of highly selective chiral stationary phases, preparative overloaded high-performance liquid chromatography can currently be seen as the most versatile technique. The high-pressure equipment required and the process complexities limit the applicability on larger scales. The breakthrough of the simulated moving bed technology contributes to further promoting chromatographic enantioseparation.

A very promising approach is the application of hybrid processes which combine the positive features of several separation processes. Although these processes are more challenging, there are nowadays rules and short-cut methods available for a rational design. It was shown within the INTENANT project that the achievable overall productivity can be increased significantly by, for example, initially generating an enrichment by using chromatography followed by highly selective crystallization processes. Based on the results obtained in the two case studies described (bicalutamide and PPX) as well as many further examples, a helpful decision tree was suggested that first considers the economically crucial question, whether a compound can be racemized efficiently or not (Figure 38).

If racemization is possible, the knowledge of the phase diagram of the specific chiral system is most instructive to a) decide about the presence of a conglomerate and afterwards b) to evaluate the eutectic composition in the chiral system. As a result of this information, different resolution strategies open up, which might be, for example, a possible combination of racemization with only crystallization (right branch in Figure 38) or based on chromatographic enrichment and subsequent crystallization steps, as proposed for PPX (second branch from the right). In cases where racemization is not successful, asymmetric synthesis might be economically most attractive, eventually combined with a subsequent final crystallization purification step (second branch from the left). If both racemization and asymmetric synthesis are not feasible, chromatography and crystallization, for example, enables resolution of such a racemate (left branch).

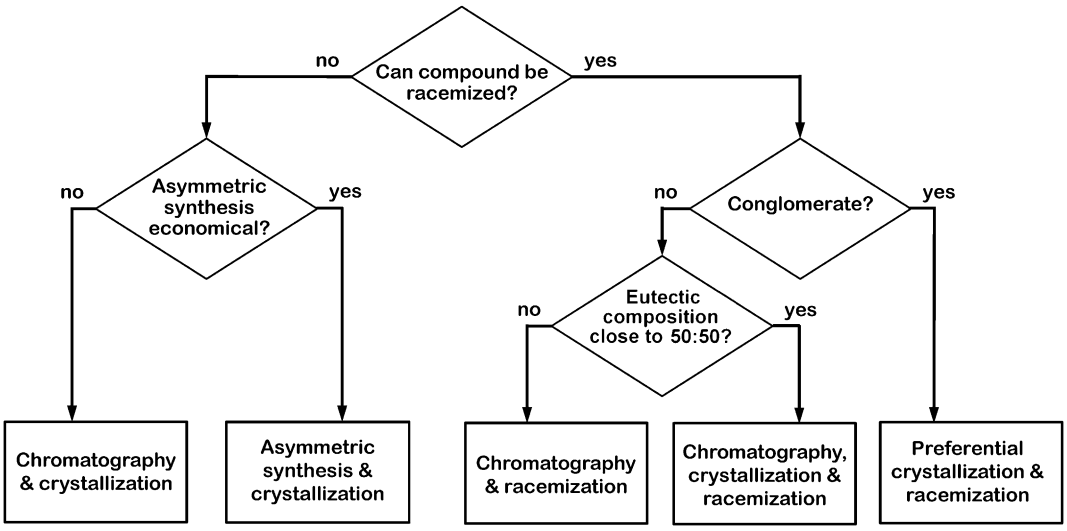


Figure 38. Decision tree based on simple qualitative criteria supporting a rational selection of a suitable process concept for the production of pure enantiomers (based on Ref. [305]).

It can be expected that in the future enantioselective separation processes will be increasingly combined in advanced integrated processes to facilitate the access to pure enantiomers.

We thank all the current and former co-workers for their contributions to this work, in particular all colleagues involved in the INTENANT project, specifically Dr. Martin Hedberg (AstraZeneca), Dr. Henning Kämmerer (Evonik), Prof. Malte Kaspereit (Nürnberg-Erlangen), Prof. Achim Kienle (Magdeberg), and Dr. Jan von Langermann (Rostock), as well as Jacqueline Kaufmann and Luise Borchert for supporting the experimental work presented. The financial support of the Deutsche Forschungsgemeinschaft (SE 586/15, SE 586/16, PAK 281), German Ministry of Education and Research (BMBF, NMT/CT/03C0319), and European Union (FP7-NMP2-SL2008-214129) is gratefully acknowledged.

Received: April 5, 2013

[1] E. L. Eliel, S. Wilen, M. Doyle, *Basic Organic Stereochemistry*, Wiley-Interscience, New York, **2001**.
[2] U. Meierhenrich, *Amino Acids and the Asymmetry of Life*, Springer, Berlin, **2008**.
[3] *Origin of Life. Chemical Approach* (Eds.: P. Herdewijn, M. V. Kisakürek), Helvetica Chimica Acta, Zürich, **2008**.
[4] P. Cintas, *Angew. Chem.* **2002**, *114*, 1187 – 1193; *Angew. Chem. Int. Ed.* **2002**, *41*, 1139 – 1145.
[5] D. P. Glavin, J. E. Elisa, A. S. Burton, M. P. Callahan, J. P. Dworkin, R. W. Hilts, C. D. K. Herd, *Meteorit. Planet. Sci.* **2012**, *47*, 1347 – 1364.
[6] A. M. Rouhi, *Chem. Eng. News* **2003**, *81*, 56 – 61.
[7] R. A. Sheldon, *Chirotechnology*, Marcel Dekker, New York, **1993**.
[8] *Chiral Drugs*: *Chemistry and Biological Action*, (Eds.: G.-Q. Lin, Q.-D. You, J.-F. Cheng), Wiley, Hoboken, **2011**.
[9] S. C. Stinson, *Chem. Eng. News* **2001**, *79*, 45 – 57.
[10] A. M. Rouhi, *Chem. Eng. News* **2003**, *81*, 45 – 55.
[11] J. Blumenstein in *Chirality in Industry II*, *Developments in the Manufacture and Applications of Optically Active Compounds* (Eds.: A. N. Collins, G. N. Sheldrake, J. Crosby), Wiley, Chichester, **1997**, pp. 11 – 18.
[12] J. Crosby in *Chirality in Industry*, *The Commercial Manufacture and Applications of Optically Active Compounds* (Eds.: A. Collins, G. Sheldrake, J. Crosby), Wiley, Chichester, **1992**, pp. 1 – 66.
[13] J. Crosby in *Chirality in Industry II*, *Developments in the Manufacture and Applications of Optically Active Compounds* (Eds.: A. Collins, G. Sheldrake, J. Crosby), Wiley, Chichester, **1997**, pp. 1 – 10.
[14] B. A. Asteleford, L. O. Weigel, *Chirality in Industry II*, *Developments in the Manufacture and Applications of Optically Active Compounds* (Eds.: A. Collins, G. Sheldrake, J. Crosby), Wiley, Chichester, **1997**, pp. 119 – 156.
[15] a) M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer, T. Zelinski, *Angew. Chem.* **2004**, *116*, 806 – 843; *Angew. Chem. Int. Ed.* **2004**, *43*, 788 – 824; b) W. Leuchtenberger, K. Huthmacher, K. Drauz, *Appl. Microbiol. Biotechnol.* **2005**, *69*, 1 – 8; c) K. Mikami, M. Lautens, *New Frontiers in Asymmetric Catalysis*, Wiley, Hoboken, **2007**; d) "Enzyme-Catalyzed Asymmetric Synthesis": H. Gröger in *Catalytic Asymmetric Synthesis* (Ed.: I. Ojima), Wiley, Hoboken, **2010**, pp. 269 – 341; e) J. van Ooyen, S. Noack, M. Bott, A. Reth, L. Eggeling, *Biotechnol. Bioeng.* **2012**, *109*, 2070 – 2081.
[16] U. Klar, B. Röhr, F. Kuczynski, W. Schwede, M. Berger, W. Skuballa, B. Buchmann, *Synthesis* **2005**, 301 – 305.
[17] *Compendium of Chiral Auxiliary Applications* (Ed.: G. Roos), Academic Press, New York, **2002**.
[18] R. Noyori, *Asymmetric Catalysis in Organic Synthesis*, Wiley, New York, **1994**.
[19] E. N. Jacobsen, A. Pfaltz, H. Yamamoto, *Comprehensive Asymmetric Catalysis*, Springer, Berlin, **1999**.
[20] K. Mikaki, M. Lauterns, *New Frontiers in Asymmetric Catalysis*, Wiley, New York, **2007**.
[21] W. S. Knowles, M. J. Sabacky, *Chem. Commun.* **1968**, 1445 – 1446.
[22] W. S. Knowles, *Angew. Chem.* **2002**, *114*, 2096 – 2107; *Angew. Chem. Int. Ed.* **2002**, *41*, 1998 – 2007.
[23] a) H. Nozaki, H. Takaya, S. Moriuti, R. Noyori, *Tetrahedron* **1968**, *24*, 3655 – 3669; b) R. Noyori, *Angew. Chem.* **2013**, *125*, 83 – 98; *Angew. Chem. Int. Ed.* **2013**, *52*, 79 – 92.
[24] a) T.-P. Dang, H. B. Kagan, *J. Chem. Soc. D* **1971**, 481; b) H. B. Kagan, T.-P. Dang, *J. Am. Chem. Soc.* **1972**, *94*, 6429 – 6433.
[25] S. Akutagawa in *Chirality in Industry*: *The Commercial Manufacture and Applications of Optically Active Compounds* (Eds.: A. N. Collins, G. N. Sheldrake, J. Crosby), Wiley, Chichester, **1992**, pp. 313 – 324.
[26] C. Giordano, M. Villa, S. Panossian, *Chirality in Industry*: *The Commercial Manufacture and Applications of Optically Active Compounds* (Eds.: A. N. Collins, G. N. Sheldrake, J. Crosby), Wiley, Chichester, **1992**, pp. 303 – 312.
[27] H. C. Kolb, M. S. van Nieuwenhze, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483 – 2547.
[28] a) A. Berkessel, H. Gröger, *Asymmetric Organocatalysis*: *From Biomimetic Concepts to Applications in Asymmetric Synthesis*, Wiley, New York, **2005**; b) K. Maruoka, T. Ooi, T. Kano, *Chem. Commun.* **2007**, 1487 – 1495; c) H. U. Blaser, H.-J. Federsel, *Asymmetric Synthesis on Industrial Scale*, 2nd ed., Wiley-VCH, Weinheim, **2010**.
[29] R. N. Patel, *Coord. Chem. Rev.* **2008**, *252*, 659 – 701.
[30] *Chirality in Industry II*, *Developments in the Manufacture and Applications of Optically Active Compounds* (Eds.: A. Collins, G. Sheldrake, J. Crosby), Wiley, Chichester, **1997**, pp. 309 – 400.
[31] a) *Chiral Separations. Applications and Technology* (Ed.: S. Ahuja), American Chemical Society Washington, **1997**; b) *Chiral Recognition in Separation Methods*: *Mechanisms and Applications* (Ed.: A. Berthod), Springer, Berlin, **2010**; c) R. Bhushan, J. Martens, *Amino Acids. Chromatographic Separation and Enantioresolution*, HNB, New York, **2010**.
[32] G. Gübitz, M. G. Schmid, *Chiral Separations*, *Methods in Molecular Biology*, *Vol. 243*, Humana, Totowa, **2004**.
[33] B. Chankvetadze, *J. Chromatogr. A* **2007**, *1168*, 45 – 70.
[34] H. J. Federsel, *Org. Process Res. Dev.* **2012**, *16*, 260 – 261.
[35] *Chiral Separation Methods for Pharmaceutical and Biotechnological Products* (Ed.: S. Ahuja), Wiley, Hoboken, **2011**.
[36] P. Franco, C. Minguillón, *Chiral Separation Techniques* (Ed.: G. Subramanian), 2nd ed., Wiley-VCH, Weinheim, **2001**, pp. 1 – 23.
[37] N. M. Maier, P. Franco, W. Lindner, *J. Chromatogr. A* **2001**, *906*, 3 – 33.
[38] *Chiral Separation Techniques. A Practical Approach* (Ed.: G. Subramanian), 3rd ed., Wiley-VCH, Weinheim, **2007**.
[39] "From racemates to single enantiomers—Chiral synthetic drugs over the last 20 years": H. Murakami in *Novel Optical Resolution Technologies*, *Top. Curr. Chem.*, *Vol. 269* (Eds.: K. Sakai, N. Hirayama, R. Tamura), Springer, Berlin, **2007**, pp. 273 – 299.
[40] J. E. Rekoske, *AIChE J.* **2001**, *47*, 2 – 5.

[41] W. Marckwald, A. McKenzie, *Ber. Dtsch. Chem. Ges.* **1899**, *32B*, 2130 – 2136.
[42] R. Noyori, T. Ikeda, T. Ohkuma, M. Widhalm, M. Kitamura, H. Takaya, S. Akutagawa, N. Sayo, T. Saito, *J. Am. Chem. Soc.* **1989**, *111*, 9134 – 9135.
[43] M. Kitamura, T. Ohkuma, M. Tokunaga, R. Noyori, *Tetrahedron: Asymmetry* **1990**, *1*, 1 – 4.
[44] a) O. Pàmies, J.-E. Bäckvall, *Chem. Rev.* **2003**, *103*, 3247 – 3262; b) O. May, S. Verseck, A. S. Bommarius, K. Drauz, *Org. Process Res. Dev.* **2002**, *6*, 452 – 457.
[45] E. Vedejs, M. Jure, *Angew. Chem.* **2005**, *117*, 4040 – 4069; *Angew. Chem. Int. Ed.* **2005**, *44*, 3974 – 4001.
[46] M. T. Reetz, W. Wiesenhöfer, G. Franciò, W. Leitner, *Adv. Synth. Catal.* **2003**, *345*, 1221 – 1228.
[47] J. D. Thornton, *Science and Practice of Liquid – Liquid Extraction*, *Vol. 1 – 2*, Clarendon, Oxford, **1992**.
[48] D. J. Cram, J. M. Cram, *Container Molecules and Their Guests*, Royal Society of Chemistry, Cambridge, **1994**.
[49] S. Zhao, Y.-M. Liu, *Electrophoresis* **2001**, *22*, 2769 – 2774.
[50] D. Kmecz, M. Simandi, E. Szekely, E. Fogassy, *Tetrahedron: Asymmetry* **2004**, *15*, 1841 – 1845.
[51] R. M. C. Viegas, C. A. M. Afonso, J. G. Crespo, I. M. Coelhoso, *Sep. Purif. Technol.* **2007**, *53*, 224 – 234.
[52] M. Togrul, Y. Turgut, H. Hosgoren, *Chirality* **2004**, *16*, 351 – 355.
[53] P. E. Hare, E. Gil-Av, *Science* **1979**, *204*, 1226 – 1228.
[54] H. Nishizawa, K. Tahara, A. Hayashida, Y. Abe, *Anal. Sci.* **1993**, *9*, 611 – 615.
[55] P. Dzygiel, T. B. Reeve, U. Piarulli, M. Krupicka, I. Tvaroska, C. Gennari, *Eur. J. Org. Chem.* **2008**, 1253 – 1264.
[56] K. Tang, J. Yi, K. Huang, G. Zhang, *Chirality* **2009**, *21*, 390 – 395.
[57] A. B. De Haan, B. Simandi *Ion Exchange and Solvent Extraction* (Eds.: Y. Marcus, M. M. Sharma, J. A. Marinsky), Marcel Dekker, New York, **2001**, pp. 255 – 294.
[58] M. Newcomb, J. L. Toner, R. C. Helgeson, D. J. Cram, *J. Am. Chem. Soc.* **1979**, *101*, 4941 – 4947.
[59] a) A. J. Hallett, G. J. Kwant, J. G. de Vries, *Chem. Eur. J.* **2008**, 2111 – 2120; b) B. Schuur, A. J. Hallett, J. G. M. Winkelman, J. G. de Vries, H. J. Heeres, *Org. Process Res. Dev.* **2009**, *13*, 911.
[60] a) N. Rubio, S. Ignatova, C. Minguillón, I. A. Sutherland, *J. Chromatogr. A* **2009**, *1216*, 8505 – 8511; b) C. Minguillón, *Chem. Eng. Technol.* **2012**, *35*, 35 – 45.
[61] E. Peréz, C. Minguillón, *Chiral Separation Techniques. A Practical Approach* (Ed.: G. Subramanian), 3rd ed., Wiley-VCH, Weinheim, **2007**, pp. 369 – 398.
[62] B. J. V. Verkuijl, PhD Thesis, University of Groningen (The Netherlands), **2009**.
[63] D. W. Armstrong, H. L. Jin, *Anal. Chem.* **1987**, *59*, 2237 – 2241.
[64] a) J. T. F. Keurentjes, F. J. M. Voermans, *Chirality in Industry II*, *Developments in the Manufacture and Applications of Optically Active Compounds* (Eds.: A. Collins, G. Sheldrake, J. Crosby), Wiley, Chichester, **1997**, pp. 157 – 180; b) M. F. Kemmere, J. T. F. Keurentjes, *Chiral Separation Techniques* (Ed.: G. Subramanian), 2nd ed., Wiley-VCH, Weinheim, **2001**, pp. 1271 – 1250.
[65] C. A. M. Afonso, J. G. Crespo, *Angew. Chem.* **2004**, *116*, 5405 – 5407; *Angew. Chem. Int. Ed.* **2004**, *43*, 5293 – 5295.
[66] J. D. Clark, B. Han, A. S. Bhown, S. R. Wickramasinghe, *Sep. Purif. Technol.* **2005**, *42*, 201 – 211.
[67] H. Maximini, H. Chmiel, N. W. Holdik, N. Maier, *J. Membr. Sci.* **2006**, *276*, 221 – 231.
[68] J. T. F. Keurentjes, L. J. W. M. Nabuurs, E. A. Vegter, *J. Membr. Sci.* **1996**, *113*, 351 – 360.
[69] A. J. B. Kemperman, D. Bargeman, T. Van Den Boomgaard, H. Strathmann, *Sep. Sci. Technol.* **1996**, *31*, 2733 – 2762.
[70] E. Yashima, J. Noguchi, Y. Okamoto, *Chem. Lett.* **1992**, 1959 – 1962.
[71] E. Yashima, J. Noguchi, Y. Okamoto, *Macromolecules* **1995**, *28*, 8368 – 8374.
[72] W. H. Pirkle, W. E. Bowen, *Tetrahedron: Asymmetry* **1994**, *5*, 773 – 776.
[73] T. Gumí, C. Minguillón, C. Palet, *Polymer* **2005**, *46*, 12306 – 12312.
[74] B. Sellergren in *Chiral Separation Techniques. A Practical Approach* (Ed.: G. Subramanian), 3rd ed., Wiley-VCH, Weinheim, **2007**, pp. 399 – 432.
[75] a) B. Gutiérrez, N. Rubio, C. Minguillón, *Desalination* **2006**, *200*, 117 – 119; b) B. Gutiérrez, PhD thesis, University of Barcelona (Spain), **2009**.
[76] J. M. D. Theodorus, T. M. M. Antonius, Z. Han, M. R. Lisette, A. G. N. Harm, K. Arue, E. M. O. Pieter, V. D. V. Albert, J. R. S. Ernst, *Chirality* **2002**, *12*, 627 – 636.
[77] F. Garnier, J. Randon, J. L. Rocca, *Sep. Purif. Technol.* **1999**, *16*, 243 – 250.
[78] A. H. Ölçeroğlu, P. Çalık, L. Yilmaz, *J. Membr. Sci.* **2008**, *322*, 446 – 452.
[79] P. E. M. Overdevest, M. H. J. Hoenders, K. van't Rief, A. van der Padt, J. T. F. Keurentjes, *AIChE J.* **2002**, *48*, 1917 – 1926.
[80] a) G. Kaupp, *Angew. Chem.* **1994**, *106*, 1519 – 1522; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1452 – 1455; G. Kaupp, *Angew. Chem.* **1994**, *106*, 768 – 770; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 728 – 729b) P. H. Au-Yeung, S. M. Resnick, P. M. Witt, T. C. Frank, F. A. Donate, L. A. Robbins, *AIChE J.* **2013**, *59*, 2603 – 2620.
[81] L. Pasteur, *Ann. Chim. Phys.* **1848**, *3*, 442 – 459.
[82] J. Jacques, A. Collet, S. H. Wilen, *Enantiomers*, *Racemates and Resolutions*, Krieger, Malabar, **1994**.
[83] A. E. Flood, *Recent Pat. Mater. Sci.* **2008**, *1*, 98 – 115.
[84] A. Collet, *Angew. Chem.* **1998**, *110*, 3429 – 3431; *Angew. Chem. Int. Ed.* **1998**, *37*, 3239 – 3241.
[85] P. J. Harrington, E. Lodewijk, *Org. Process Res. Dev.* **1997**, *1*, 72 – 76.
[86] A. J. Blacker, S. Brown, B. Clique, B. Gourlay, C. E. Headley, S. Ingham, D. Ritson, T. Screen, M. J. Stirling, D. Taylor, G. Thompson, *Org. Process Res. Dev.* **2009**, *13*, 1370 – 1378.
[87] T. Vries, H. Wynberg, E. van Echten, J. Koek, W. ten Hoeve, R. M. Kellogg, Q. B. Broxterman, A. Minnaard, B. Kaptein, S. van der Sluis, L. Hulshof, J. Kooistra, *Angew. Chem.* **1998**, *110*, 2491 – 2496; *Angew. Chem. Int. Ed.* **1998**, *37*, 2349 – 2354.
[88] J. S. C. Loh, W. J. P. van Enckevort, E. Vlieg, C. Gervais, R. F. P. Grimbergen, B. Kaptein, *Cryst. Growth Des.* **2006**, *6*, 861 – 865.
[89] B. Kaptein, T. R. Vries, J. W. Nieuwenhuijzen, R. M. Kellog, R. F. P. Grimbergen, Q. B. Broxterman, *Handbook of Chiral Chemicals* (Ed.: D. Ager), 2nd ed., Taylor&Francis, CRC, Boca Raton, **2006**, pp. 97 – 116.
[90] E. Pálovics, J. Schindler, F. Faigl, E. Fogassy, *Tetrahedron: Asymmetry* **2010**, *21*, 2429 – 2434.
[91] *CRC Handbook of Optical Resolutions via Diastereomeric Salt Formation* (Ed.: D. Kozma), CRC, Boca Raton, **2002**.
[92] H. Nohira, K. Sakaiin, *Enantiomer Separation. Fundamentals and Practical Methods* (Ed.: F. Toda), Kluwer, Dordrecht, **2004**.
[93] J. W. Schroer, C. Wibowo, K. M. Ng, *AIChE J.* **2001**, *47*, 369 – 387.
[94] A. Collet, *Enantiomer* **1999**, *4*, 157 – 172.
[95] H.-H. Tung, E. L. Paul, M. Midler, J. A. McCauley, *Crystallization of Organic Compounds: An Industrial Perspective*, Wiley, Hoboken, **2009**.
[96] L. Synoradzki, H. Hajmowicz, J. Wisialski, A. Mizerski, T. Rowicki, *Org. Process Res. Dev.* **2008**, *12*, 1238 – 1244.
[97] M. Leeman, F. Querniard, T. R. Vries, B. Kaptein, R. M. Kellogg, *Org. Process Res. Dev.* **2009**, *13*, 1379 – 1381.
[98] A. Collet, M.-J. Brienne, J. Jacques, *Chem. Rev.* **1980**, *80*, 215 – 230.

[99] "Preferential crystallization": G. Coquerel in *Novel Optical Resolution Technologies, Top. Curr. Chem., Vol. 269* (Eds.: K. Sakai, N. Hirayama, R. Tamura),**2007**, pp. 1 – 51.
[100] G. Levilain, G. Coquerel, *CrystEngComm* **2010**, *12*, 1983 – 1992.
[101] P. A. Levkin, V. Y. Torbeev, D. A. Lenev, R. G. Kostyanovski, *Top. Stereochem.* **2006**, *25*, 81 – 134.
[102] L. Pérez-García, D. B. Amabilino, *Chem. Soc. Rev.* **2007**, *36*, 941 – 967.
[103] A. A. Bredikhin, Z. A. Bredikhina, D. V. Zakharychev, L. V. Konoshenko, *Tetrahedron: Asymmetry* **2007**, *18*, 1964 – 1970.
[104] a) Z. A. Bredikhina, F. S. Akhatova, D. V. Zakharychev, A. A. Bredikhin, *Tetrahedron: Asymmetry* **2008**, *19*, 1430 – 1435; b) A. A. Bredikhin, Z. A. Bredikhina, D. V. Zakharychev, *Mendeleev Commun.* **2012**, *22*, 171 – 180.
[105] A. Galland, V. Dupray, B. Berton, S. Morin-Grognet, M. Sanselme, H. Atmani, G. Coquerel, *Cryst. Growth Des.* **2009**, *9*, 2713 – 2718.
[106] L. Addadi, S. Weinstein, E. Gati, I. Weissbuch, M. Lahav, *J. Am. Chem. Soc.* **1982**, *104*, 4610 – 4617.
[107] M. Lahav, L. Leiserowitz, *J. Phys. D* **1993**, *26*, B22 – B31.
[108] L. Addadi, Z. Berkovitch-Yellin, I. Weissbuch, J. van Mil, L. J. W. Shimon, M. Lahav, L. Leiserowitz, *Angew. Chem.* **1985**, *97*, 476 – 496.
[109] D. Zbaida, M. Lahav, K. Drauz, G. Knaup, M. Kottenhahn, *Tetrahedron* **2000**, *56*, 6645 – 6649.
[110] D. Polenske, M. P. Elsner, H. Lorenz, A. Seidel-Morgenstern, *Chem. Ing. Tech.* **2006**, *78*, 1101 – 1110.
[111] H. Lorenz, D. Polenske, A. Seidel-Morgenstern, *Chirality* **2006**, *18*, 828 – 840.
[112] F. Czapla, H. Lorenz, M. P. Elsner, A. Seidel-Morgenstern, *Chem. Ing. Tech.* **2007**, *79*, 281 – 286.
[113] D. Polenske, H. Lorenz, A. Seidel-Morgenstern, *Chirality* **2009**, *21*, 728 – 737.
[114] A. Seidel-Morgenstern, H. Lorenz, D. Polenske (Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.), WO 2007/023129 A2, 01.03., **2007**; US 7,820,860 B2, 26. 10. **2010**.
[115] H. Lorenz, D. Polenske, L. Klukas, A. Seidel-Morgenstern (Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.), EP 2292306 A1 (9.3., **2011**); US 2012/0197040 A1, filed: 08.02.**2012**.
[116] M. P. Elsner, G. Ziomek, A. Seidel-Morgenstern, *AIChE J.* **2009**, *55*, 640 – 649.
[117] L. Klukas, D. Polenske, H. Lorenz, A. Seidel-Morgenstern, *Proceedings 16th Int. Workshop on Industrial Crystallization (BIWIC 2009)* (Eds.: M. Louhi-Kultanen, H. Hatakka), Lappeenranta University of Technology, Lappeenranta, **2009**, pp. 218 – 224 ISBN 978-952-214-806-3.
[118] G. Coquerel, M.-N. Petit, R. Bouaziz (The University of Rouen), U.S. Patent US006.022.409-A, **2000**.
[119] R. K. Mughal, R. J. Davey, N. Blagden, *Cryst. Growth Des.* **2007**, *7*, 218 – 224.
[120] R. K. Mughal, R. J. Davey, S. N. Black, *Cryst. Growth Des.* **2007**, *7*, 225 – 228.
[121] N. Wermester, O. Lambert, G. Coquerel, *CrystEngComm* **2008**, *10*, 724 – 733.
[122] K. Würges, K. Petrusevska-Seebach, M. P. Elsner, S. Lütz, *Biotechnol. Bioeng.* **2009**, *104*, 1235 – 1239.
[123] C. Viedma, *Phys. Rev. Lett.* **2005**, *94*, 065504.
[124] W. L. Noorduin, E. Vlieg, R. M. Kellog, B. Kaptein, *Angew. Chem.* **2009**, *121*, 9778 – 9784; *Angew. Chem. Int. Ed.* **2009**, *48*, 9600 – 9606.
[125] W. L. Noorduin, W. J. P. van Enckevort, H. Meekes, B. Kaptein, R. M. Kellog, J. C. Tully, J. M. McBride, E. Vlieg, *Angew. Chem.* **2010**, *122*, 8613 – 8616; *Angew. Chem. Int. Ed.* **2010**, *49*, 8435 – 8438.
[126] J. E. Hein, B. H. Cao, C. Viedma, R. M. Kellog, D. G. Blackmond, *J. Am. Chem. Soc.* **2012**, *134*, 12629 – 12636.
[127] W. L. Noorduin, H. Meekes, W. J. P. van Enckevort, B. Kaptein, R. M. Kellog, E. Vlieg, *Angew. Chem.* **2010**, *122*, 2593 – 2595; *Angew. Chem. Int. Ed.* **2010**, *49*, 2539 – 2541.
[128] M. W. van der Meijden, M. Leeman, E. Gelens, W. L. Noorduin, H. Meekes, W. J. P. van Enckevort, B. Kaptein, E. Vlieg, R. M. Kellog, *Org. Process Res. Dev.* **2009**, *13*, 1195 – 1198.
[129] W. L. Noorduin, P. van der Asdonk, A. A. C. Bode, H. Meekes, W. J. P. van Enckevort, E. Vlieg, B. Kaptein, M. W. van der Meijden, R. M. Kellog, G. Deroover, *Org. Process Res. Dev.* **2010**, *14*, 908 – 911.
[130] G. Levilain, C. Rougeot, F. Guillen, J.-C. Plaquevent, G. Coquerel, *Tetrahedron: Asymmetry* **2009**, *20*, 2769 – 2771.
[131] T. Buhse, D. K. Kondepudi, B. Hoskins, *Chirality* **1999**, *11*, 343 – 348.
[132] A. Lüttringhaus, D. Berrer, *Tetrahedron Lett.* **1959**, *1*, 10 – 12.
[133] M. B. Groen, H. Schadenberg, H. Wynberg, *J. Org. Chem.* **1971**, *36*, 2797 – 2809.
[134] N. Doki, M. Yokota, S. Sasaki, N. Kubota, *Cryst. Growth Des.* **2004**, *4*, 1359 – 1363.
[135] S. K. Tulashie, H. Lorenz, L. Hilfert, F. T. Edelmann, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2008**, *8*, 3408 – 3414.
[136] S. K. Tulashie, H. Lorenz, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2009**, *9*, 2387 – 2392.
[137] S. K. Tulashie, H. Lorenz, C. Malwade, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2010**, *10*, 4023 – 4029.
[138] S. K. Tulashie, J. von Langermann, H. Lorenz, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2011**, *11*, 240 – 246.
[139] G. Coquerel, *Enantiomer* **2000**, *5*, 481 – 498.
[140] H. W. B. Roozeboom, *Z. Phys. Chem.* **1899**, *28*, 494.
[141] Z. J. Li, M. T. Zell, E. J. Munson, D. J. W. Grant, *J. Pharm. Sci.* **1999**, *88*, 337 – 346.
[142] M. Klussmann, H. Iwamura, S. P. Mathew, Jr., D. H. Wells, U. Pandya, A. Armstrong, D. G. Blackmond, *Nature* **2006**, *441*, 621 – 623.
[143] M. Klussmann, A. J. P. White, A. Armstrong, D. G. Blackmond, *Angew. Chem.* **2006**, *118*, 8153 – 8157; *Angew. Chem. Int. Ed.* **2006**, *45*, 7985 – 7989.
[144] A. I. Kitaigorodski, *Molekülkristalle*, Akademie-Verlag, Berlin, **1979**.
[145] A. I. Kitaigorodsky, *Mixed Crystals*, Springer, Berlin, **1984** (Springer Series in Solid-State Sciences 33).
[146] H. Lorenz in *Crystallization: Basic Concepts and Industrial Applications* (Ed.: W. Beckmann), Wiley-VCH, Weinheim, **2013**, pp. 35 – 74.
[147] D. Polenske, H. Lorenz, A. Seidel-Morgenstern, *J. Pharm. Sci.* **2010**, *99*, 1762 – 1773.
[148] N. Wermester, E. Aubin, M. Pauchet, S. Coste, G. Coquerel, *Tetrahedron: Asymmetry* **2007**, *18*, 821 – 831.
[149] H. Kaemmerer, H. Lorenz, S. Black, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2009**, *9*, 1851 – 1862.
[150] Z. J. Li, D. J. W. Grant, *Int. J. Pharm.* **1996**, *137*, 21 – 31.
[151] a) H. Kaemmerer, H. Lorenz, A. Seidel-Morgenstern, *Chem. Ing. Tech.* **2009**, *81*, 1955 – 1965; b) H. Kaemmerer, *New Concepts for Enantioselective Crystallization*, Shaker, Aachen, **2012**; c) H. Lorenz, T. Le Minh, H. Kaemmerer, H. Buchholz, A. Seidel-Morgenstern, *Chem. Eng. Res. Design*, **2013**, *91*, 1890 – 1902.
[152] H. Lorenz, H. Kaemmerer, D. Polenske, A. Seidel-Morgenstern (Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.), US 2011/0263896 A1, 27.10., **2011**.
[153] H. Lorenz, D. Sapoundjiev, A. Seidel-Morgenstern, *Eng. Life Sci.* **2003**, *3*, 132 – 136.
[154] D. Sapoundjiev, H. Lorenz, A. Seidel-Morgenstern, *J. Chem. Eng. Data* **2006**, *51*, 1562 – 1566.
[155] K. Petruševska-Seebach, K. Würges, A. Seidel-Morgenstern, S. Lütz, M. P. Elsner, *Chem. Eng. Sci.* **2009**, *64*, 2473 – 2482.

[156] D. Polenske, H. Lorenz, *J. Chem. Eng. Data* **2009**, *54*, 2277 – 2280.
[157] H. Kaemmerer, A. Seidel-Morgenstern, H. Lorenz, *Chem. Eng. Process.* **2013**, *67*, 80 – 88.
[158] H. Kaemmerer, S. K. Tulashie, H. Lorenz, A. Seidel-Morgenstern, *J. Chem. Eng. Data* **2010**, *55*, 1131 – 1136.
[159] S. K. Tulashie, H. Lorenz, A. Seidel-Morgernstern, *Proceedings 15th Int. Workshop on Industrial Crystallization (BIWIC 2008)* (Eds.: H. Lorenz, H. Kaemmerer), Shaker, Aachen, **2008**, pp. 192 – 196 ISBN 978-3-8322-7505-1.
[160] H. Lorenz, D. Sapoundjiev, A. Seidel-Morgenstern, *J. Chem. Eng. Data* **2002**, *47*, 1280 – 1284.
[161] H. Lorenz, A. Seidel-Morgenstern, *Thermochim. Acta* **2004**, *415*, 55 – 61.
[162] S. K. Tulashie, H. Kaemmerer, H. Lorenz, A. Seidel-Morgenstern, *J. Chem. Eng. Data* **2010**, *55*, 333 – 340.
[163] T. Le Minh, J. von Langermann, H. Lorenz, A. Seidel-Morgenstern, *J. Pharm. Sci.* **2010**, *99*, 4084 – 4095.
[164] N. Taratin, H. Lorenz, E. N. Kotelnikova, A. E. Glikin, A. Galland, V. Dupray, G. Coquerel, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2012**, *12*, 5882 – 5888.
[165] J. Worlitschek, M. Bosco, M. Huber, V. Gramlich, M. Mazzotti, *Helv. Chim. Acta* **2004**, *87*, 279 – 291.
[166] D. Polenske, H. Lorenz, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2007**, *7*, 1628 – 1634.
[167] H. Kaemmerer, M. J. Jones, H. Lorenz, A. Seidel-Morgenstern, *Fluid Phase Equilib.* **2010**, *296*, 192 – 205.
[168] H. Kaemmerer, Z. Horvath, J. W. Lee, M. Kaspereit, R. Arnell, M. Hedberg, B. Herrschend, M. J. Jones, K. Larson, H. Lorenz, A. Seidel-Morgenstern, *Org. Process Res. Dev.* **2012**, *16*, 331 – 342.
[169] J. von Langermann, M. Kaspereit, M. Shakeri, H. Lorenz, M. Hedberg, M. J. Jones, K. Larson, B. Herrschend, R. Arnell, E. Temmel, J.-E. Bäckvall, A. Kienle, A. Seidel-Morgenstern, *Org. Process Res. Dev.* **2012**, *16*, 343 – 352.
[170] H. Lorenz, J. von Langermann, G. Sadiq, C. S. Seaton, R. J. Davey, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2011**, *11*, 1549 – 1556.
[171] J. von Langermann, T. Le Minh, H. Lorenz, A. Seidel-Morgenstern, *Chem. Ing. Tech.* **2010**, *82*, 93 – 99.
[172] A. A. Rodrigo, H. Lorenz, A. Seidel-Morgenstern, *Chirality* **2004**, *16*, 499 – 508.
[173] H. Lorenz, A. Perlberg, D. Sapoundjiev, M. P. Elsner, A. Seidel-Morgenstern, *Chem. Eng. Process.* **2006**, *45*, 863 – 873.
[174] F. Czapla, D. Polenske, L. Klukas, H. Lorenz, A. Seidel-Morgenstern, *Chem. Eng. Process.* **2010**, *49*, 22 – 28.
[175] D. Polenske, Dissertation, Otto-von-Guericke-Universität, Magdeburg, Germany, **2010**.
[176] a) F. Czapla, H. Haida, M. P. Elsner, H. Lorenz, A. Seidel-Morgenstern, *Chem. Eng. Sci.* **2009**, *64*, 753 – 763; b) F. Czapla, H. Lorenz, A. Seidel-Morgenstern, *Chem. Ing. Tech.* **2009**, *81*, 839 – 848.
[177] T. Menahem, Y. Mastai, *J. Polym. Sci. Part A* **2006**, *44*, 3009 – 3017.
[178] a) M. P. Elsner, G. Ziomek, A. Seidel-Morgenstern, *Chem. Eng. Sci.* **2007**, *62*, 4760 – 4769; b) M. P. Elsner, G. Ziomek, A. Seidel-Morgenstern, *Chem. Eng. Sci.* **2011**, *66*, 1269 – 1284; c) G. Levilain, M. Eicke, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2012**, *12*, 5396 – 5401; d) M. J. Eicke, G. Levilain, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2013**, *13*, 1638 – 1648.
[179] J. E. F. Reynolds, *Martindale: The Extra Pharmacopoeia*, *30th ed.*, The Pharmaceutical Press, London, **1993**.
[180] *Ullmann's Encyclopedia of Industrial Chemistry* (Ed.: B. Elvers), VCH, Weinheim, **1989**.
[181] Y. Yamazaki, S. Kajiwara, *Bioindustry* **1988**, *5*, 261 – 268.
[182] A. M. Rouhi, *Chem. Eng. News* **2004**, *82*, 47 – 62.
[183] G. J. Quallich, *Chirality* **2005**, *17*, S120 – S126.
[184] Y. Lu, X. Wang, C. B. Ching, *Ind. Eng. Chem. Res.* **2009**, *48*, 7266 – 7275.
[185] L. Gou, H. Lorenz, A. Seidel-Morgenstern, *Cryst. Growth Des.* **2012**, *12*, 5197 – 5202.
[186] J. Falbe, M. Regitz, *Römpp Lexikon Chemie*, *Vol. 4*, *10th ed.*, Georg Thieme, Stuttgart, **1998**.
[187] Y. Wang, R. LoBrutto, R. W. Wenslow, I. Santos, *Org. Process Res. Dev.* **2005**, *9*, 670 – 676.
[188] B. G. Bag, *Curr. Sci.* **1995**, *68*, 279 – 288.
[189] B. Baldeyrou, C. Tardy, C. Bailly, P. Colson, C. Houssier, F. Charmantray, M. Demeunynck, *Eur. J. Med. Chem.* **2002**, *37*, 315 – 322.
[190] S. H. Wilen, J. Z. Qi, P. G. Williard, *J. Org. Chem.* **1991**, *56*, 485 – 487.
[191] M. Amanullah, M. Mazzotti, *J. Chromatogr. A* **2006**, *1107*, 36 – 45.
[192] R. W. Souter, *Chromatographic Separation of Stereoisomers*, CRC, Boca Raton, **1985**.
[193] M. Zief, L. J. Crane, *Chromatographic Chiral Separations*, Marcel Dekker, New York, **1988**.
[194] *Chiral Separations by HPLC* (Ed.: A. M. Krstulovic), Ellis Horwood, New York, **1989**.
[195] S. G. Allenmark, *Chromatographic Enantioseparation: Methods and Applications*, Ellis Horwood, Chichester, **1991**.
[196] *A Practical Approach to Chiral Separations by Liquid Chromatography* (Ed.: G. Subramanian), VCH, Weinheim, **1994**.
[197] T. E. Beesley, R. P. W. Scott, *Chiral Chromatography*, Wiley, Chichester, **1998**.
[198] H. Y. Aboul-Enein, A. Imran, *Chiral Separations by Liquid Chromatography and Related Technologies*, Marcel Dekker, New York, **2003**.
[199] *Preparative Enantioselective Chromatography* (Ed.: G. B. Cox), Blackwell, Oxford, **2005**.
[200] M. Lämmerhofer, N. M. Maier, W. Lindner, *Introduction to Modern Liquid Chromatography*, (Eds.: L. R. Snyder, J. J. Kirkland, J. W. Dolan), 3rd ed., Wiley, Hoboken, **2010**, pp. 665 – 724.
[201] Special Issues on "Chiral Separations", B. Chankvetadze, *J. Chromatogr. A* **2001**, *906*, 1 – 481; **2010**, *1217*, 925 – 1182; **2012**, *1269*, 1 – 387.
[202] T. E. Beesley, *Review of Chiral Stationary Phase Development and Chiral Applications*, *Vol. 24*, LC-GC Europe, **2011**, pp. 270 – 276.
[203] E. Gil-Av, B. Feibush, R. Charles-Sigler, *Tetrahedron Lett.* **1966**, 1009 – 1015.
[204] V. Schurig, *Inorg. Chem.* **1972**, *11*, 736 – 738.
[205] W. A. König, *Chromatographia* **1976**, *9*, 72 – 73.
[206] H. Frank, G. J. Nicholson, E. Bayer, *J. Chromatogr. Sci.* **1977**, *15*, 174.
[207] *Introduction to Modern Liquid Chromatography* (Eds.: L. R. Snyder, J. J. Kirkland, J. W. Dolan), 3rd. ed., Wiley, Hoboken, **2010**.
[208] M. S. Villeneuve, L. A. Miller, *Preparative Enantioselective Chromatography* (Ed.: G. B. Cox), Blackwell, Oxford, **2005**, pp. 205 – 223.
[209] K. De Klerck, G. Parewyck, D. Mangelings, Y. Vander Heyden, *J. Chromatogr. A* **2012**, *1269*, 336 – 345.
[210] K. Unger, R. Ditz, E. Machtejevas, R. Skudas, *Angew. Chem.* **2010**, *122*, 2350 – 2363; *Angew. Chem. Int. Ed.* **2010**, *49*, 2300 – 2312.
[211] M. Lämmerhofer, *J. Chromatogr. A* **2010**, *1217*, 814 – 856.
[212] C. Pettersson, *J. Chromatogr. A* **1984**, *316*, 553 – 567.
[213] V. A. Davankov, A. A. Kurganov, A. S. Bochkov, *Adv. Chromatogr.* **1983**, *22*, 71.
[214] G. Guiochon, B. Lin, *Modeling for Preparative Chromatography*, Elsevier, Amsterdam, **2003**.

[215] *Preparative Chromatography* (Eds.: H. Schmidt-Traub, M. Schulte, A. Seidel-Morgenstern), 2nd ed., Wiley-VCH, Weinheim, **2012**.
[216] G. Guiochon, A. Felinger, D. G. Shirazi, A. M. Katti, *Fundamentals of Preparative and Nonlinear Chromatography*, Elsevier, Amsterdam, **2006**.
[217] M. Negawa, F. Shoji, *J. Chromatogr.* **1992**, *590*, 113 – 117.
[218] D. B. Broughton, C. G. Gerhold, US Patent 2 985 589, **1961**.
[219] E. R. Francotte, *J. Chromatogr. A* **2001**, *906*, 379 – 397.
[220] E. Francotte in *Preparative Enantioselective Chromatography* (Ed.: G. B. Cox), Blackwell, Oxford, **2005**, pp. 48 – 77.
[221] C. E. Dalglish, *J. Chem. Soc.* **1950**, *132*, 3940.
[222] K. B. Lipkowitz in *A Practical Approach to Chiral Separations by Liquid Chromatography* (Ed.: G. Subramanian), VCH, Weinheim, **1994**, pp. 19 – 56.
[223] C. Zhao, N. M. Cann, *J. Chromatogr. A* **2007**, *1149*, 197 – 218.
[224] a) H.-W. Tsui, J. N. Willin, R. B. Kasat, N.-H. L. Wang, E. I. Franses, *J. Phys. Chem. B* **2011**, *115*, 12785 – 12800; b) S. Ravichandran, J. R. Collins, N. Singh, I. W. Wainer, *J. Chromatogr. A* **2012**, *1269*, 218 – 225.
[225] I. W. Wainer, *TrAC Trends Anal. Chem.* **1987**, *6*, 125 – 134.
[226] G. Hesse, R. Hagel, *Justus Liebigs Ann. Chem.* **1976**, 996 – 1008.
[227] Y. Okamoto, M. Kawashima, K. Hatada, *J. Am. Chem. Soc.* **1984**, *106*, 5357 – 5359.
[228] T. Zhang, P. Franco in *Chiral Separation Techniques. A Practical Approach* (Ed.: G. Subramanian), 3rd ed., Wiley-VCH, Weinheim, **2007**, pp. 99 – 134.
[229] B. Chankvetadze, *J. Chromatogr. A* **2012**, *1269*, 26 – 51.
[230] J. N. Kinkel in *A Practical Approach to Chiral Separations by Liquid Chromatography* (Ed.: G. Subramanian), VCH, Weinheim, **1994**, pp. 217 – 278.
[231] S. Anderson, S. Allenmark, *J. Chromatogr. A* **1994**, *666*, 167 – 179.
[232] J. Haginaka, *J. Chromatogr. B* **2008**, *875*, 12 – 19.
[233] a) D. Armstrong, W. DeMond, *J. Chromatogr. Sci.* **1984**, *22*, 411; b) D. W. Armstrong, T. J. Ward, R. D. Armstrong, T. E. Beesley, *Science* **1986**, *232*, 1132 – 1135.
[234] Y. Xiao, S.-C. Ng, T. T. Tan, Y. Wang, *J. Chromatogr. A* **2012**, *1269*, 52 – 68.
[235] a) D. W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J.-R. Chen, *Anal. Chem.* **1994**, *66*, 1473 – 1484; b) D. W. Armstrong, Y. liu, K. H. Ekborgott, *Chirality* **1995**, *7*, 474 – 497; c) Z. Pataj, I. Ilisz, R. Berkecz, A. Misicka, D. Tymecka, F. Fülöp, D. W. Armstrong, A. Péter, *J. Sep. Sci.* **2008**, *31*, 3688 – 3697.
[236] L. R. Sousa, G. G. Y. Sogah, D. H. Hoffmann, D. J. Cram, *J. Am. Chem. Soc.* **1978**, *100*, 4569 – 4576.
[237] W. H. Pirkle, M. H. Hyun, A. Tsiporous, B. C. Hampe, B. Banks, *J. Pharm. Biomed. Anal.* **1984**, *2*, 173 – 181.
[238] W. H. Pirkle, C. J. Welch, *Tetrahedron: Asymmetry* **1994**, *5*, 777 – 780.
[239] M. Lämmerhofer, W. Lindner, *J. Chromatogr. A* **1996**, *741*, 33 – 48.
[240] C. V. Hoffmann, M. Lämmerhofer, W. Lindner, *J. Chromatogr. A* **2007**, *1161*, 242 – 251.
[241] V. A. Davankov, *Enantiomer* **2000**, *5*, 209 – 223.
[242] G. Gübitz, M. G. Schmidt in *Chiral Separation Techniques. A Practical Approach* (Ed.: G. Subramanian), 3rd ed., Wiley-VCH, Weinheim, **2007**, pp. 155 – 180.
[243] P. Franco, T. Zhang, *LC-GC Europe* **2010**, *23*, 302 – 309.
[244] R. Sancho, C. Minguillon, *Chem. Soc. Rev.* **2009**, *38*, 797 – 805.
[245] *Preparative and Production Scale Chromatography* (Eds.: G. Ganetsos, P. E. Barker), Marcel Dekker, New York, **1993**.
[246] *Process Scale Liquid Chromatography* (Ed.: G. Subramanian), VCH, Weinheim, **1995**.
[247] C. M. Grill, L. M. Miller in *Preparative Enantioselective Chromatography* (Ed..: G. B. Cox), Blackwell, Oxford, **2005**, pp. 149 – 175.
[248] G. Cox in *Introduction to Modern Liquid Chromatography* (Eds.: L. R. Snyder, J. J. Kirkland, J. W. Dolan), 3rd ed., Wiley, Hoboken, **2010**, pp. 725 – 755.
[249] C. J. Welch in *Preparative Enantioselective Chromatography* (Ed.: G. B. Cox), Blackwell, Oxford, **2005**, pp. 1 – 18.
[250] A. Seidel-Morgenstern, *J. Chromatogr. A* **2004**, *1037*, 255 – 272.
[251] C. Heuer, P. Hugo, G. Mann, A. Seidel-Morgenstern, *J. Chromatogr. A* **1996**, *752*, 19 – 29.
[252] T. Sainio, M. Kaspereit, *Sep. Purif. Technol.* **2009**, *66*, 9 – 18.
[253] D. B. Broughton, *Sep. Sci. Technol.* **1984**, *19*, 723 – 736.
[254] J. A. Johnson, R. G. Kabza in *Preparative and Production-scale Chromatography* (Eds.: G. Ganetsos, P. E. Barker), Marcel Dekker, New York, **1993**, pp. 257 – 271.
[255] E. R. Francotte, P. Richert, *J. of Chromatogr. A* **1997**, *769*, 101 – 107.
[256] M. Hamende in *Preparative Enantioselective Chromatography* (Ed.: G. Cox), Blackwell, Oxford, **2005**, pp. 253 – 276.
[257] O. Dapremont in *Preparative nantioselective Chromatography* (Ed.: G. Cox), Blackwell, Oxford, **2005**, pp. 277 – 302.
[258] M. Juza, M. Mazzotti, M. Morbidelli, *Trends Biotechnol.* **2000**, *18*, 108 – 118.
[259] M. Schulte, J. Strube, *J. Chromatogr. A* **2001**, *906*, 399 – 416.
[260] P. Sá Gomes, M. Minceva, A. E. Rodrigues, *Adsorption* **2006**, *12*, 375 – 392.
[261] P. S. Gomes, M. Minceva, L. S. Pais, A. E. Rodrigues, *Chiral Separation Techniques. A Practical Approach* (Ed.: G. Subramanian), 3rd ed., Wiley-VCH, Weinheim, **2007**, pp. 181 – 202.
[262] A. Rajendran, G. Paredes, M. Mazzotti, *J. Chromatogr. A* **2009**, *1216*, 709 – 738.
[263] M. Kaspereit in *Advances in Chromatography* (Eds.: E. Grushka, N. Grinberg), Taylor & Francis, CRC, Boca Raton, **2009**, pp. 165 – 192.
[264] H. K. Rhee, R. Aris, N. R. Amundson, *First-order Partial-differential Equations*, *Vol. 1,2*, Prentice Hall, Englewood Cliffs, **1986** and **1989**.
[265] M. Mazzotti, G. Storti, M. Morbidelli, *J. Chromatogr. A* **1997**, *769*, 3 – 24.
[266] C. Migliorini, M. Mazzotti, M. Morbidelli, *AIChE J.* **2000**, *46*, 1384 – 1399.
[267] A. Seidel-Morgenstern, L. C. Kessler, M. Kaspereit, *Chem. Eng. Technol.* **2008**, *31*, 826 – 837.
[268] L. C. Kessler, A. Seidel-Morgenstern, *J. Chromatogr. A* **2008**, *1207*, 55 – 71.
[269] R. C. R. Rodrigues, R. J. S. Silva, J. P. B. Mota, *J. Chromatogr. A* **2010**, *1217*, 3382 – 3391.
[270] S. Katsuo, M. Mazzotti, *J. Chromatogr. A* **2010**, *1217*, 1354 – 1361.
[271] G. Terfloth, *J. Chromatogr. A* **2001**, *906*, 301 – 307.
[272] A. Rajendran, S. Peper, M. Johannsen, M. Mazzotti, M. Morbidelli, G. Brunner, *J. Chromatogr. A* **2005**, *1092*, 55 – 64.
[273] S. Abel, M. Juza, *Chiral Separation Techniques. A Practical Approach* (Ed.: G. Subramanian), 3rd ed., Wiley-VCH, Weinheim, **2007**, pp. 203 – 273.
[274] *Advances in Large-scale Biopharmaceutical Manufacturing and Scale-up Production* (Ed.: E. S. Langer), 2nd ed., ASM, Herndon, **2007**.
[275] M. Kaspereit, P. Jandera, M. Skavrada, A. Seidel-Morgenstern, *J. Chromatogr. A* **2002**, *944*, 249 – 262.
[276] M. Kaspereit, *Separation of Enantiomers by a Process Combination of Chromatography and Crystallization*, Shaker, Aachen, **2006**.
[277] Results of calculations provided by Prof. Malte Kaspereit, extending Ref. [276].
[278] G. Hesse, R. Hagel, *Chromatographia* **1973**, *6*, 277 – 280.
[279] G. Blaschke, *J. Liq. Chromatogr.* **1986**, *9*, 341 – 368.
[280] M. Pedeferri, G. Zenoni, M. Mazzotti, M. Morbidelli, *Chem. Eng. Sci.* **1999**, *54*, 3735 – 3748.

[281] C. Suteuphy in *Preparative Enantioselective Chromatography* (Ed.: G. B. Cox), Blackwell, Oxford, **2005**, pp. 78 – 109.
[282] S. Sergeyev, F. Diederich, *Chirality* **2006**, *18*, 707 – 712.
[283] D. Didier, S. Sergeyev, *Targets Heterocycl. Syst.* **2007**, *11*, 258 – 283.
[284] S. Sergeyev, *Helv. Chim. Acta* **2009**, *92*, 415 – 444.
[285] J. N. Kinkel, K. Reichert, P. Knöll, *GIT* **1998**, Supplement 3/89-Chromatographie, 104.
[286] R. Isaakson, P. Erlandsson, L. Hansson, A. Holmber, S. Berner, *J. Chromatogr. A* **1990**, *498*, 257 – 280.
[287] A. Seidel-Morgenstern, G. Guiochon, *Chem. Eng. Sci.* **1993**, *48*, 2787 – 2797.
[288] T. L. Hill, *An Introduction to Statistical Thermodynamics*, Addison-Wesley, Reading, **1960**.
[289] A. Seidel-Morgenstern, G. Guiochon, *J. Chromatogr. A* **1993**, *631*, 37 – 47.
[290] S. Jacobson, A. Seidel-Morgenstern, G. Guiochon, *J. Chromatogr. A* **1993**, *637*, 13 – 18.
[291] A. Seidel-Morgenstern, S. Jacobson, G. Guiochon, *J. Chromatogr. A* **1993**, *637*, 19 – 28.
[292] C. J. Radke, J. M. Prausnitz, *AIChE J.* **1972**, *18*, 761 – 768.
[293] A. Seidel-Morgenstern, *Mathematische Modellierung der präparativen Flüssigchromatographie*, Deutscher Universitätsverlag, Wiesbaden, **1995**.
[294] K. Mihlbachler, A. Seidel-Morgenstern, G. Guiochon, *J. Chromatogr. A* **2002**, *955*, 35 – 52.
[295] K. Mihlbachler, M. A. De Jesús, K. Kaczmarski, M. J. Sepaniak, A. Seidel-Morgenstern, G. Guiochon, *J. Chromatogr. A* **2006**, *1113*, 148 – 161.
[296] K. Mihlbachler, A. Seidel-Morgenstern, G. Guiochon, *AIChE J.* **2004**, *50*, 611 – 624.
[297] S. Katsuo, M. Mazzotti, *J. Chromatogr. A* **2010**, *1217*, 3067 – 3075.
[298] K. Petrusevska, M. A. Kuznetsov, K. Gedicke, V. Meshko, S. M. Staroverov, A. Seidel-Morgenstern, *J. Sep. Sci.* **2006**, *29*, 1447 – 1457.
[299] G. F. Gauze, M. G. Brazhnikova, A. V. Laiko, M. A. Sveshnikova, T. P. Preobrazhenskaia, *Antibiot. Med. Biotechnol.* **1987**, *32*, 571 – 576.
[300] L. Zhang, K. Gedicke, M. A. Kuznetsov, S. M. Staroverov, A. Seidel-Morgenstern, *J. Chromatogr. A* **2007**, *1162*, 90 – 96.
[301] B.-G. Lim, C.-B. Ching, R. B. H. Tan, S.-C. Ng, *Chem. Eng. Sci.* **1995**, *50*, 2289 – 2298.
[302] J. Blehaut, R.-M. Nicoud, *Anal. Magazine* **1998**, *26*, M60 – M70.
[303] H. Lorenz, P. Sheehan, A. Seidel-Morgenstern, *J. Chromatogr. A* **2001**, *908*, 201 – 214.
[304] M. Kaspereit, K. Gedicke, V. Zahn, A. W. Mahoney, A. Seidel-Morgenstern, *J. Chromatogr. A* **2005**, *1092*, 43 – 54.
[305] M. Kaspereit, S. Swernath, A. Kienle, *Org. Process Res. Dev.* **2012**, *16*, 353 – 363.
[306] K. Y. Fung, K. M. Ng, C. Wibowo, *Ind. Eng. Chem. Res.* **2005**, *44*, 910 – 921.
[307] K. Gedicke, W. Beckmann, A. Brandt, D. Sapoundjiev, H. Lorenz, U. Budde, A. Seidel-Morgenstern, *Adsorption* **2005**, *11*, 591 – 596.
[308] K. Gedicke, M. Kaspereit, W. Beckmann, U. Budde, H. Lorenz, A. Seidel-Morgenstern, *Chem. Eng. Res. Des.* **2007**, *85*, 928 – 936.
[309] a) L. Gou, S. Robl, K. Leonhard, H. Lorenz, M. Sordo, A. Butka, S. Kesselheim, M. Wolff, A. Seidel-Morgenstern, K. Schaber, *Chirality* **2011**, *23*, 118 – 127; b) S. Robl, L. Gou, A. Gere, M. Sordo, H. Lorenz, A. Mayer, C. Pauls, K. Leonhard, A. Bardow, A. Seidel-Morgenstern, K. Schaber, *Chem. Eng. Process.* **2013**, *67*, 80 – 88.
[310] S. R. Perrin, W. Hauck, E. Ndzie, J. Blehaut, O. Ludemann-Hombourger, R.-M. Nicoud, W. H. Pirkle, *Org. Process Res. Dev.* **2007**, *11*, 817 – 824.
[311] V. Meyer in *Chiral Separations by Liquid Chromatography* (Ed.: S. Ahuja), ACS Symposium Series, Washington, **1991**, pp. 217 – 227.
[312] a) I. Ali, V. K. Gupta, H. Y. Aboul-Enein, P. Singh, B. Sharma, *Chirality* **2007**, *19*, 453 – 463; b) E. J. Ebbers, G. J. A. Ariaans, J. P. M. Houbiers, A. Bruggink, B. Zwanenburg, *Tetrahedron* **1997**, *53*, 9417 – 9476; c) L. K. Thalén, D. Zhao, J.-B. Sortais, J. Paetzold, C. Hoben, J.-E. Bäckvall, *Chem. Eur. J.* **2009**, *15*, 3403 – 3410; d) T. Jerphagnon, A. J. A. Gayet, F. Berthiol, V. Ritleng, N. Mršić, A. Meetsma, M. Pfeffer, A. J. Minnaard, B. L. Feringa, J. de Vries, *Chem. Eur. J.* **2009**, *15*, 12780 – 12790; e) L. K. Thalén, N. Hermanns, J.-E. Bäckvall, *Tetrahedron Lett.* **2010**, *51*, 6802 – 6802.
[313] a) M. Bechtold, S. Makart, M. Heinemann, S. Panke, *J. Biotechnol.* **2006**, *124*, 146 – 162; b) S. Makart, M. Bechtold, S. Panke, *Chem. Eng. Sci.* **2008**, *63*, 5347 – 5355.
[314] U. Felfer, M. Goriup, M. F. Koegl, U. Wagner, B. Larissegger-Schnell, K. Faber, W. Kroutil, *Adv. Synth. Catal.* **2005**, *347*, 951 – 961.
[315] K. Würges, K. Petrusevska, S. Serci, S. Wilhelm, C. Wandrey, A. Seidel-Morgenstern, M. P. Elsner, S. Lütz, *J. Mol. Catal. B* **2009**, *58*, 10 – 16.

