Processes to separate enantiomers

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Short text for Table of Content

The progress achieved in the area of enantioseparation processes is reviewed focusing on enantioselective crystallization and preparative chromatography applied both individually or coupled. The incorporation of racemization steps is also considered.

The provision of pure enantiomers is of increasing importance not only for the pharmaceutical industry but also for agrochemistry and in biotechnology. In general there are two rivaling approaches to provide pure enantiomers. The chiral approach is based on developing an asymmetric synthesis of just one of the enantiomers. In contrast, the racemic approach is based on separating mixtures of the two enantiomers. In the last years remarkable progress has been achieved in the latter area. Below a review is given focusing in particular on enantioselective crystallization processes and preparative chromatography, including also hybrid processes and the incorporation of racemization steps. For illustration serve several examples studied in the laboratory of the authors. The manuscript illustrates the large potential for improved provision of pure enantiomers by joint efforts of synthesis chemistry and separation science.

1. Introduction

Enantiomers are stereoisomers that are non-superimposable mirror images of each other. They are designated by classical notations as (D) or (L), as (*R*) or (*S*) or as (+) or (-).[e.g. 1] There is still no final answer to the question, 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 activities.[e.g. 2-5] Independent of the outcome, there is a tremendous interest to produce pure enantiomers in the food and agrochemical industries, and in particular in the pharmaceutical industry.[6-8] Nowadays, there is clear evidence that in chiral drugs often only one enantiomer 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 optically pure form.[9-11] This has intensified efforts of industrial and academic research devoted to develop techniques which are capable to produce 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 or B) the "*racemic approach"* based on *separating mixtures* of the two enantiomers.[12-14]

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

Fermentation methods

Methods based on exploiting the selective natural metabolism of microorganisms are applied very successfully in a very large scale to produce optically pure amino acids, in particular as food additives. E.g. the production of L-Lysine by fermentation is realized exploiting genetically adapted cell lines of *Corynebacterium glutamicum.* There are many excellent reviews available

describing both the mechanisms of various successful biotransformations and related industrial applications.[15]

Chiral pool synthesis

In this easiest approach available chiral starting materials are manipulated through successive reactions using achiral reagents retaining chirality to obtain desired target molecules. This concept is especially attractive for target molecules having a chirality similar to a relatively inexpensive naturally occurring building block, such as e.g. 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 material, which should be sufficiently enantiopure and, thus, could be rather expensive.[e.g. 16]

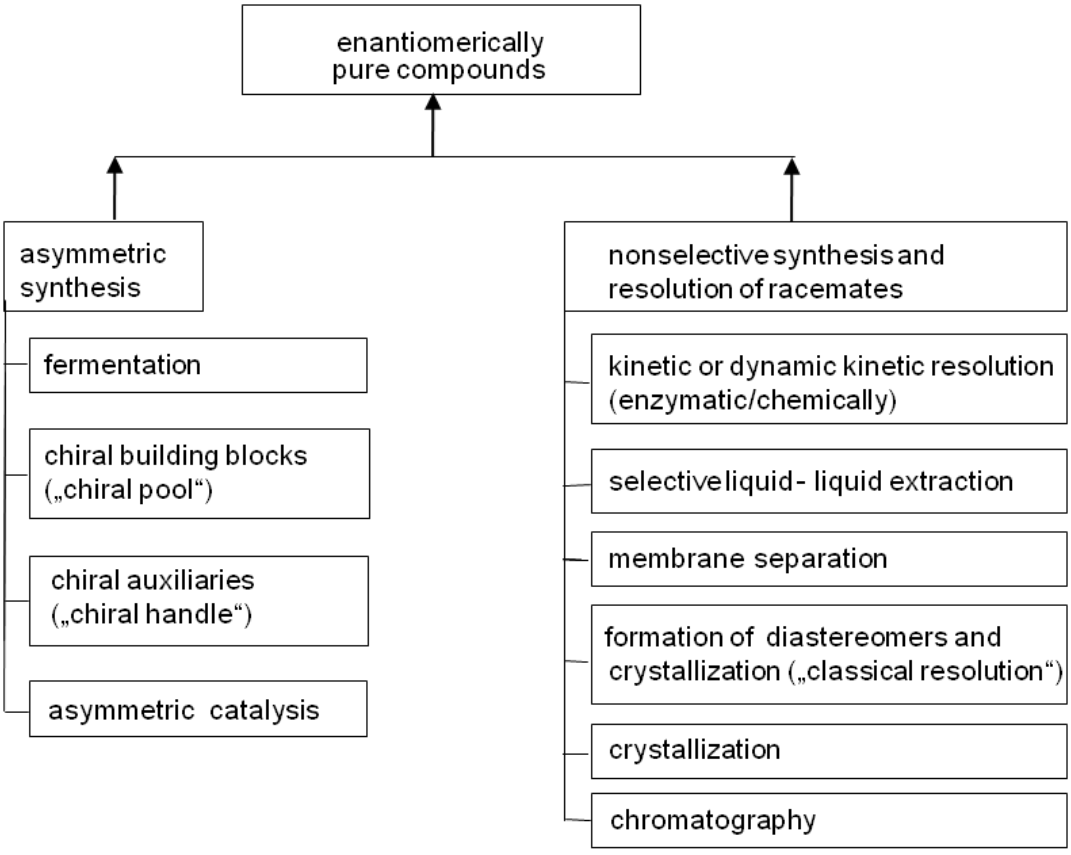

Figure 1: Illustration of possible pathways leading to pure enantiomers.

Use of chiral auxiliaries

What many strategies in asymmetric synthesis have in common is asymmetric induction to transform enantiomers into diastereomers, which have different reactivities. One asymmetric induction strategy is e.g. 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 path ways 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 (enzymatic processes)

The first methods were pioneered by William S. Knowles and Ryoji Noyori (Nobel Prize in Chemistry 2001) and Henri Kagan. Knowles replaced in 1968 the achiral triphenylphosphine ligands in Wilkinson's 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 resulting in an enantiomeric excess of 15%. The methodology was ultimately used in an asymmetric hydrogenation step in the industrial production of L-DOPA. In the same year Noyori[23] published his chiral ligand for a cyclopropanation reaction of styrene. In common with the observations Knowles made, the enantiomeric excess for this first generation ligand was with 6% still disappointingly low. A major breakthrough was achieved by Kagan in 1971/1972 developing 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 well the hydrogenation of functionalized alkenes on only one face of the molecule, 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 vicinaldiol (Sharpless dihydroxylation and aminohydroxylation).[27]

There are excellent reviews available summarizing the progess achieved in the fields of enantioselective organocatalysis[28]. Comprehensive overviews regarding the current status of asymmetric catalysis including also fermentation and biocatalysis give.[29,30] Various examples of successful industrial application are described. In some cases combinations of all three catalytic approaches mentioned are used in order to optimize productivities.

Nonselective synthesis and resolution of racemates

Considering the broad spectrum of desired target molecules, despite of tremendous progress achieved in the last decades, the number of highly selective reactions supplying pure enantiomers in industrially relevant scales is still limited. Consequently, there is a large 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 enantiomer separation techniques. Hereby the focus is set on techniques which could be applied in a preparative and industrial scale, i.e. the large arsenal of techniques developed for analytical purposes is not considered. The latter aspect is extensively discussed in various reviews and textbooks focusing mostly on analytical chromatography and electrophoresis.[31-33]

After shortly introducing in the next section important concepts which allow performing separations of enantiomers, a particular focus will be set on selective crystallization and chromatographic methods. Significant progress has been achieved regarding these two methods and they are currently seen as the most flexible and productive ones. Various concepts will be illustrated 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 capable to separate enantiomers

Due to their importance and difficulty the separation of enantiomers has been studied intensively in the last decades. Comprehensive overviews were given recently[35-39] and excellent descriptions of selected process concepts are available.[e.g. 31, 40]

Usually the provision of an initial asymmetry is needed to permit enantioseparation. In the various separation methods developed this is provided by specific chiral partners, which could be e.g. reactants, solvents, carriers or solid surfaces. If available, also some of the target enantiomers could be initially invested to recover 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 will 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. Based on this difference an excess of the less reactive enantiomer is

created, 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 was used successfully in the organic synthesis of various chiral molecules. It was first observed by Marckwald and McKenzie in 1899 in the esterification reaction of racemic mandelic acid with optically active (-)-menthol to a pair of diastereomeric esters.[41]In this reaction the (*R*)-enantiomer of mandelic acid displays 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 called dynamic kinetic resolution (DKR). It tackles the obvious 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 engage in a chemical equilibrium and exchange. In this way, the faster-reacting enantiomer is replenished in 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 is used successfully applying both enzymatic and chemical reactions. Several examples of enzymatic DKR processes are given in [44]. An overview on nonenzymatic kinetic resolution is provided in.[45]

The combination of kinetic resolution in ionic liquids (IL) 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 regarding product purity still restrict wider application of the attractive kinetic resolution approach.

Enantioselective liquid-liquid extraction

In general liquid-liquid extraction is a mature separation method that can be operated in a productive countercurrent mode to fractionate continuously racemates 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 to operate at different scales makes the use of liquid-liquid extraction interesting for enantioseparation.

There are many reports about successful enantioselective liquid-liquid extractions in a laboratory scale. Most of these studies deal with the identification and characterization of extraction systems. Hereby the most frequently used carriers are cyclodextrin derivatives,[e.g. 49] tartaric acid derivatives[e.g. 50, 51] and crown ethers.[e.g. 52] Metal complexes and metalloids have

been also applied successfully as reactive extractants.[53, 54] The resolution of racemic *N*-benzyl-α–amino acids using a chiral cobalt (III) salen complex was studied in[55]. Recently, successful attempts were reported combining the effects of two carriers.[56] A review regarding technological aspects was given in[57].

The development of efficient processes requires multistage schemes with back-extraction sections to recover the host. Many types of extraction columns are available with a broad range of internals 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 coworkers.[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[60]. Overviews regarding enantioselective liquid-liquid extraction were given recently in[61, 62]. 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 in order 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 introducing different possibilities are given in[63-65]. Membrane separations offer, in general, attractive options for clean, energy efficient, easy to perform and continuous operation.

Two basic types of membrane processes can be distinguished for the separation of racemic mixtures: a direct separation using enantioselective chiral membranes or a 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 rather immobilized by capillary and interfacial tension forces in a porous matrix (supported liquid membrane).[66, 67] An interesting concept suggested is based on a combination of countercurrent fractionation and liquid membrane technology and applies two liquids, which are oppositely chiralized by the addition of the (*R*)- or the (*S*)-enantiomer of a chiral selector and separated by another non-miscible liquid immobilized in a porous membrane.[68]A main disadvantage of liquid membrane systems is their instability over longer 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 were suggested.[e.g. 70-73] Alternatively, molecularly imprinted polymers,

i.e. polymers having enantiospecific cavities in their bulk phase, have been suggested as a basis for chiral membranes. During the polymer synthesis one of the enantiomers is used as a template.[74] A detailed characterization of enantioselective membranes applying various L-proline derivates as chiral carriers was given in.[75]

Regarding the application of nonselective achiral membranes there is significant progress in applying cheap and widely accessible ultrafiltration membranes. The selectivity is achieved by preferably creating in the feed phase micelles or complexes with enzymes formed with only one of the enantiomers. Then the separation is simply based on retaining the larger complexes and allowing permeation of the unbound enantiomer.[76-78]

In order to reach high purities, due to the limited separation factors, membrane processes have to be carried out typically in several stages.[79] General drawbacks of membrane technology, limiting currently 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[35,36]. Introduced are e.g. enantioselective foam flotation, preparative gel electrophoresis and distillation. Enantioselective distillation is discussed e.g. in[80]. However, all techniques mentioned and other alternatives have not yet acquired larger industrial relevance.

In the next two main sections of this paper we will concentrate in more detail on enantioselective crystallization and chromatography. Hereby, 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 of separating the sodium ammonium tartrate enantiomers by direct crystallization,[81] extensive activities have been devoted to study and to 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. To carry out crystallization, just standard equipment is required which is readily available in the pharmaceutical and fine chemical industries. Crystallization techniques are not restricted to resolution of racemates but can also be applied for further purification of non-racemic mixtures of enantiomers resulting from other techniques such as partially selective synthesis, chromatographic or membrane separation. Compared to asymmetric synthesis and chromatographic separations, crystallization processes often bear a "low-tech-image" or are considered sometimes as "out-of-date technology".

Though, when reviewing manufacturing methods applied to provide pure enantiomer drugs, it becomes obvious that the majority of drugs are produced via classical resolution, i.e. crystallization.[39] An overview on crystallization methods for resolution of racemates is given in the textbook of Jacques and coworkers.[82] A review on current patents in the field of optical resolution by crystallization methods with focus on amino acids has been published recently.[83]
In principle, Pasteur's technique of hand sorting crystals of the 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 solid-solid separation. The challenging task of developing such methods might be 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 at industrial scale and increasingly also the method of choice in the API section.[39] E.g., thousands of tons per year of (*S*)-naproxen, D-phenylglycine and D-4-hydroxyphenylglycine are produced via the formation and separation of diastereomeric salts, as reported in a "Highlights" article in Angew. Chem. Int. Ed. in 1998.[84] More recently launched drugs produced via classical resolution are Frovatriptan (2002), Duloxetine and Eszopiclone (both 2004).[39]
When racemization of the unwanted enantiomer is feasible, the low yield inherent in a diastereomeric resolution can be overcome offering significant increases in product amount and thus, process performance. Exemplarily, it is reported that implementing chemical racemization within the (*S*)-naproxen production can enhance the overall yield to ≥95%.[85] A more recent example of a so-called crystallization-induced diastereomeric transformation refers to the API Sertraline where a semi-continuous resolution-racemization process is performed.[86] Most frequently used acidic and basic resolving agents are natural L-tartaric acid, its derivatives such as L-/D-dibenzoyl- and L-/D-ditolouyltartaric acid, (*R*)-/(*S*)-mandelic acid and (+)-/(-)-α-methylbenzylamine.[39] The sometimes elaborate search for an optimal resolving agent that forms easily to separate diastereomeric salts with the compound to be resolved has clearly been improved by the introduction of a combinatorial approach.[87]Hereby, a family of resolving agents is simultaneously added to a solution of a racemate causing a rapid precipitation of a crystalline diastereomeric salt with high purity and yield. Since up to now the mechanism behind

this so-called “Dutch resolution” is not fully understood, work is 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 in a book contribution.[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[82, 91, 92]. Suggestions for the conceptual design of enantioselective crystallization processes implementing classical resolution and other alternatives are provided in [93].

Preferential crystallization of conglomerates

Direct crystallization of an enantiomer from a racemic solution is only feasible in case that the enantiomers in their mixtures form separate but pure crystals and, thus, the corresponding racemates are just conglomerates, i.e. mixtures of crystals of both enantiomers. However, only 5 to 10% of the chiral substances belong to this group of conglomerates.[94] For racemates of such substances preferential crystallization is an attractive technology that allows for direct crystallization of the desired enantiomer without needing any chiral auxiliary. Generally it can be distinguished between two techniques of direct crystallization, a) the entrainment process and b) simultaneous crystallization, both 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. Actually, it is a kinetically driven separation relying on different crystallization rates of the enantiomers in presence of homochiral seeds. The entrainment process is usually applied at smaller scales in a batch mode. Examples of industrial use refer particularly to the production of broad-spectrum antibiotics such as chloramphenicol, thiamphenicol and β-lactames.[94] For larger scales the second type, i.e. simultaneous crystallization, is an option. There 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 mode and has been industrially realized e.g. for the production of (-)-menthol (via the benzylester, Haarmann & Reimer, 1400 t a^{-1}) and an L-α-methyldopa intermediate (Merck, >100 t a^{-1}). Further, over a period of ten years Ajinomoto produced L-glutamic acid at about 13000 t a^{-1} by simultaneous crystallization.[82] From an industrial perspective, 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] There, in particular the application of fluidized bed crystallizers and the implementation of ultrasonic cleavage of seed particles to maintain the

population balance are emphasized. Also very recently, the advantages of preferential crystallization were proven for resolving racemic calcium pantothenate (with the (*R*)-enantiomer an important commercial precursor of vitamin B_5) and an industrially required resolving agent, both in lab and technical scale (35 L and 35 L) reaching high yields.[96, 97]

Detailed information on preferential crystallization can be found in several overview articles[e.g. 94, 98] and book contributions.[82, 99] Capabilities and limitations ("pitfalls and rewards") have just 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 stererochemistry"[101] and several review articles are devoted to that issue.[e.g. 101, 102] Efforts to systematically derive possible conglomerates among a group of derivatives of a chiral substance of interest were described by the group of Bredikhin.[e.g. 103, 104] Recently, the usage of the Second Harmonic Generation effect has been proven as an alternative and efficient prescreening technique for 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 group of Lahav and Leiserowitz.[e.g. 106-108] In a recent work[109] the authors describe a cyclic separation process in the presence of a tailor-made single-enantiomer polymer that causes differences in growth and dissolution between the enantiomers and thus assists in resolution.

In the last years different innovative process modifications have been suggested in order to enhance the performance of the classical isothermal process. These include amongst others polythermal crystallization procedures, auto-seeding strategies and innovative reactor concepts.[99, 110-117] Selected applications of preferential crystallization will be presented and discussed in chapter 3.3 below. There, advanced process concepts such as an auto-seeded polythermal process mode, first introduced by Coquerel and coworkers,[118] innovative crystallizer configurations and the extension of applying 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 work refers to enhancing yields in preferential crystallization by increasing the racemic mixture solubility via addition of appropriate modifiers.[121]

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

Attrition-enhanced deracemization (Viedma ripening). Attrition-enhanced deracemization is a just recently emerged technique based on pathbreaking work of Viedma[123] who discovered that a 1:1 mixture of enantiomorphous $NaClO_3$ crystals in contact with a saturated solution "deracemizes" when grinding the crystals in the suspension. This technique could be verified to be applicable in cases where the racemic substance to be resolved is 1) a conglomerate and 2) quickly racemizes in liquid state.[124] Just recently, 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 been reported.[129] Further it could be shown, that implementation of grinding and in-situ racemization in 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 applicability of preferential crystallization to racemic compounds are expected to increase the attractiveness of both techniques for industrial scale enantiomer separation.

Optically active solvents

A further method for resolution of racemates is the application of optically active solvents which might also be achiral solvents containing definite amounts of a pure enantiomer as solute. In principle, diastereomeric interactions can occur when enantiomers are dissolved in an optically active solvent. As a result these diastereomeric complexes should possess different physico-chemical 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 of enantioseparation since the beginning of the 20th century, only a few studies are reported[82] and there is a lack of systematic studies allowing for generalizable results.

On the other hand, a couple of successful chiral resolutions employing kinetic effects for enantioselective crystallization with the help of optically active solvents have been described. For example, the resolution of racemic glutamic acid using a chiral solvent made from lysine and water was demonstrated.[131] Small amounts of L- or D-lysine were applied to retard the crystallization rate of the corresponding glutamic acid enantiomer leading to a transient optical resolution during crystallization. Additionally, 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 work has been done by applying tailor-made additives in kinetic resolutions as already mentioned above (e.g. [106] or more recently [134]). All examples given only

apply to conglomerate systems. Comprehensive studies performed in our group were devoted to evaluate both thermodynamic and kinetic effects of chiral solvents on enantioselective crystallization. Specifically chosen solvents were applied also to verify the feasibility of resolving racemic compounds.[135-138] Selected results will be depicted in more detail in chapter 3.3.2.

3.1 Solid-liquid equilibria and related possibilities for enantioseparation

The application of crystallization for 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 in 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 phase diagrams relevant with regard to crystallization-based enantioseparation are 1) the binary melt phase diagram of the two enantiomers describing the melting behavior in the binary system and 2) the ternary solubility phase diagram of the two enantiomers in a specific solvent depicting the solubility behavior of the enantiomers and their mixtures in presence of this solvent. Overviews regarding identified types of phase diagrams and their description have been published elsewhere.[82,94,139,140] Examples for systematic studies of binary and ternary phase diagrams are given in articles from the groups of Grant [e.g. 141] and Klussmann.[142, 143] To the knowledge of the authors, up to now there is no monograph available focusing mainly on phase equilibria of organic compounds. A comprehensive overview to 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]

The fundamental types of phase diagrams occurring in chiral systems are schematically shown in Fig. 2.[146] Generally, since the enantiomers exhibit identical physical properties as melting points, melting enthalpies and solubilities, both the binary melt phase diagrams and the ternary solubility phase diagrams (upper and lower parts of Fig. 2, resp.) 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 that arise from the particular characteristics of the crystalline racemate in the system and which have been firstly described in a pioneering work by Roozeboom.[140] In the 1st 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, what is the main requirement for preferential crystallization. However, as already mentioned, just 5 to 10% of the chiral substances belong to that group.[94]

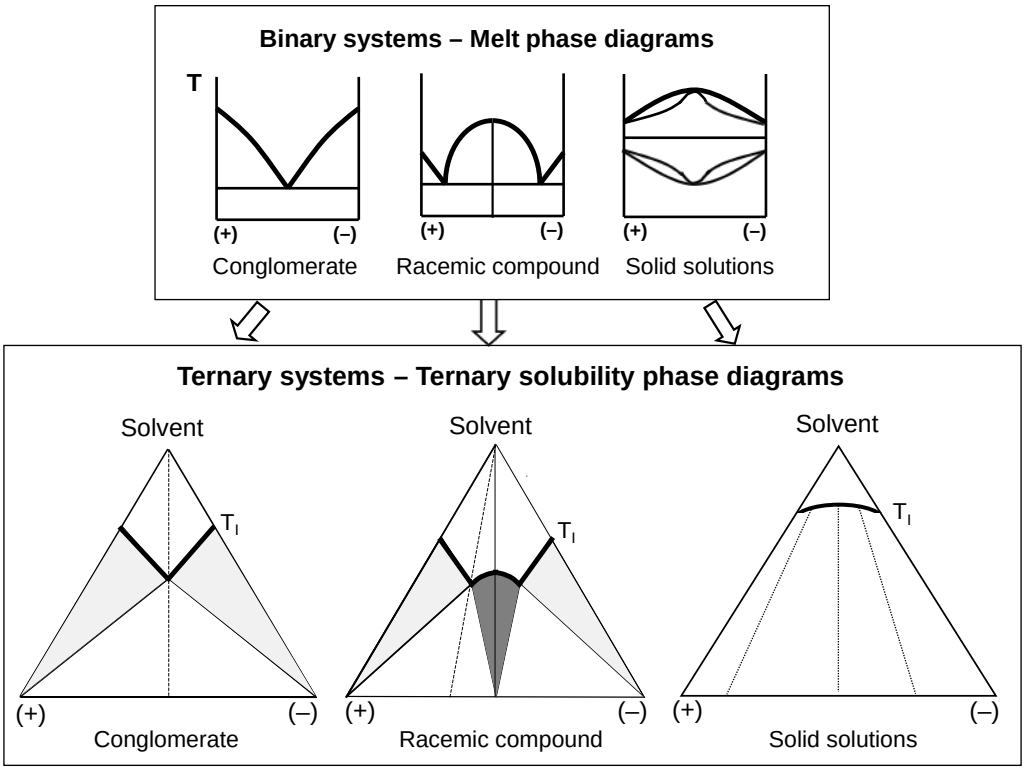

Figure 2: Fundamental types of binary melt phase diagrams and ternary solubility phase diagrams for chiral systems. The latter are represented as isothermal slices depicting the SLE at a certain temperature, T_I. Fat black lines mark related liquidus (saturation) lines. In the ternary phase diagram the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

In the 2nd case, i.e. when the enantiomers form an intermediate stoichiometric 1:1 compound, there is only one solid phase present, the racemic compound. The majority of chiral systems (90-95%) form racemic compounds. Here, from a thermodynamic point of view, direct crystallization is not feasible to provide pure enantiomers from the racemates. An important property of such systems is the composition of the two eutectics occurring 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 applicable to provide the desired pure enantiomer from enriched mixtures.

In the 3rd case, complete miscibility at solid state occurs, i.e. 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 from an enriched solution no pure enantiomer can be crystallized. Therefore, this type of systems 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 case of molecular crystals in general and enantiomers in particular (<1%). Not considered in Fig. 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 sparsely reported. Examples referring to partial solid solutions on the enantiomer side are given in [147, 148], cases depicting limited miscibility at solid state for the racemic compound in [149] and examples with partial solid solutions for both the enantiomers and the racemate in [150].

The ternary solubility phase diagrams shown in Fig. 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 vertical axis perpendicular to the prism base. Light grey areas represent the existence regions of the corresponding enantiomers, where in equilibrium these enantiomers as solid phases can be crystallized. The dark grey area relates to the existence region of the racemic compound, which is the solid phase crystallizing 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 below in chapter 3.3.2. Differing from the binary chiral systems, in the solubility phase diagrams solvates can occur as additional solid phases. For that 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.

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 work and requires a sufficient amount of substance which is often not available in an early stage of development. In addition, usually a combination of different analytical techniques is 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 already the type of the crystalline racemate and the location of the eutectic composition in the chiral system. Moreover, based on the melting data (temperature and heat of fusion) ideal solubilities can be calculated providing a first rough estimate of the solubility curve using classical thermodynamic equations (simplified Schröder-van Laar equation).[82, 146]

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

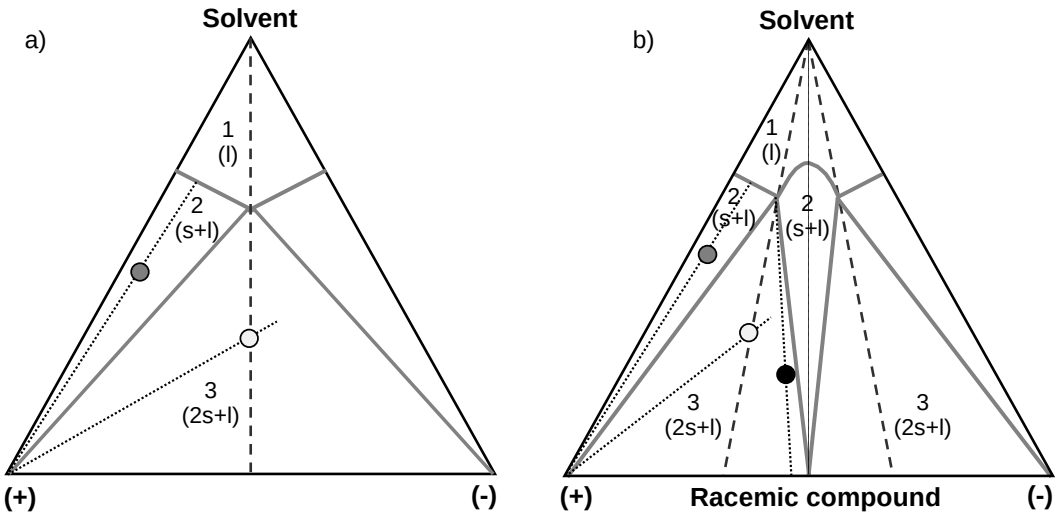

Figure 3: General possibilities of crystallization-based enantioseparation without a chiral auxiliary illustrated for a) conglomerates and b) racemic compound-forming systems. The numbers and states of aggregation of the phases present in equilibrium are depicted in the particular regions (s: solid;l: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

If there are already enantiomerically enriched mixtures of the enantiomers available (characterized by a composition in the existence region of the pure enantiomer, e.g. dark grey points in Fig. 3), the target enantiomer can directly be 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 for conglomerates both enantiomers, and for racemic compound-forming systems one enantiomer and the racemic compound 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 grey point in Fig. 3a), preferential crystallization can be applied. By adding seeds of just one enantiomer to a solution supersaturated with both antipodes, it is possible to selectively crystallize just the desired (seeded) enantiomer for a certain period of time. However, since in equilibrium both enantiomers are stable solid phases, this is a non-equilibrium kinetically driven process, where nucleation of the counter-enantiomer has to be avoided. As mentioned before, for resolution of racemates preferential crystallization 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 the eutectic one (e.g. light grey point in Fig. 3b).[111, 113] 2) Further, as indicated in Fig. 3b by the black point, an initial step might be applied exploiting enrichment in the liquid phase. Hereby just a slight initial enantiomeric enrichment is sufficient to provide a liquid phase, which under certain conditions allows for 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 in an integrated process via partially selective synthesis or a prior alternative separation step.

3.2 Illustration of selected melt and solution equilibria

In the last years in our group more than 30 chiral substances originating from the fields of fine chemicals (chemical intermediates, amino acids) and pharmaceuticals have been characterized by their binary and/or ternary phase diagrams, considering also diastereomeric salts. In Table 1 a selection of 14 chiral substances is compiled specifying the type of the crystalline racemate and the eutectic composition in the chiral system determined from the melt phase and ternary solubility phase diagrams. In some cases different solvents have been studied, e.g. for N-methylephedrine as a conglomerate and mandelic acid as a racemic compound-forming system, solubilities in eight andeleven, respectively, different achiral and chiral solvents were measured. Table 1 further contains thermodynamic information not acquired in our lab for Tröger's base 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, nine racemic compounds. Just threonine and the pharmaceutical intermediate A turned out to belong to the simplest type of phase diagrams, i.e. being a conglomerate and showing no polymorphs, solvates or solid solutions in 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 case of 3-chloromandelic acid for both the enantiomers and the racemic compound monotropic modifications exist.[163] For 2-chloromandelic acid (Table 2) a metastable conglomerate was found[170]. For the relatively simple compound malic acid an additional monotropic polymorph of the racemic compound as well as partial miscibility of the enantiomer and the racemic compound were observed. 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 in many investigations not considered.

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

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	Refe-rences
Amino acids[a]					
Threonine	C	-	0.5 (w, w/EtOH)	-	[153, 154]
Asparagine	C	-	0.5 (w)	C: 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 (API)					
Compound A	C	0.5	0.5 (acetonitrile)	-	[153]
Propranolol hydro-chloride	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 (just given for $x \geq 0.5$); EtOH: ethanol; MeOH: methanol; w: water; ss: solid solutions

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

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

3.3 Demonstration of various concepts of crystallization-based enantioseparation

Below classical and innovative crystallization processes capable to separate enantiomers will be illustrated using four of the compounds listed in Table 1 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 results given below have been collected in our laboratory in the last decade.

3.3.1 Conglomerates

Among the four compounds considered, threonine is the only one that belongs to the group of conglomerates. Threonine, an essential amico acid, occurs in nature as the (2*S,* 3*R*)-form designated as L-threonine. Since it contains two chiral centres, four stereoisomers are possible, 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 Fig. 4.

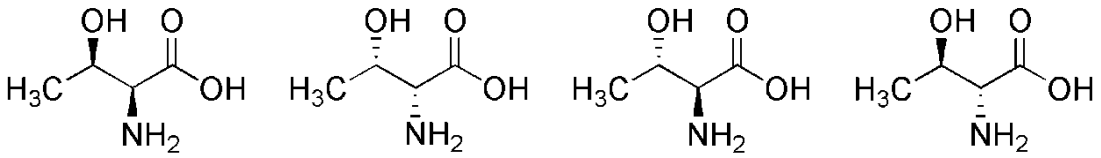

Figure 4: L-(2*S,*3*R*)-(-)-threonine, D-(2*R,*3*S*)-(+)-threonine, L-(2*S,*3*S*)-(+)-*allo*-threonine, D-(2*R,*3*R*)-(-)-*allo*-threonine

Separations will be demonstrated for the example of the L- and D-threonine pair. The ternary solubility phase diagram with water as solvent is presented in Fig. 5 providing 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. Further, solubility isotherms run almost parallel to the opposite triangle sides, i.e. the solubility of one enantiomer is not affected by the presence of the counter-enantiomer which indicates close to ideal solution behavior.

In Fig. 6 is illustrated the principle of preferential crystallization for conglomerates. Starting from a racemic composition in the three-phase region of the phase diagram (black point in Fig. 6a), in presence of homochiral seeds (here the (-)-enantiomer), it is possible to crystallize just the seeded enantiomer for a certain period of time. The crystallization trajectory is indicated by an arrow. When finally reaching the metastable solubility line of the crystallizing enantiomer, 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 providing both enantiomers in a periodic manner (Fig. 6b). There, trajectories A→B and C→D represent the selective crystallization pathways of the (-)- and (+)-enantiomer initiated by addition of respective seeds. In between (B→C, D→A) the crystallized products are filtered off and new racemic feed is added. Online monitoring of the separation progress (measuring the solution composition by 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 Focussed Beam Reflectance Measurement (FBRM®) or videomicroscopy) allows for monitoring

the evolution of the solid phase with regard to particle size, particle size distribution, particle shape etc.[174-176]

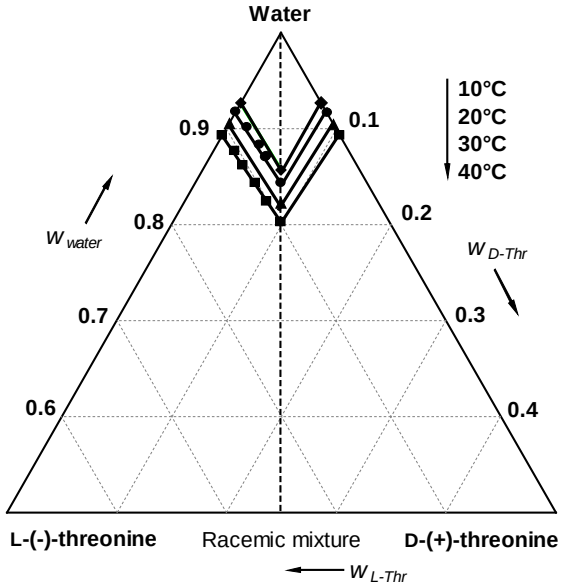

Figure 5: Ternary solubility phase diagram of L- and D-Thr in water. Since the solubilities of the threonine species in aqueous solution are comparatively small, only the upper half of the solubility phase diagram is shown (axes in weight fractions, *w*; isotherm lines are guides to the eyes).

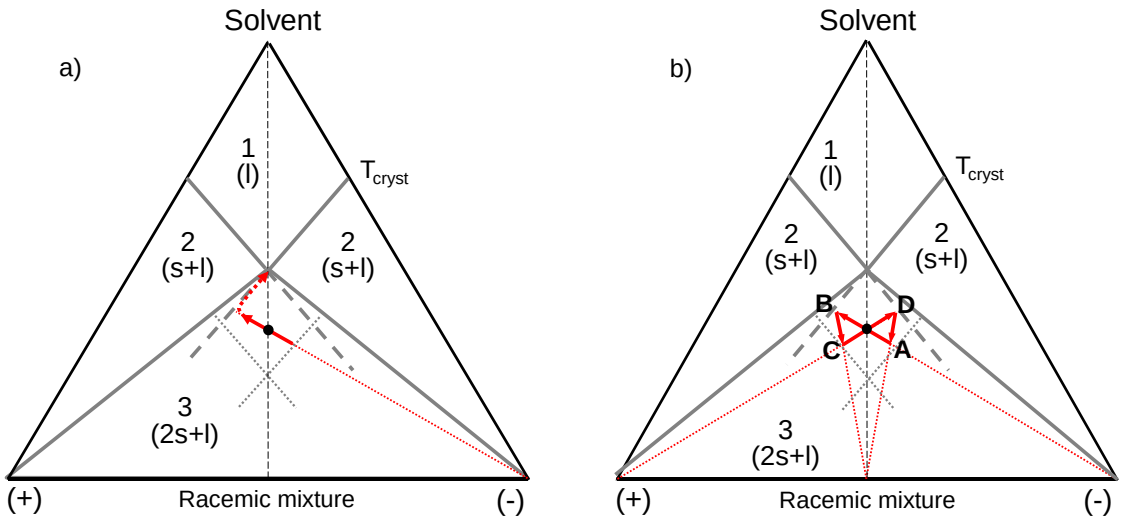

Figure 6: Illustration of the principle of preferential crystallization in the ternary solubility phase diagram. Shown is a) a trajectory of preferentially crystallizing a single enantiomer and b) a cyclic process producing periodically both enantiomers in an isothermal process at a crystallization temperature,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

Results of this classically isothermal process are presented e.g. in [110]. To enhance the process performance with respect to robustness, productivity and product purity, different more sophisticated process modes have been suggested, namely polythermal crystallization procedures, auto-seeding strategies and alternative crystallizer concepts. Exemplarily, the

potential of a promising auto-seeded polythermal mode of preferential crystallization[118] shall be briefly illustrated using the threonine case study. The process comprises a cooling crystallization that allows for enhanced yields and improved product purities and, further, a particular auto-seeding supplying well-conditioned seeds for reliable separation. Fig. 7 illustrates a typical temperature profile applied for a complete separation cycle. In Fig. 8 the resulting profiles of the optical rotation angle as a function of time are represented for five consecutive separation cycles of DL-threonine using aqueous solutions.

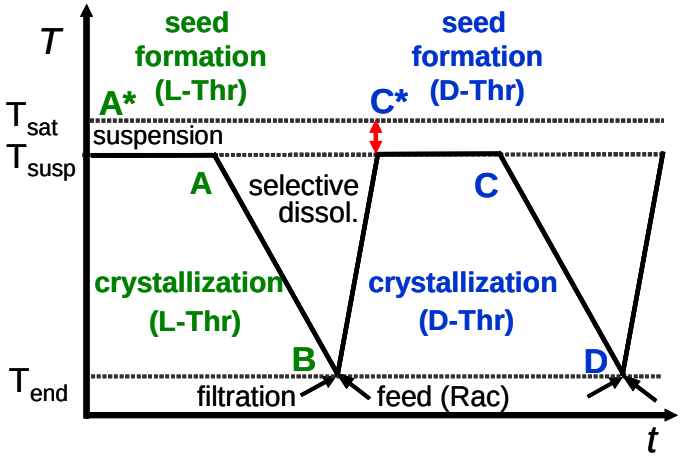

Figure 7: Schematic temperature profile applied in cyclic auto-seeded polythermal preferential crystallization. (T_{sat}, T_{susp} and T_{end}, saturation, suspension and end temperature of the cooling crystallization process; *t*, time; points A, B, C, D characterize the production periods of the separation cycle providing periodically L-Thr and D-Thr, compare with Fig. 8).[based on 175]

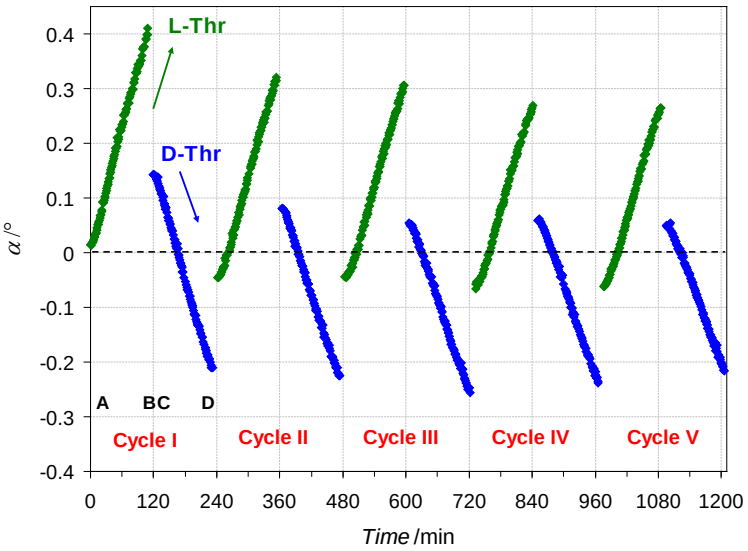

Figure 8: Profiles of the optical rotation angle, α, for five consecutive separation cycles in auto-seeded polythermal preferential crystallization of threonine. Just the sole crystallization periods A→B and C→D are shown.Crystallization conditions: 1000 g solution; 20 Kh^{-1} cooling rate; T_{sat},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

Unlike the isothermal process described before, each new half cycle now starts after addition of racemic feed with adjusting the temperature somewhat below the saturation temperature of the mixture (suspension temperature, T_{susp}, Fig. 7) in order to facilitate selective dissolution of just one of the enantiomers. E.g. at point B in Fig. 8 (i.e. after filtering off the gained 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, Fig. 7), they exhibit well-formed faces and a smooth, healed surface and serve as seed crystals for the subsequent cooling crystallization providing D-Thr as product (C→D, Figs. 7, 8). In the runs shown, the purity of L- and D-Thr gained was in the 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]

As for many processes that are based on recycling, 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 preferential crystallization of D- and L-threonine was discussed and evaluated in [174]. There, also the application of a modified auto-seeding strategy is presented. It is shown that, within certain limits, in dependence of 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]For resolution of racemic threonine, L-glutamic acid is known to selectively suppress nucleation and crystallization of L-Thr. Experiments showed that in presence of L-glutamic acid both the process robustness and the yield for crystallization of D-Thr as target product is enhanced.[112] Further, in literature 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, for DL-Thr in presence of 1 mg/ml of poly-L-leucine high chiral discrimination at the early crystallization stages has been observed.[177]

Different innovative concepts of alternative crystallizer configurations are presented in [110]. One concept considers simultaneous preferential crystallization in two crystallizers coupled via the liquid phase. The enhanced performance provided by this concept is illustrated in Fig. 9, where 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.

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

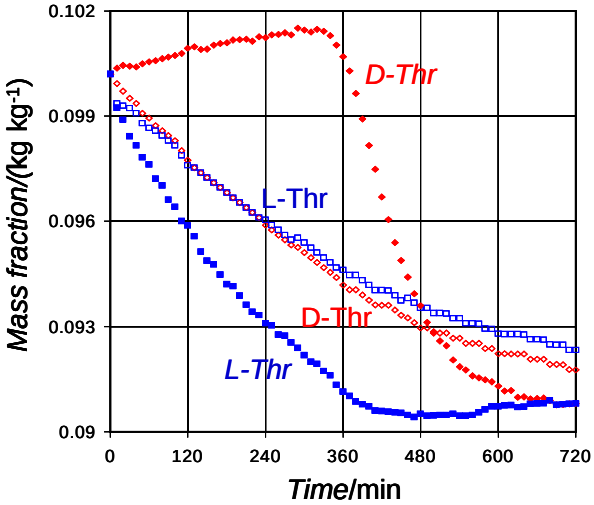

Figure 9: Concentration profiles of the enantiomers in the liquid phase for simple batch and coupled crystallization mode.[116] Full symbols and italic letters represent the simple batch mode, open symbols and regular letters represent the mode using two crystallizers coupled via their liquid phases.

3.3.2 Racemic compound-forming systems

a) Mandelic acid

Mandelic acid (MA) and its derivatives have several pharmaceutical applications, such as the treatment of urinary tract infections due to their bacteriostatic properties[179] and as a pharmaceutical constituent due to analgesic, antirheumatic and spasmolytic effects.[180] Furthermore, pure (*R*)-mandelic acid is used as a precursor for synthesis of cephalosporin and penicillin.[181] Both enantiomers, (*S*)- and (*R*)-mandelic acid (Fig. 10), belong to the most frequently applied acidic resolving agents utilized in classical resolution.[39] E.g. mandelic acid is used at an intermediate step of Duloxetine synthesis ((*S*)-MA, 2004) and in manufacturing Sertraline ((*R*-)-MA, 1990).[39] The latter one is among 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 diastereomeric resolution with (*R*)-MA implementing racemization of the undesired enantiomer providing both good economics and high-quality products are under development.[85]

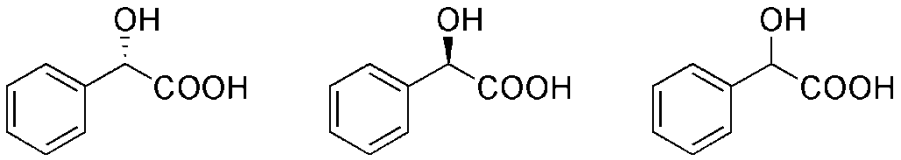

Figure 10: (*S*)-(+)-mandelic acid, (*R*)-(-)-mandelic acid, (*RS*)-(±)-mandelic acid.

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. In Fig. 11 the

ternary solubility phase diagrams of the mandelic acid enantiomers are shown 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 character of mandelic acid as racemic compound-forming.[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 presence of both solvents involved. Even when applying the chiral solvent DET (and also other optically active solvents, e.g. lactates[137]), no recognizable asymmetry is introduced in the MA phase diagram, that could be applied for separation purposes. In both solvents shown, the solubility of the mandelic acid species increases with temperature and is higher for the racemic compound than for the enantiomers.

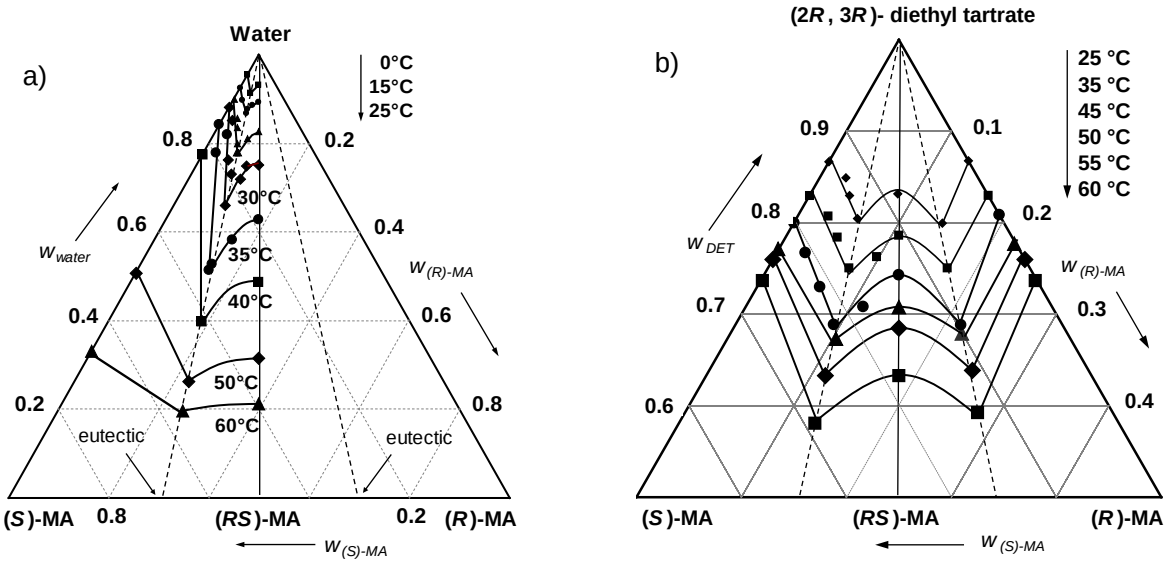

Figure 11: Ternary solubility phase diagrams of the mandelic acid enantiomers in a) water[153] and b) (2*R*, 3*R*)-diethyl tartrate.[162] Isotherm lines are guides to the eyes.

Though no quantifiable impact on solution thermodynamics in terms of different solubilities of the mandelic acid enantiomers in the chiral solvents was observed,[135] in 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 in (2*R*, 3*R*)-diethyl tartrate to be smaller for (*R*)-mandelic acid than for (*S*)- and racemic mandelic acid, facilitating preferential nucleation of the (*R*)-enantiomer and, thus, its selective crystallization. In Fig. 12 a typical result of a preferential nucleation experiment for racemic mandelic acid in (2*R*, 3*R*)-diethyl tartrate is presented. As expected from MZW data, with occurrence of nucleation, a decrease in solution density and increase in optical rotation of the solution was observed 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, application of certain mandelic acid esters as chiral “task-specific”solvents provided evidence of chiral recognition in the solution phase, indicated by clear solubility differences of the mandelic acid enantiomers.[138]

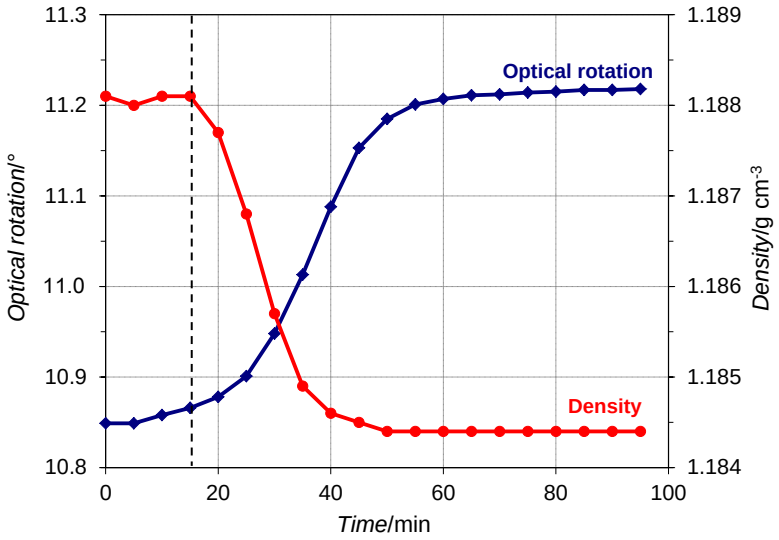

Figure 12: Profiles of the optical rotation of the mother liquor and the solution density during a preferential nucleation experiment starting with racemic mandelic acid in (2*R*, 3*R*)-diethyl tartrate at T_{sat}=55°C. The initial optical rotation of about 10.85° characterizes the optical rotation of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

As shown on the example of threonine, preferential crystallization can be used to resolve racemic mixtures of conglomerates. Here, it should be demonstrated that this method is also applicable to racemic compound-forming systems when starting from solutions being 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 as the two enantiomers for conglomerates.

Characteristic trajectories of a cyclic auto-seeded polythermal preferential crystallization for a racemic compound-forming system are illustrated in Fig. 13. In Fig. 14 the applicability of this technique for enantioseparation in the mandelic acid system is demonstrated. The measured trajectories principally look similar to the threonine case (Fig. 8), but differ in the fact, that the process is not any more symmetrical around the eutectic line. The maximum difference in enantiomeric excess (*Δee*) attainable by preferential crystallization can be unequal for the racemic compound and the enantiomer (e.g. *Δee*~6% vs. 4%, Fig. 14), since it depends on the particular eutectic composition of the chiral system. For mandelic acid, racemate and enantiomer can be gained in a ratio of 3:2 according to the eutectic at an *ee* of 40% (ee_{eut}, Fig. 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 via the enrichment step. Moreover, as demonstrated already for threonine, simultaneous preferential crystallization in the coupled crystallizer mode,

offers also for mandelic acid further improvement of process performance in terms of yield and reliability.[115, 117] Just recently, the application of hydroquinine-4-methyl-2-quinolylether as a chiral additive in preferential crystallization of mandelic acid was described. [185] Due to its particular effect on nucleation kinetics this additive also allows for significant enhancements in yield.

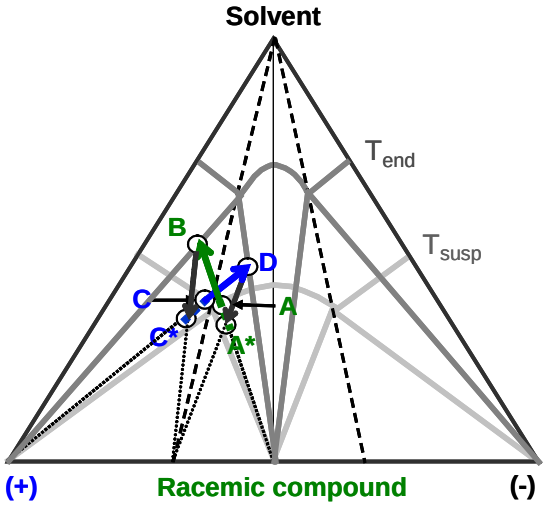

Figure 13: Illustration of a cyclic auto-seeded polythermal preferential crystallization process providing periodically the racemic compound and the (+)-enantiomer of a racemic compound-forming system.[113] The notation refers to the temperature profile given in Fig. 7.

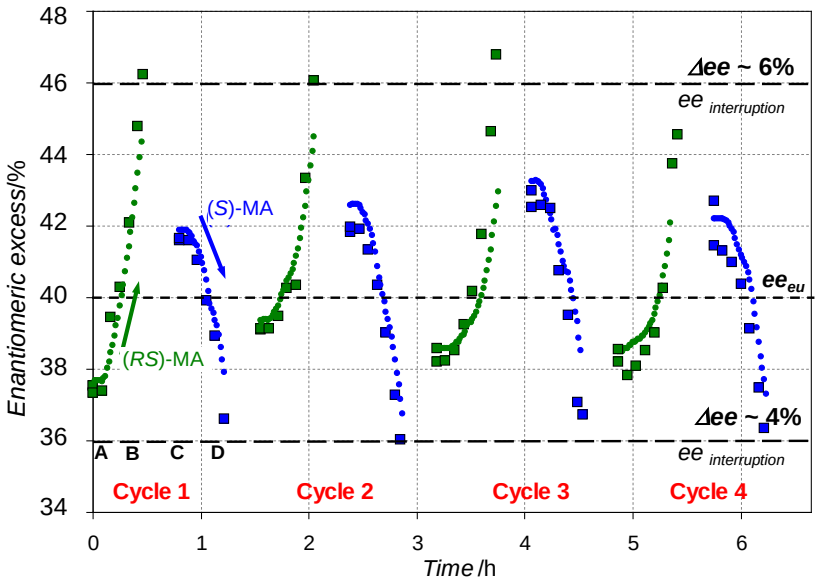

Figure 14: Profiles of enantiomeric excess for four consecutive separation cycles in auto-seeded polythermal preferential crystallization of mandelic acid.[113] Just the sole cooling crystallization periods A→B and C→D are shown. Selected crystallization conditions: 300 g solution; 15 Kh^{-1} cooling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

b) Methionine and Tröger's base

The amino acid methionine (Fig. 15) is an essential sulphur containing amino acid that (as all proteinogenic amino acids) is found in nature as the L-enantiomer. It is of particular relevance as animal feed additive. For that purpose, it is principally not necessary to resolve racemic

methionine, since it can be transformed into the desired L-enantiomer by the animal organisms. Pharmaceutical application is given as liver protection agent and in infusion solutions.[186] Methionine was used in our study as a model system to discuss separation strategies applicable for substances with similar phase diagram characteristics.

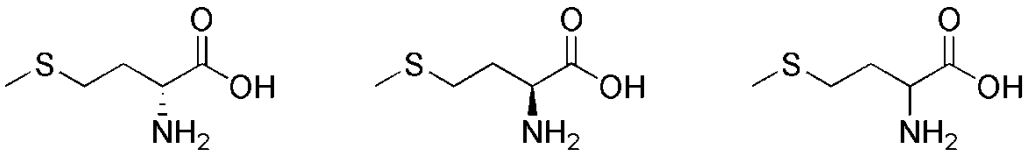

Figure 15: D-(*R*)-(+)-methionine, L-(*S*)-(-)-Methionine, DL-(*RS*)-(±)-methionine

Fig. 16 shows the ternary solubility phase diagram of the methionine enantiomers in water, which is clearly of racemic compound-forming type. The solubilities are found to be comparatively low, in particular for the racemic compound. The eutectic composition in the chiral system is close to the pure enantiomer side and varies with temperature. Actually, 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 and coworkers[187] and Tröger's base.[165]

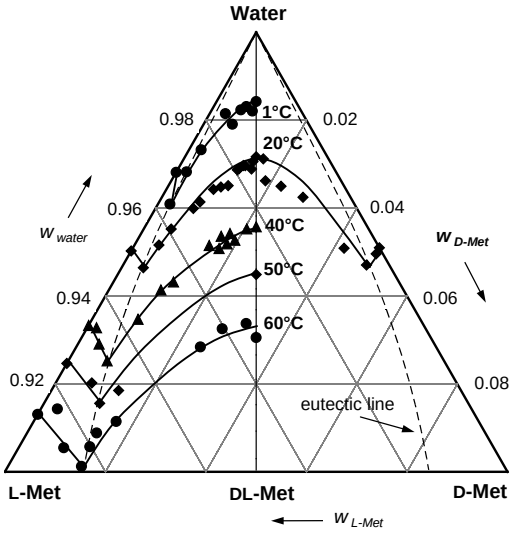

Figure 16: Ternary solubility phase diagram of the methionine (Met) enantiomers in water. Just the upper 10% of the phase diagram are shown due to the low solubilities in the system. Isotherm lines are guides to the eyes.[156]

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 an intermediate compound at racemic composition, which at same temperature has significantly lower solubility than the enantiomers. As result, the eutectic composition left and right from the racemic compound occur close to the enantiomer sides in the phase diagram. Further, as a function of temperature, the eutectic lines show convex

profiles, i.e. a high eutectic composition at low temperature shifts to lower values at elevated temperatures. For Tröger's base this shift in the eutectic composition is with *w*=0.92 at 25°C to 0.885 at 50°C[165] close to the methionine data.

Tröger's base ((±)-2,8-dimethyl-6H,12H-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 (Fig. 17). It is frequently used as a model system to study enantioseparation, e.g. in investigations of chiral chromatography (see chapter 4.4). Further, 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 NRTL model to account for non-ideality in solution. Crystallization-based resolution of racemic Tröger's base has been achieved via diastereomer-mediated resolution with a strongly acidic resolving agent. The resolution was found to be attended by a crystallization-induced asymmetric transformation of the salt containing the (+)-enantiomer of Tröger's base providing high yield.[190] A hybrid chromatography-crystallization process for resolution of Tröger's base has been studied by Amanullah and Mazzotti.[191]

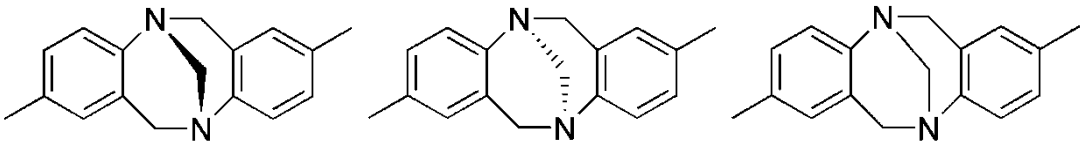

Figure 17: Structures of (-)-Tröger's base, (+)-Tröger's base and (±)-Tröger's base.

Exploiting the shift of the eutectic composition in the chiral system with temperature, an alternative two-step process for enantioseparation can be suggested. It is illustrated for methionine in Fig. 18.[151, 152] Starting at a low temperature, T_1, from a (just slightly) enantiomerically enriched solution (point 1), evaporation of solvent provides a solution of a composition inside the three-phase region of the phase diagram (point 2). Equilibration results in a solid phase of composition (3) depleted in the target enantiomer and a saturated solution of eutectic composition, $x_{eu,1,}$(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) supplying it enantiopure (6). Maximum yield is provided when the solution composition meets the phase boundary between the two- and adjacent three-phase region (point 5').

The process suggested can be performed in a cyclic manner as illustrated in the flow scheme in Fig. 19. The crystalline phase of the 1st step, the solvent and the mother liquor of the 2nd step can be fully or partially recycled attaining an efficient utilization of the process educts. The potential of this process was successfully demonstrated.[151]

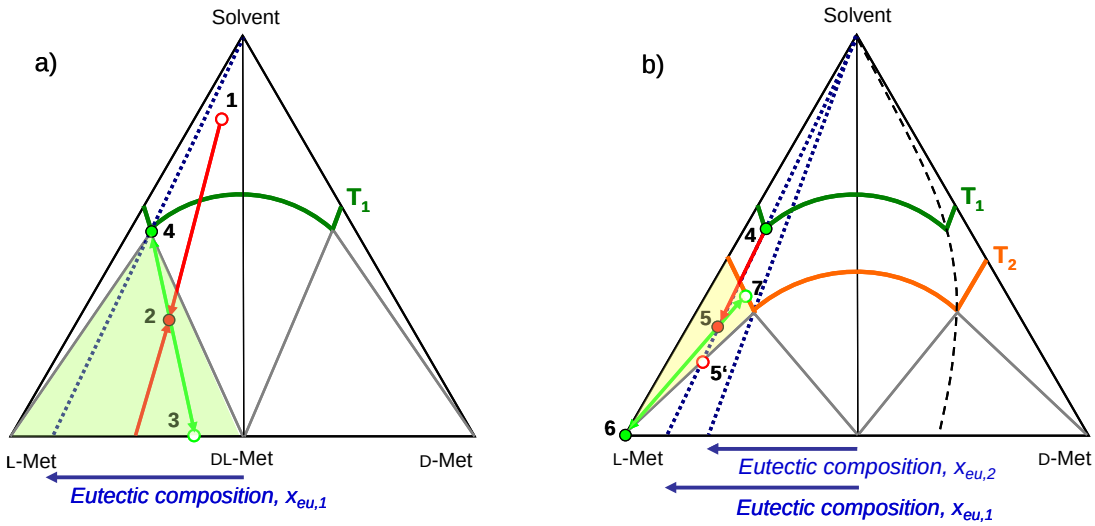

Figure 18: Two-step process for enantioseparation in the methionine system exploiting the shift in the eutectic composition with temperature; a) step 1: enrichment in the liquid phase at a low temperature, T_1; b) step 2: evaporation of solvent and selective crystallization of L-Met at a higher temperature,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

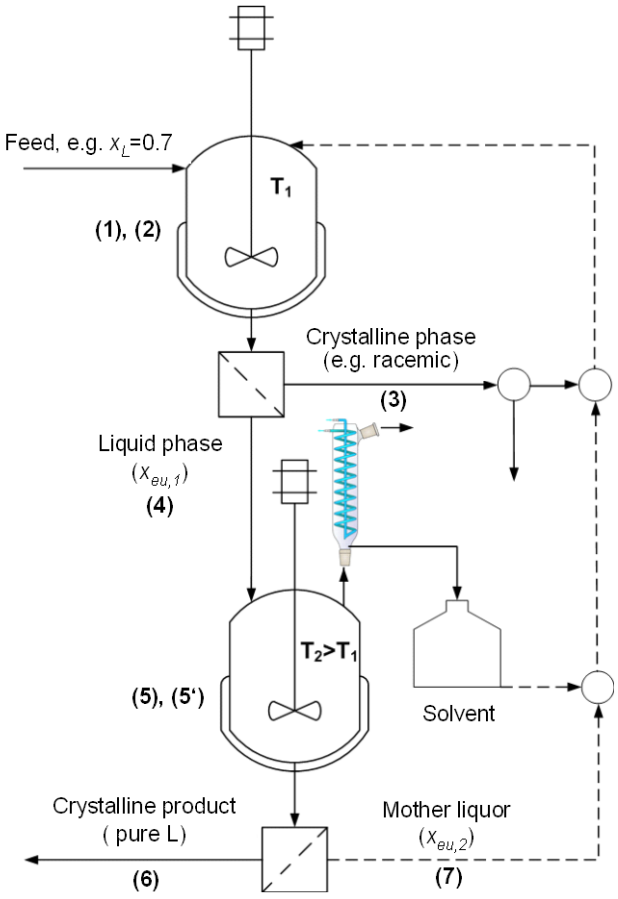

Figure 19: Flow scheme of the two-step separation process for methionine considering partial or full recycling of the solid separated in the 1st step, the solvent and the mother liquor of the 2nd step (target enantiomer: L-Met).[151] Characteristic numbers correspond to points in Fig. 18.

The next chapter 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 to resolve racemates is described in several excellent books and reviews.[31, 36, 38, 192-202]

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

Column chromatography using 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.

Depending on the properties of the feed mixture, gases, liquids and supercritical fluids are applied as the mobile phase. Pioneers in chiral GC are Gil-Av[203], Schurig[204], König [205] and Franck et al.[206]. However, for preparative applications this technique has still almost no importance. For nonvolatile and/or thermally labil compounds the wide spectrum of liquid chromatographic techniques provides the platform for numerous chiral HPLC methods. The current state of the art was recently reviewed in [207]. A renewed interest in supercritical fluid chromatography (SFC) led in the last years to the development of successful enantioselective separations using supercritical CO_2 as the main constituent of the mobile phase.[208, 209]

In conventional High Performance Liquid Chromatography (HPLC) it can be often chosen between several separation methods, i.e. combinations of mobile and stationary phases.[207, 210] This advanced state has not yet achieved regarding the task of separating enantiomers. To solve such complicated separation problems suitable chromatographic methods are still often developed empirically. However, considerable progress has been achieved in the last years in understanding important principles of chiral recognition.[211]Supported by the development of specific and highly productive process concepts and the provision of the corresponding design tools, nowadays chromatographic techniques allow separating virtually any mixture of enantiomers.

Regarding the selection, which of the two phases should be chiral to efficiently perform the chromatographic separation, there have been several efforts made to develop methods using chiral mobile phases or adding specific chiral additives to achiral mobile phases. An example is the separation of acid enantiomers by forming ion pairs with chiral bases such as quinines.[212] Ligand exchange methods have been reviewed in [213]. However, the use of chiral mobile phases appears to be not 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 compared to systems based on chiral stationary phases (CSP).

The application of 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. Hereby, 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 arsenal of selective CSP commercially available, which allows separating a broad spectrum of racemates. Important CSP will be introduced in section 4.1.

Chromatographic techniques are widely used in industry to purify smaller amounts of target enantiomers in early development stages. However, these techniques become increasingly a tool for production of larger quantities.[38, 199] Since large scale (industrial) chromatographic processes are expensive, they require a careful design and optimization. In the last years the theoretical understanding of preparative chromatography operated under overloaded conditions has improved significantly.[214-216] Theoretical analysis of band propagation processes occuring in packed columns revealed that in the design of preparative chromatographic processes the nonlinearities in the underlying distribution equilibria have to be taken into account.[216] Compared to conventional analytical chromatography, isocratic batch elution concepts are frequently not adequate and various alternative concepts have been developed in order to increase productivities and yields and to decrease solvent consumptions, e.g. several recycling techniques.[215, 216]A breakthrough in increasing the productivity was achieved by applying for chromatographic enantioseparations the multi-column simulated moving bed (SMB) technology[217]developed in the petrochemistry 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 CSP 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 CSP are applied with achiral mobile phases. During migration of the sample through the column diastereomeric complexes are formed within the stationary phases retaining the individual enantiomers in a specific manner.

The principle of chiral recognition has been rationalized earlier frequently using the classical “three point interaction model” suggested by Dalglish.[221] The numerous more detailed

mechanisms suggested 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 mobile phases and CSP.[e.g. 8, 223, 224]

An essential aspect for a successful preparative separation is to find a combination of CSP with a mobile phase that allows for high solubility of the enantiomers and for “reasonable” retention times in the columns. Hereby, both too long and too short retentions are not desirable. Despite of the considerable improvement in understanding separation mechanisms, due to the large number of degrees of freedom the standard technique to identify for a specific enantioseparation problem a suitable CSP is still based on screening a smaller are larger set of available stationary phases and various mobile phases. Regarding the latter typically solvent mixtures are applied in order to adjust retention times. This screening is frequently supported by the manufacturers of the CSP.

A broad variety of chiral selectors, of both natural and synthetic origin, have been applied in CSP. More than hundred CSPs are offered nowadays commercially, among which probably around 20 are most frequently used.

There are various classifications for CSP.[200, 220, 225] We follow in the short overview given below the grouping of the most important selectors for CSP applied in[200].

Macromolecular selectors of semi-synthetic origin (polysaccharide-based CSPs)

Polysaccharide selectors have a long tradition. Hesse and Hagel introduced in 1973 microcrystalline cellulose triacetate (Fig. 20) as a polymeric selector material (without support).[226] By coating different cellulose derivatives (e.g. amylose tris(3,5-dimethylphenylcarbamate, Fig. 20) to marcoporous silica beads Okamoto and coworkers made highly enantioselective materials with higher pressure stability and chromatographic efficiency which were successfully commercialized by Daicel Chemical Industries.[227]

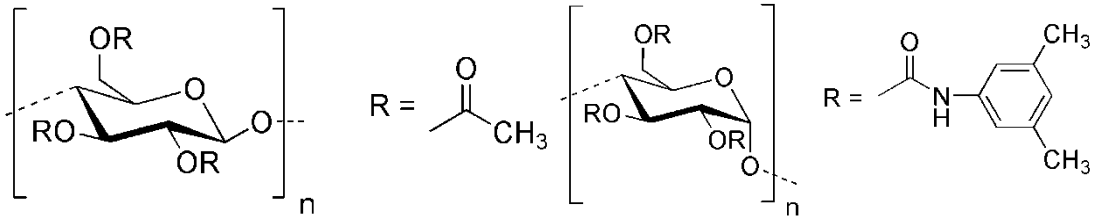

Figure 20: Polymer backbone of polysaccharide based CSPs, left: *Cellulose triacetate* (CTA), right: *ChiralpakAD*.

Currently there are phases available, which can be used for a wide spectrum of solvents due to improved selector immobilization. This allows choosing mobile phases offering a maximum in solubility which, together with the typically high enantioselectivities, makes this group of CSP 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 semi-synthetic polysaccharides. A review regarding polyacrylamide/silica composites was given by Kinkel.[230] Probably due to much less ordered structures of the polymer chains these materials often do not achieve the same enantiorecognition as the polysaccharide phases.

Macromolecular selectors of natural origin (protein phases)

After the pioneering work of Allenmark and coworkers[231] and others, who investigated the steroeselective binding of chiral components to proteins, nowadays a variety of protein-based CSPs is well documented[232] and commercially available.[200] A drawback of protein phases is their limited sample loadability, making 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 CSP are based on α-, β, or γ-cyclodextrins, i.e. 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 via ether linkage or via carbamate linkage.

Inspired from the stereoselective inclusion capabilities of the macrocyclic cyclodextrins, Armstrong and coworkers developed a new very successful class of CSP: the macrocyclic antibiotic CSPs. The first type of this class was vancomycin-modified silica.[235] In the meantime the company *ASTEC* (now *Sigma Aldrich*, USA) has commercialized several other CSP based on glycpeptide antibiotics[235]. The structure of Teicoplanin, which is the selector of the commercially available CSP *Chirobiotic T*, is given in Fig. 21 as an example. High loadibilities were achieved for Teicoplanin Aglycone (CHIROBIOTIC TAG)[235d].

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

Synthetic neutral entitities of low molecular weight (Donor-acceptor phases/Pirkle phases)

The first commercialized CSP with entirely synthetic selectors were developed by Pirkle and coworkers.[200] A DNB-phenylglycine derivative immobilized ionicly onto silica was used as the selector. Later a larger collection of donor-acceptor type of CSPs was developed,[237] which exploit frequently 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 that has pre-organized clefts for solute insertion and allows for simultaneous face-to-face and face-to-edge π-π-interactions. An interesting advantage of these CSP is the fact that, due to the availability of the selectors in both enantiomeric forms, the elution order can be selected.[238]

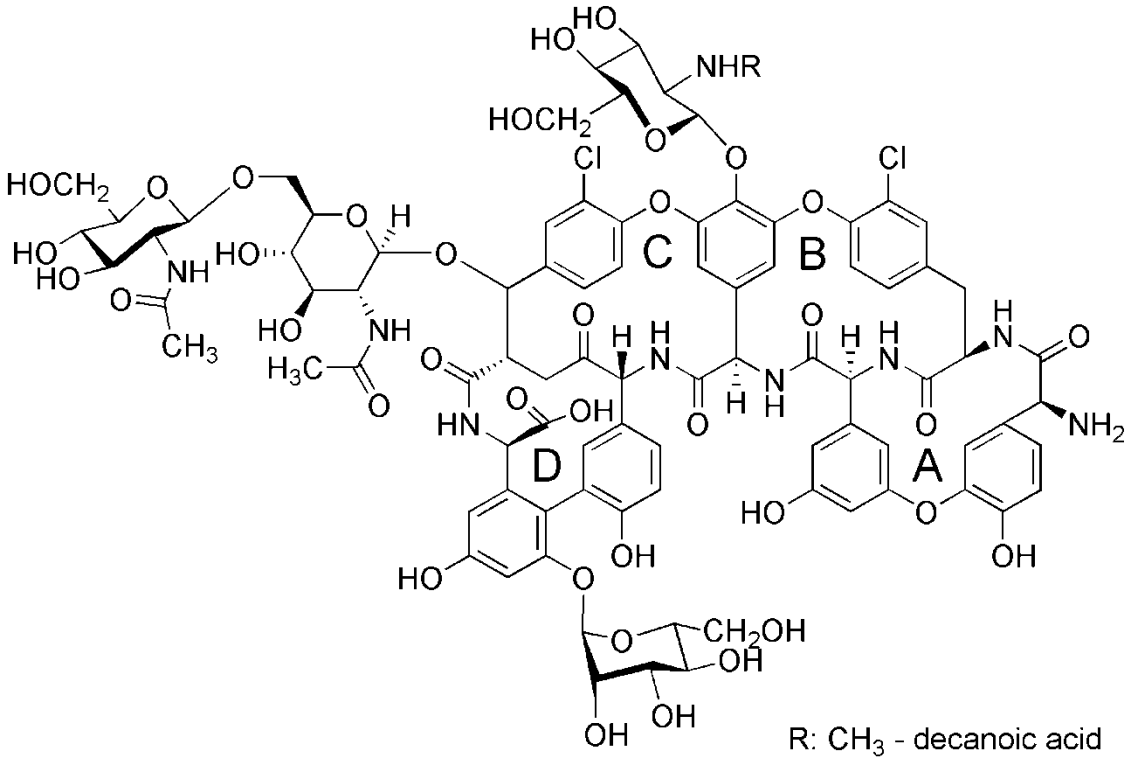

Figure 21: Structure of the glycopeptide antibiotic Teicoplanin, which is the selector in theCSP *Chirobiotic T* (with 4 inclusion cavities A, B, C and D).

Synthetic ionic entities of low molecular weight possessing ion-exchange properties

Several ion-exchangers have been developed for the separation of ionisable chiral compounds: e.g. 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 as the cinchona alkaloids quinine and quinidine as backbone of the selectors. The corresponding columns appear to be very promising in particular due to 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 e.g. praline onto a polystyrene support and used this enantioselective matrix in combination with Cu(II)-ion containing mobile phases for the enantiomer separation of amino acids[241]. An overview about the method is available in[242].

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

The intensive research activities in the area of developing improved CSP will for sure 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 relative 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 peaks shapes. Besides the selectivities, now the loadabilities of the stationary phases become very important. To increase productivities and recoveries and to reduce the solvent consumptions several alternatives to batch chromatography have been developed and are used in industry. Examples are given for example in [245, 246].One class of processes improving conventional batch chromatography exploits the principle of recycling not sufficiently resolved fractions, as e.g. the so-called steady-state recycling.[247] The most important chromatographic techniques used for enantioseparation are reviewed in [38, 199, 248]. Useful hints and rules of thumb to develop a preparative chromatographic enantioseparation are given in [249].

Due to 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, nowadays sufficiently accurate model equations and the tools required to solve them are available.[214, 215] Thus, important features of nonlinear overloaded chromatography, as e.g. displacement and tag along effects and the mentioned

peak distortions, are well understood. Due to the lack of sufficiently accurate prediction methods, an important problem is still the fact that the adsorption equilibrium isotherms required 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 well predicted and optimized. The theoretical framework available can be used to scale-up laboratory results to industrial scale columns.[251] A powerful modeling approach capable to simulate and design steady-state recycling chromatography was developed recently.[252]

Due to the availability of both high capacity and selectivity CSP's and mathematical tools allowing the design of various modes of operation, the separation of racemic mixtures using preparative liquid chromatography is currently in many cases the most rapid route to produce smaller quantities of pure enantiomers. In the last years a specific process applying 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 already in 1961.[218] It was initially applied in the petroleum and sugar industries to separate e.g. the xylene isomers and fructose from glucose.[253, 254]

In order to explain the SMB process it is expedient to explain at first a hypothetical true moving bed (TMB) process. As illustrated schematically in Fig. 22, the process is based on a countercurrent 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, i.e. 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*, e.g. two enantiomers. Under successful conditions one of the outgoing streams is enriched in the less adsorbable component A (the raffinate stream) and one is enriched in 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 flowrates 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 in order to desorb component *B* and thus to regenerate the solid phase. Component *A* is adsorbed on the solid phase in zone IV in order to regenerate the eluent. The process reaches a steady-state in which the positions of the internal concentration profiles do not change. Provided the flowrates have been adjusted correctly, a complete separation of the feed mixture can be achieved as illustrated in Fig. 22.

The drawback of the TMB process described is the fact that a continuous transport of fragile solid particles without unwanted backmixing is hardly possible. This led to the suggestion of the simulated moving bed process (Fig. 23). Hereby the counter-current movements of 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 mainting its attractive features. Due to the discrete switching events, the SMB process does not reach a steady state, which would be characterized e.g. 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 problems, 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. 255-257]. Several comprehensive reviews describe the success story.[258-263] Typical productivities achievable with the technology are in the range of 1-10 $kg_{Enantiomer}kg_{CSP}^{-1}$ day^{-1}. The breakthrough of 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.

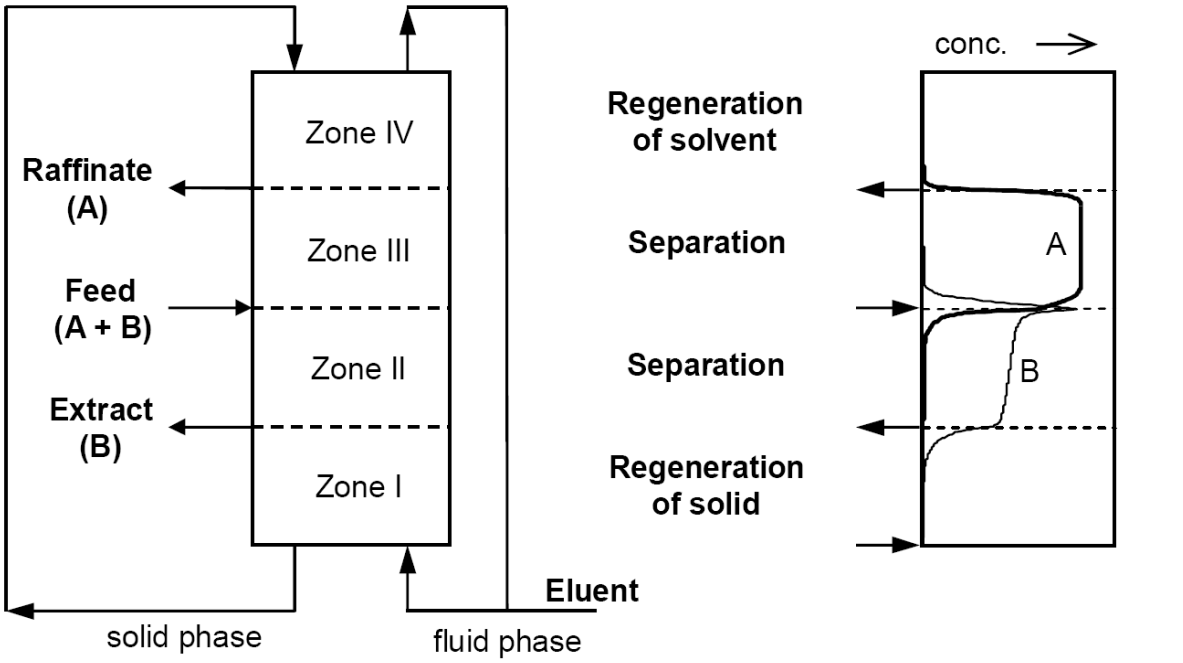

Figure 22: Principle of true moving bed (TMB) chromatography and typical internal concentration profiles for a successful binary separation of a binary mixture of components A and B.

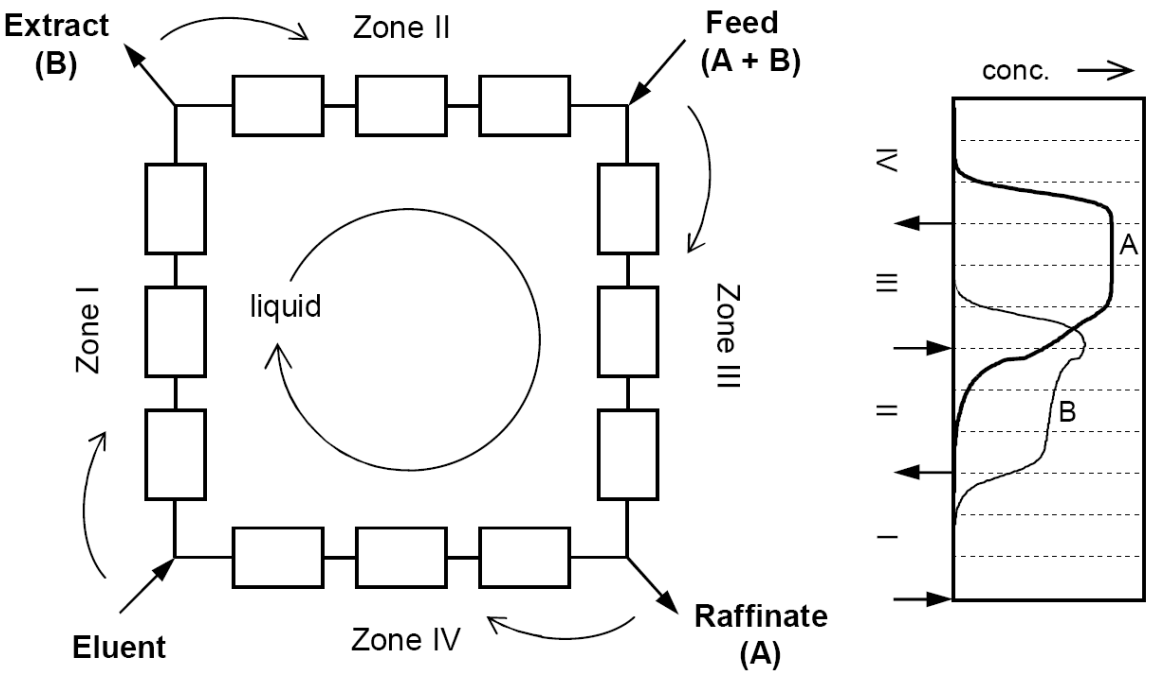

Figure 23: Principle of simulated moving bed (SMB) chromatography and typical internal concentration profiles at the end of a shift period for a successful binary separation.

Besides the conventional 4-zone closed-loop SMB-process explained above, several improved modifications have been developed in the last years,[e.g. 267-270]considering also the application of supercritical fluids as the mobile phase.[271, 272] The availability of these variants broadened the spectrum of application[273] and led already to 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 already in the section above describing options for enantioselective crystallization.

The large arsenal of available chiral stationary phases (section 4.1) provides in most cases several possibilities to perform a chromatographic enantioseparation. Unfortunately, there is still no reliable theoretical framework available in order 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 injecting analytical (small) sample sizes on small lab scale columns filled with potential CSP candidates. Hereby sufficient solubility in the scanned solvents or solvent mixtures should be available. As an outcome of this empirical search, selectivities between the two enantiomers valid for diluted conditions can be evaluated based on the ratios of rentention times. Typically, in a second empirical stage more systematic work is carried out for the most promising chromatographic system(s). Hereby the adsorption isotherms, as the most essential information required for predicting chromatograms, have to be determined

in a wider concentration range. These thermodynamic functions quantify the column capacities, allow optimizing operating conditions and discriminating between rivaling operating regimes and, thus, form finally the essential basis for the quantitative design of the chosen chromatographic separation process.

a) Threonine

An example for a successful chromatographic separation of a racemic mixture under analytical conditions is given in Fig. 24 for the amino acid DL-threonine. A*Chirobiotic T* stationary phase (see Fig. 24) was used with a 60:40 ethanol-water mixture as the mobile phase. There is obviously a considerable selectivity that can be exploited for preparative purposes injecting periodically larger samples of DL-threonine. However, the development and application of a large scale chromatographic method appears to be not useful for the separation of these threonine enantiomers, because this amino acid belongs to the relative small group of conglomerates for which the preferential crystallization (Chapter 3) could be applied most efficiently 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.

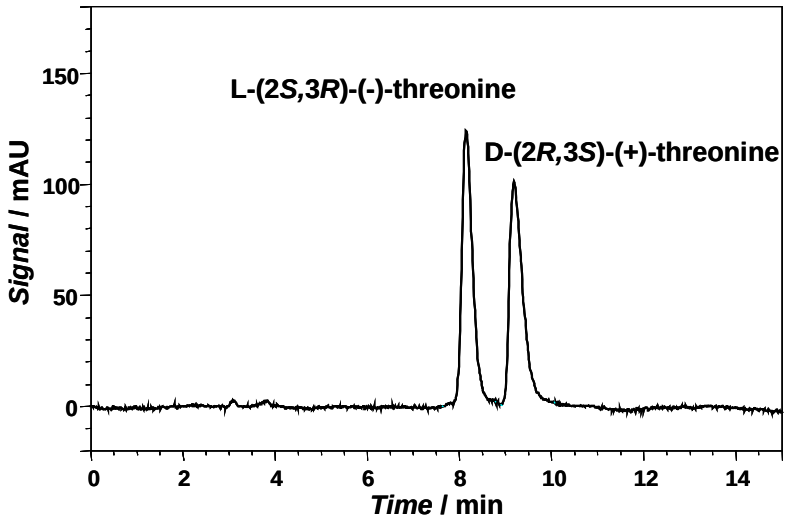

Figure 24: Chromatographic separation of DL-threonine. Chiral stationary phase: *Chirobiotic T* (5 μm, L=250, d=4.6 mm, ASTEC (Sigma Aldrich, USA), mobile phase: ethanol/water (60/40, v/v). Injection volume: 5 μl, injection concentration: 1% of DL-threonine in water, flowrate 0.5 ml min^{-1}, *T*=24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

b) Mandelic acid

Fig. 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*, using triethylammoniumacetate/methanol as the mobile phase, CSP II: a cyclodextrin based CSP using 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. Obviously, CSP I provides the higher selectivity for this separation.

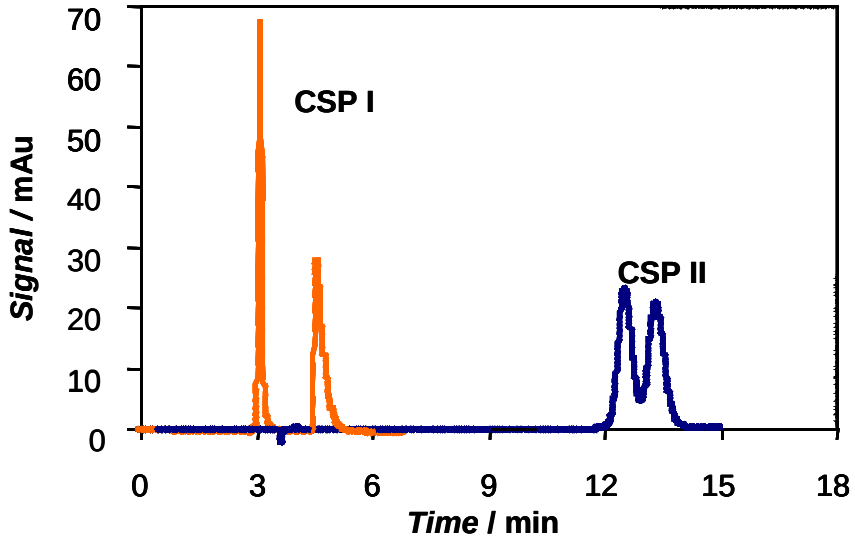

Figure 25: Chromatographic separations of racemic mandelic acid using two different chiral stationary phases. First peaks: (*S*)-(+)-mandelic acid, second peaks:(*R*)-(-)-mandelic acid. Injection volume: 1 µl, injection concentration: 1% of (*RS*)-(±)--mandelic acid in water, flowrate 0.5 ml min^{-1}, temperature: 20 °C, detection: UV at 254 nm. CSP I:*Chirobiotic T* (ASTEC/Sigma Aldrich, USA, 5 µm, 150 x 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 x 4 mm), mobile phase: 0.05 M NH_4Ac/acetonitrile (95/5, v/v), pH = 3, T=20°C. [173]

Besides the selectivity also the loadabilities of the stationary phase are of large importance. Frequently the Langmuir adsorption isotherm model can be applied to describe the typically nonlinear distribution equilibria.[216]

$$q = q_{sat}\frac{bc}{1+bc} \qquad \textbf{[Eq. 1]}$$

In this equation the 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. Parameters of Eq.1, as determined by frontal analysis, are given in Table 4 for the two CSP applicable to separate the mandelic acid enantiomers.[250, 275-277] There, in addition two more mobile phases (Table 3) allowing 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. The table further further provides maximum concentrations c_{max}, which mark the range of applicability of the adsorption isotherm model and roughly limits of solubility as an important constraint in preparative chromatography.

An optimization study was performed for the different chromatographic systems using this thermodynamic information as well as the numbers of theoretical plates provided by the specific columns in a standard dynamic model capable to predict the performance of a conventional chromatographic column operated in the batch mode.[216, 275, 276] In Fig. 26 are presented 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 (α=1.40). Although mobile phase B provides a much higher solubility and with CSP I similar saturation capacities as system I.A the lower separation factor can not be compensated. Obviously, CSP II can not be considered as an alternative for solving this separation problem.

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

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

MeOH – methanol, TEAAc – triethylammonium acetate, EtOH – ethanol, MeCN – acetonitrile, HOAc – acetic acid, AAc – ammonium acetate

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

System	Enan-tiomer	*T* °C	q_{sat} gL^{-1}	*b* Lg^{-1}	$\alpha = (q_{sat}\, b)_R / (q_{sat}\, b)_S$	c_{max} gL^{-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

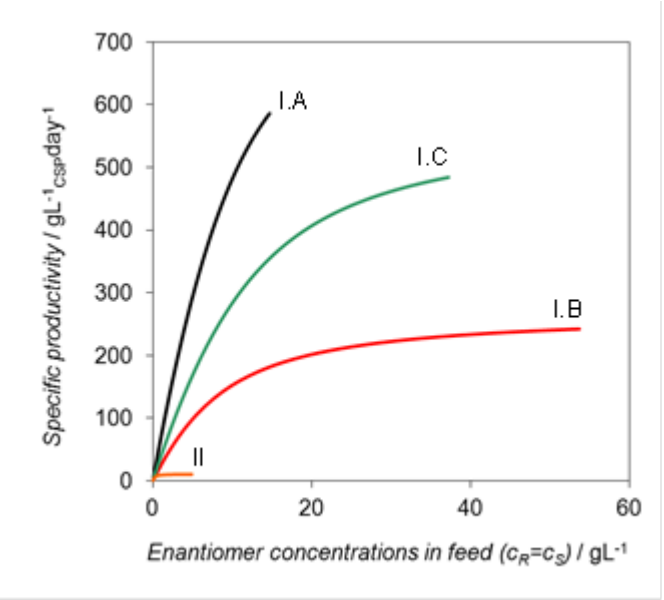

Figure 26: Productivities of performing a chromatographic separation of racemic mandelic acid as a function of the injection concentration for the four chromatographic systems summarized in Tables 3and 4.[276]

c) Tröger's base

The separation of racemic Tröger's base is a classical test problem 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 characterized were published in [279]. The successful application of cellulose triacetate in a continuous 8 column SMB process was described in [280]. Later many other CSP were found capable to perform the separation. Results of screening 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 using various CSP were reported in [282-284].

First reports about unusual shapes of chromatographic peaks of the Tröger's base enantiomers were published in [285, 286].In own work these strange peak forms were confirmed.[287] Fig. 27 shows single component elution profiles of the two pure enantiomers of Tröger's base for microcrystalline cellulose triacetate (*CTA)* as the stationary phase and pure ethanol as the mobile phase. Whereas in a series of overloading the column for the (-)-enantiomers the development of asymmetric peaks with an increasing tailing can be observed, the peak shapes of the (+)-enantiomers follow another trend. In the course of successive column overloading the elution profiles of (+)-Tröger's base are characterized by an initial orientation of the peak maximum to longer retention times. Further overloading changes this trend and the orientation of the maximum turns to shorter retention times, as typical for Langmuirian system following Eq. 1.[216]

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

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

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

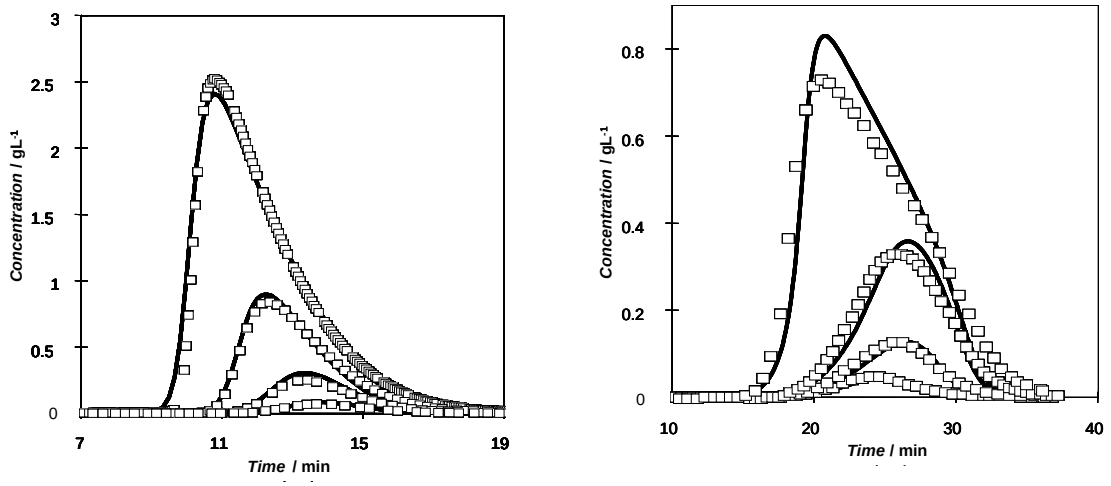

Figure 27: Series of overloading a microcrystalline cellulose triacetate column with injections of the pure enantiomers of Tröger's base. Mobile phase: ethanol. Flowrate: 0.5 ml min^{-1}. Temperature: 20 °C. Injection concentration: 15 gL^{-1}.Injection volumes: 10, 30, 90, 250µl.Left: (-)-Tröger's base.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

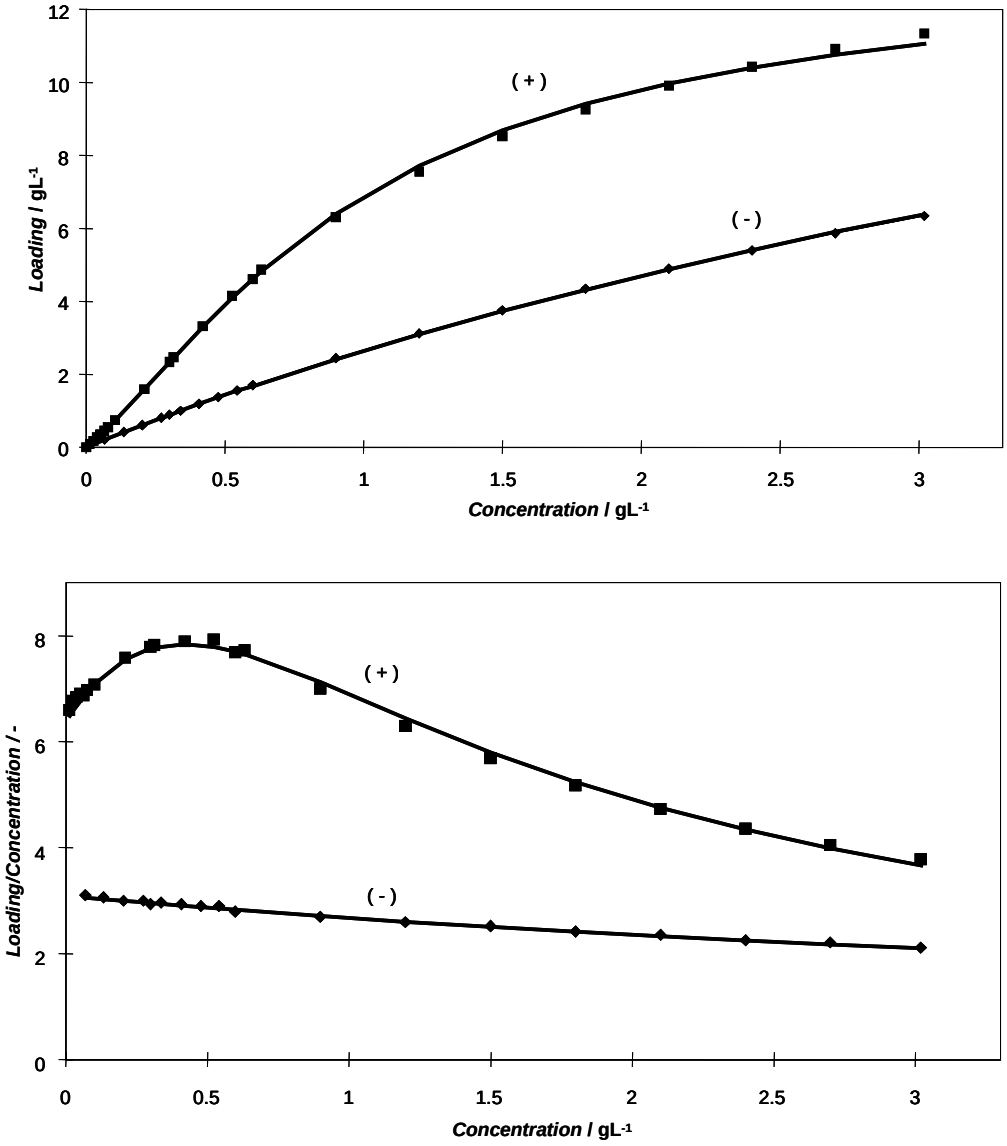

Figure 28: Adsorption isotherms (top) at 20°C and corresponding chords (bottom) for the adsorption of the enantiomers of Tröger's base in ethanol on microcrystalline cellulose triacetate.[293]

Applying a) the parameters derived in [289-291], b) the ideal adsorbed solution theory[292], which is capable to describe multicomponent adsorption equilibria for arbitrary shapes of the single component isotherms and c) the equilibrium dispersive model of a chromatographic column[216], elution profiles of Tröger's base could be rather well represented for the single enantiomers (Fig. 27) and racemic mixtures (Fig. 29).[293]

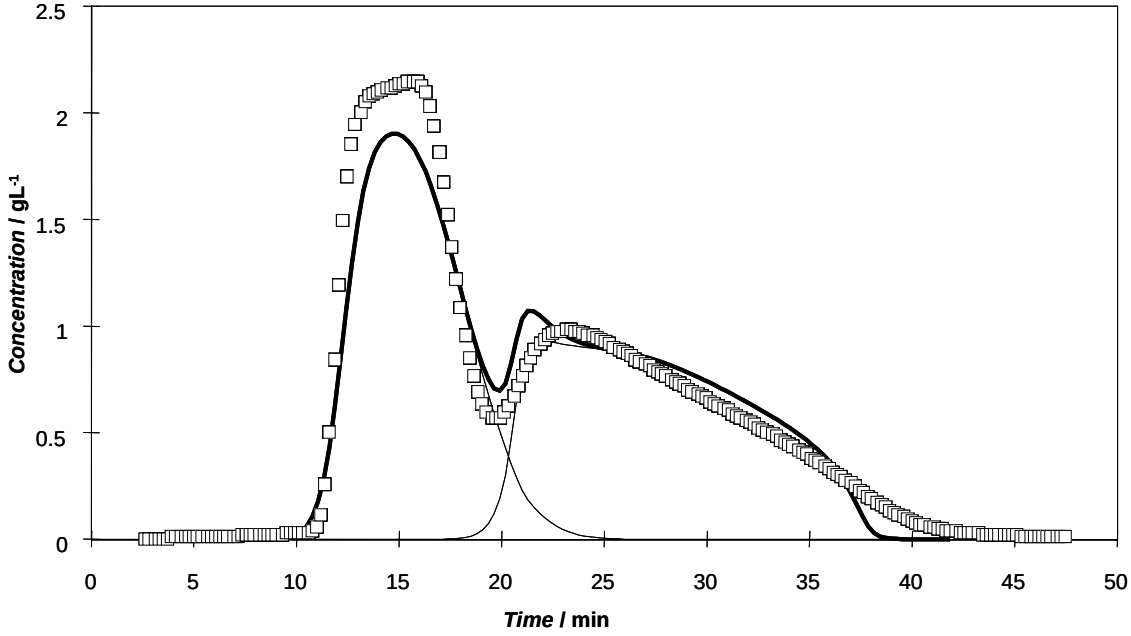

Figure 29: Measured and simulated chromatogram for the separation of the enantiomers of Tröger's base under strongly overloaded conditions. Symbols: Experiment, lines: equilibrium dispersive model using within the ideal adsorption solution theory Eq.2. Flowrate 0.5 ml min^{-1}, temperature: 20 °C, injection volumes: 1 µl, injection concentrations: 1.5 gL^{-1}.[293]

Further work regarding the thermodynamic characterization of this challenging chromatographic system and first attempts devoted to explain the behavior based on molecular simulations are given in [294, 295].

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

d) Methionine

In order to carry out a comparative study, in [298] two CSP 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). The latter (M=1540 g*mol^{-1}[299]) contains 22 chiral centers, three cavities, three sugar moieties, five aromatic rings, one carboxylic group, nine hydroxyl groups, seven amido groups and three amino groups.

In Fig. 30 are given the adsorption isotherms of the two methionine enantiomers at 25°C for these CSP and the corresponding mobile phases compositions indicated in the figure caption. The selectivities are significant and do not differ much for the two CSP.

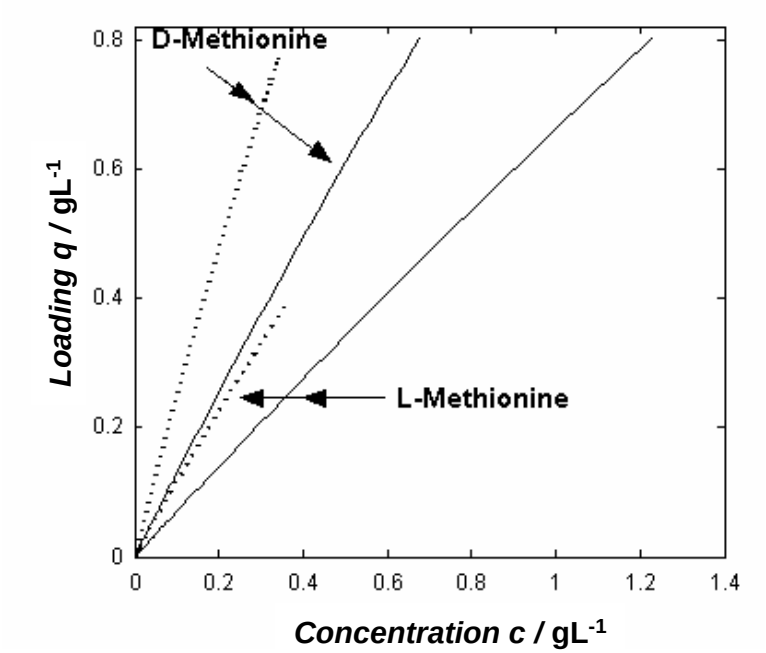

Figure 30: Adsorption isotherms at 25 °C of L- and D-methionine for *Chirobiotic T* (dotted) with EtOH:H_2O=40/60 (v/v) and *Chirasel E* (line) with MeOH: H_2O (0.1 M NaH_2PO_4)=20/80 (v/v).[298]

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

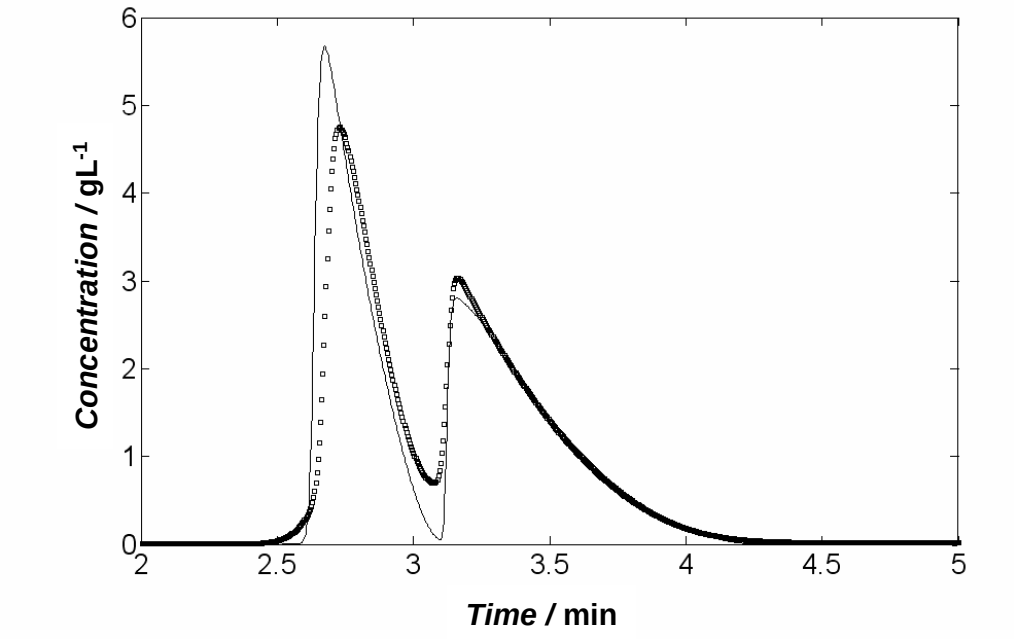

Figure 31: Experimental (symbols) and calculated (dotted) elution profiles of DL-methionine for the *Chirasel E* column, injection concentrations for both enantiomers: 6.1 gL^{-1}, the first peak corresponds to L-methionine.[298]

Fig. 32 summarizes the results of intensive numerical simulations performed with the validated model. For the applied lab scale batch column the influence of the amount injected on the product of productivity (*PR*) and recovery yield (*RY*) was evaluated. The figure clearly reveals the better performance of the *Chirasel E* CSP, and provides information about the most beneficial injection amounts. 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]

Because of the fact that *Chirasel E* performed better and appeared to be also more stable than *Chirobiotic T*, it was used in an experimental SMB study, where 8 connected conventional HPLC columns were used (each *L*=10 cm, *d*=0.46 cm). Feed-concentrations of up to 10 gL^{-1} were applied, located 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 appr. 0.75 kg $kg^{-1}_{CSP}day^{-1}$.[300]

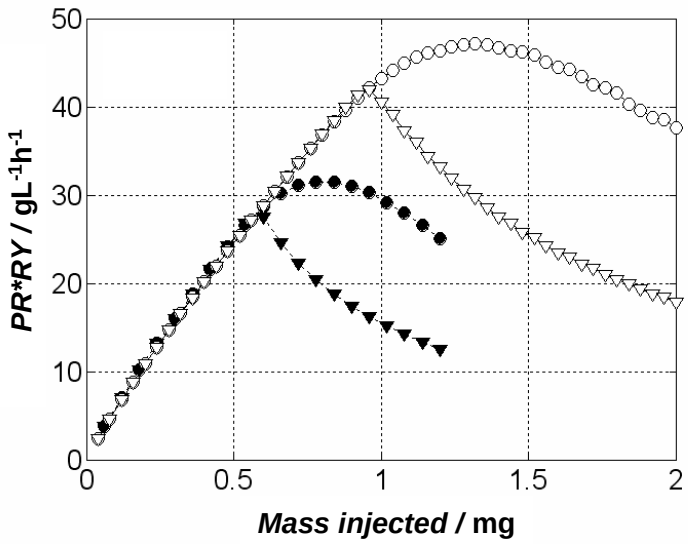

Figure 32: Simulated dependence of Productivity (*PR*) times Recover Yield (*RY*) of L- (*circles*) and D-methionine (*triangles*) on *mass injected* for 2 CSP (*Chirobiotic T,* EtOH/H_2O=40/60 (v/v), solid symbols; and *Chirasel E,* MeOH/H_2O (0.1 M NaH_2PO_4)=20/80 (v/v), open symbols). Specified purity requirements: 99%.[300]

5. Coupling of chromatography and crystallization

After introducing enantioselective crystallization and chromatography individually a straightforward and very promising concept will be described in this section based on combining these two techniques. 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 e.g. SMB chromatography, it is obvious that the process must be designed and operated much more careful and conservative if the purity of the streams leaving the

corresponding unit must be very high, compared to a constellation in which lower purities are acceptable. In addition, compared to preparative chromatography, crystallization is a cheaper and in general simpler process. However, as discussed above, for racemic compound-forming systems successful application of standard enantioselective crystallization techniques requires the provision of a certain initial enrichment. Thus, a reasonable approach, suggested in [301-303], is to use the expensive chromatographic step to achieve, prior to crystallization, just the required start composition. 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 of combining chemical and physical approaches capable to deliver pure enantiomers.[34]

Within the INTENANT-project in a comprehensive case study a racemic mixture of bicalutamide, a drug substance used in the treatment of prostate cancer, was separated by simulated moving bed chromatography, with the objective of maximizing throughput at a reduced outlet purity[168]. Fig. 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 using 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 due to 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 Fig. 34. More details regarding the realized exploitation of the shift of the position of the eutectic composition in the bicalutamide phase diagram with temperature and addition of water as an anti-solvent are given elsewhere.[168] The two simple crystallization steps increased progressively the enantiomeric purity, and the final product was 99.99% pure. The experimentally determined yield was found to be 45%.

In order to analyze and evaluate the potential of such hybrid chromatography-crystallization processes more systematically and efficiently reliable short-cut methods were developed recently.[275, 304, 305] A comparison of competing concepts is shown for the bicalutamide case in Fig. 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 chromatograph alone.[168]

A more general analysis revealed, that the described type of a hybrid process is in particular likely to be beneficial for systems possessing a eutectic composition rich in the single enantiomer.

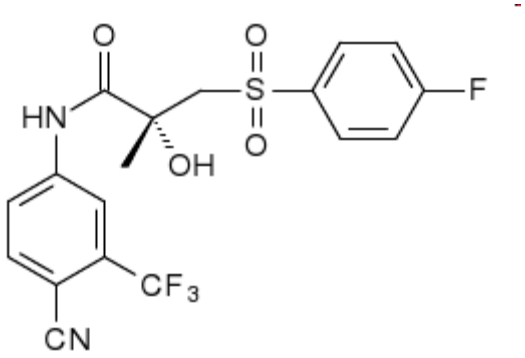

Figure 33: Structure of (*R*)-bicalutamide[168].

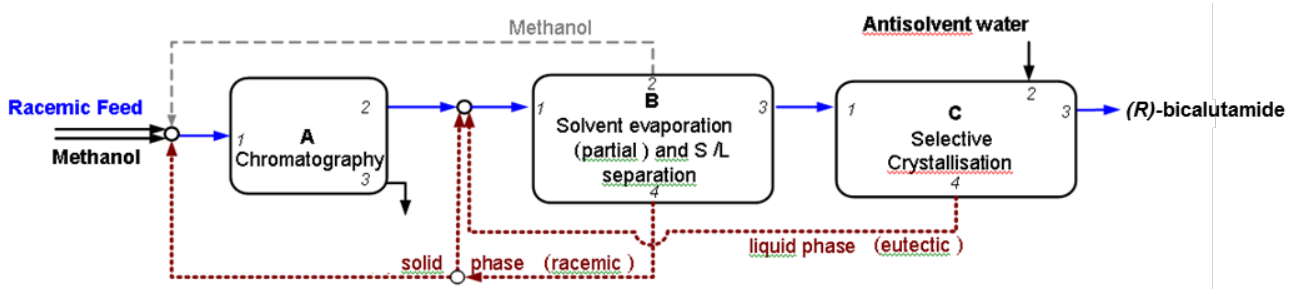

Figure 34: Flowsheet of the coupled process realized in order to isolate (*R*)-bicalutamide (target enantiomer). The racemic feed mixture is first enriched by SMB chromatography (A) and further purified in two subsequent crystallization steps (B and C). Fractional recycling of the solid phase from (B) and the entire mother liquor from (C) increases the yield (adopted from [168])

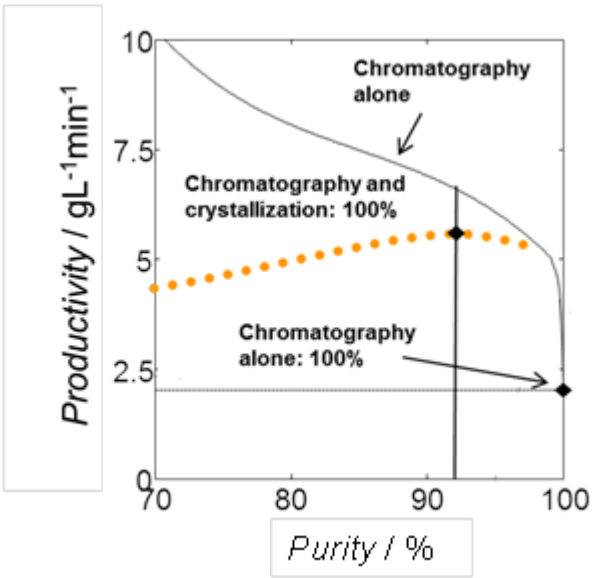

Figure 35: Optimized *(R)*-bicalutamide productivities of the stand-alone SMB chromatographic process (right diamond marks productivity for 100% purity, upper solid line marks productivity for reduced purity). The gain in productivity for the coupled process compared to SMB alone is reflected by the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

Similar results as for bicalutamide were obtained already earlier 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). For this particular example an optimal transition

concentration between chromatography and crystallization of approximately 93% was determined, providing a performance increase (compared to SMB chromatography alone) of approximately 45%.

Considering the separation of the enantiomers of Tröger's base, a genetic algorithm was used in [191] to optimize the combined process, using proper definitions of objective functions. Multi-objective optimization of productivity and evaporation cost 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 increased SMB performance and recycle of the mother liquor. Further examples devoted to study quantitatively combinations of chromatography and crystallization were reported in [306-308]. All the results summarized here clearly indicate that the combined process can achieve better performance than the SMB process alone.

Alternative provision of an initial enrichment prior to crystallization was suggested recently in [309]. It is based on using an enantiomerically selective membrane within a pertraction process. Obviously, other combinations of different separation processes with subsequent crystallization are imaginable.

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

6. Process concepts considering racemization

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

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

To reuse "counter-enantiomers" there are numerous activities devoted to develop selective and sufficiently active racemization catalysts. Both chemical and enzymatic approaches are followed. Concepts and recent examples of racemization using metal catalysis are described in [312].

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

Referring to an example already discussed extensively above, interesting progress regarding the development of an efficient mandelate racemase is reported in [314]. In [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 [155]. The racemase was further successfully used for in situ racemization during preferential crystallization of the enantiomers of asparagine. Crystallization experiments accompanied by enzymatic racemization led to a significant increase of crystallized L-asparagine.[122, 155]

Within the mentioned INTENANT-project an integrated process for the separation of the enantiomers of the industrially relevant substance 2′,6′-pipecoloxylidide (PPX, Fig. 36) was investigated.[169] *(S)*-PPX is an intermediate in the manufacture of a number of local anesthetics.

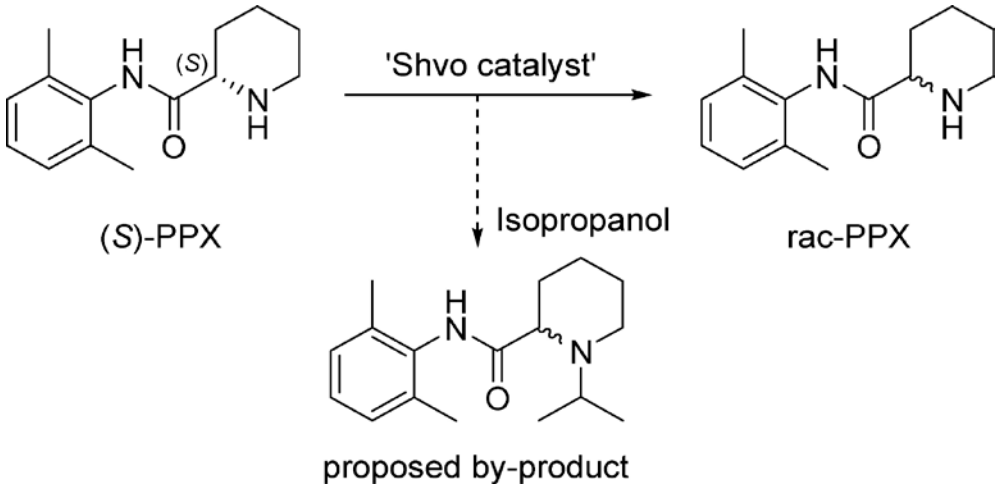

Figure 36: Structure of *(S)*-pipecoloxylidide (PPX) and reaction scheme for racemization[169].

The integrated chiral resolution process developed combines chromatography, crystallization, and racemization. The flowsheet of the entire process is illustrated in Fig. 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 2-phase region could be then used to isolate the pure *(S)*-PPX with a purity of ≥99.5%, while the remaining, enriched mother liquor is recycled into the chromatographic separation. The *(R)*-PPX-rich chromatography stream was then racemized using a homogeneous Ruthenium catalyst (Shvo-catalyst)[169, 312e], which provided efficiently a racemic mixture of PPX (Fig. 36), that could be recycled into the chromatography step together

with fresh feed. The presence of alcohols (isopropanol or ethanol) led to an undesired side reaction, which necessitated removing them out of the chromatography effluent prior to racemization by distillation. The rationally designed and successfully applied integrated process exploits dibutylether as a common basic solvent system and realizes a full recycling of all solvents and the undesired *(R)*-PPX. It could be experimentally validated on lab and pilot scale (gram and several 100 g, respectiviely) and provided both high productivity and yield.[169]

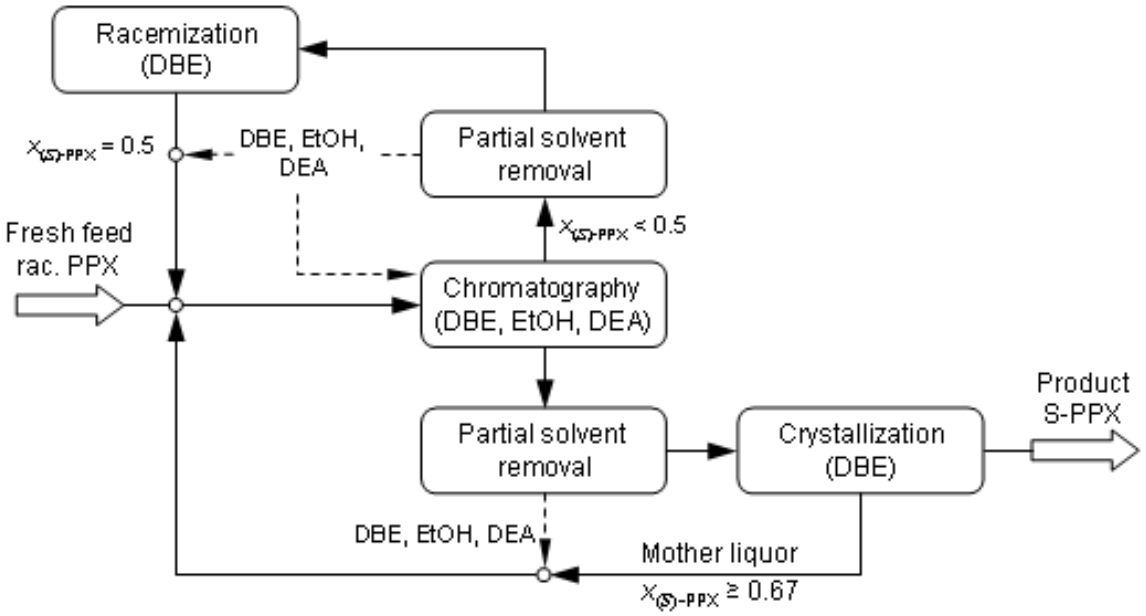

Figure 37: Integrated process scheme to separate racemic PPX. Initially the racemic feed is enriched by chromatography. The resulting (*S*)-PPX-rich stream is after creating supersaturation subjected to crystallization. The mother liquor is returned to the chromatographic enrichment. The (*R*)-PPX-rich stream is racemized and then also returned to the chromatographic separation. The function of intermediate distillations is to adjust the solvent compositions.[169]

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

7. Summary

Several possible concepts capable to separate enantiomers in a preparative manner have been described in this process oriented review demonstrating that the racemic approach provides a powerful alternative to enantioselective synthesis.

Crystallization-based methods and preparative chromatography have been presented in more detail based on various case studies. Currently these methods appear to be most advanced.

The selection, design and optimization of crystallization techniques require knowledge regarding the underlying solid-liquid equilibria which has to be gathered experimentally. Based on the specific type of phase diagram the most productive mode of operation can be identified.
Due to the availability of highly selective chiral stationary phases, preparative overloaded high performance liquid chromatography can be currently seen as the most versatile technique. The high pressure equipment required and the process complexities limit the applicability in larger scales. The breakthrough of the simulated moving bed technology contributes to further promoting chromatographic enantioseparation.
As a very promising approach is seen the application of hybrid processes combining the positive features of several separation processes. Although these processes are more challenging, there are nowadays rules and short-cut methods for a rational design available. it was shown within the INTENANT project that the achievable overall productivity can be increased significantly by e.g. generating initially an enrichment using chromatography followed by highly selective crystallization processes. Based on the results obtained regarding the two case studies described (bicalutamide and PPX) and many further examples a helpful decision tree was suggested considering at first the economically crucial question whether a compound can be racemized efficiently or not (Fig. 38).

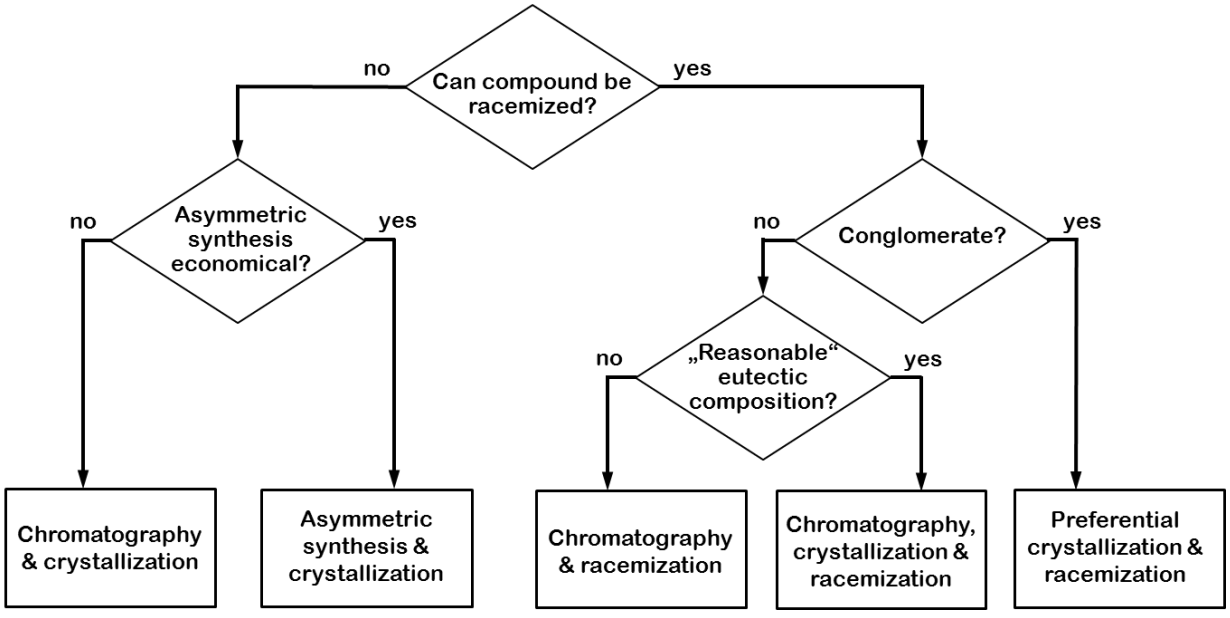

Figure 38: Decision tree based on simple qualitative criteria supporting a rational selection of a suitable process concept for the production of pure enantiomers.[based on 305]

If racemization is possible, the knowledge of the phase diagram of the specific chiral system is most instructive to decide a) about the presence of a conglomerate and to evaluate afterwards b) the eutectic composition in the chiral system. As a result of this information different resolution strategies open up, which might be e.g. a possible combination of racemization with just crystallization (right branch in Fig. 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. If both racemization and asymmetric synthesis are not feasible, e.g. chromatography and crystallization allows for resolution of such a racemate (left branch).

For the future can be expected, that enantioselective separation processes will be increasingly coupled in advanced integrated processes in order facilitate the acces to pure enantiomers.

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