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Review

Enantioselective chromatography as a powerful alternative for the preparation of drug enantiomers

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Abstract

The preparative separation of enantiomers by chromatography on chiral stationary phases (CSPs) has been recognized as being a useful alternative to the more conventional approaches such as enantioselective synthesis and enzymatically catalyzed transformations. The possible contribution of enantioselective chromatography with respect to the preparation of enantiomerically pure compounds is reviewed in the context of the competitive approaches and depending on the application scale, with a special emphasis on the recent progresses achieved in this particular field of separation.

Keywords: Reviews; Enantiomers separation; Chiral stationary phases, LC; Drugs

Contents

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1. Introduction

Chiral considerations are now integral parts of drug research and development and of the regulatory process. There is no choice! The enantiomers of all chiral bioactive molecules have to be isolated and to be tested. Besides the ethical or environmental reasons for developing single enantiomers, it may be a real therapeutic benefit and in some cases, it has been used as a strategy for extending patent life.

In this context, enantioselective chromatography on chiral stationary phases (CSPs) has become the most important tool for determining the optical purity of organic molecules and it is now a well established technique [1–7]. The method is routinely applied for analytical purposes, but the chromatographic separation of enantiomers on a preparative scale has also been recognized as a powerful alternative of supplying pure enantiomers of bioactive compounds and chiral synthons [8–11]. In the field of preparative chromatography it has even been the driving force for the acceptance of the technology as an industrial option, in particular since the introduction of the simulated moving bed (SMB) technology.

As the preparative separation of stereoisomers has been reviewed in several articles during the last ten years [8–10], this paper is focused on the progresses recently achieved in this particular field, emphasizing the state of the art of the technology and of the strategies currently applied.

To obtain the single enantiomers of chiral compounds, two approaches can basically be envisaged (Fig. 1).

The first one, ‘the chiral approach’, consists in designing an enantioselective synthesis of the desired enantiomer. If both enantiomers are needed, it is necessary to develop two independent syntheses. The chiral approach includes enantioselective synthesis using chiral synthons and auxiliaries, enzymes or stereoselective catalytic processes. In contrast to the chiral approach, the ‘racemic approach’ implies the preparation of the racemate which is subsequently resolved into the corresponding enantiomers. This preparation is usually achieved by a reaction sequence which generally presents a much lower

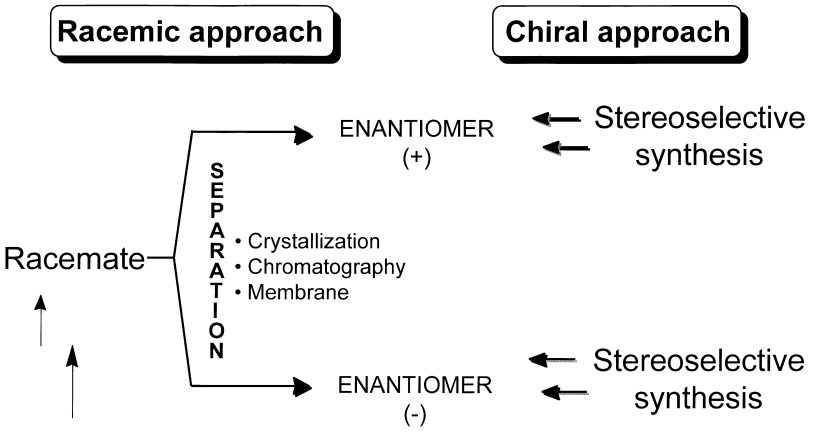

Fig. 1. Approaches to optically active compounds.

degree of difficulty than for the corresponding optically active forms. In the racemic approach the enantiomers are obtained via the separation of diastereoisomers (salt crystallization or chromatography) or the direct separation of enantiomers by chromatography on chiral stationary phases. The chromatographic method usually furnishes both enantiomers in high optical purity. This is particularly convenient and important for preliminary comparative biological testing where both enantiomers are obviously needed.

On the other hand, the current requirements for preparing the single enantiomers of chiral substances, strongly differ with the stage of development of the compound of interest, the goal being to produce a certain amount of pure enantiomers in a certain time frame and under certain cost limitations (Fig. 2).

At the discovery stage, time is the most important factor. The process must be rapid and generally applicable. In early development, the time frame is still relatively short and scale-up feasibility should already be considered. At the stage of full development, the process must be established, it must be robust and cost becomes an important factor. At the production scale, cost is a major concern and scale-up feasibility is obviously a prerequisite. Among the diverse options which can be applied to reach the goal of producing the single enantiomers, the best one will depend on the relative importance of the three mentioned factors, namely time lines, costs and scale-up feasibility.

2. Chiral stationary phases

2.1. Available chiral stationary phases

Nowadays, numerous chiral stationary phases are available for the preparative separation of enantiomers and the characteristics of most of these phases have been discussed in details in previous reviews [8–11]. However, based on the applications published over the last then years and on our own experience, it appears that the most successfully and broadly applied phases comprise the cellulose- and amylose-based phases developed by Okamoto (Chiralcel and Chiralpak) [12,13], some brush-type phases introduced by Pirkle and Welch [14], some polyacrylamide (Chiraspher) [15] and crosslinked di-

Amount	mg - 50 g	100 g - 10 kg	5 - 100 kg	tons
Needed isomer	both enantiomers	both enantiomers	active enantiomer(s)	active enantiomer(s)
Time frame	days	weeks	months	
Cost importance	minor	minor	middle	major
Scale-up feasibility	minor importance	middle importance	major importance	prerequisite

Options

- Synthesis (chiral pool, auxiliary)
- Catalytic process (ligand, enzyme)
- Diastereomers (crystallization)
- Chromatography

Fig. 2. Requirements for the preparation of chiral drugs.

allyltartaramide (Kromasil CHI) [16–18] derived phases, and to a much lesser extent some cyclodextrin-based phases [19,20]. Other phases recently developed, such as those designed from the macrocycles vancomycin and teicoplanin [21,22] have occasionally be used for the chromatographic resolution of a few milligrams of racemates [23], but the feasibility of large scale separations with these phases has not yet been demonstrated. If just a few micrograms of enantiomerically pure material is required, further commercially available CSPs may be used, and most of these micro-scale applications have been achieved on analytical columns. However, this type of application will not be discussed in this paper as it is unlikely that scale-up, even at the few gram scale is feasible. Recently, Lindner and coworkers developed several CSPs based on quinine and quinidine which also show a potential for preparative purpose [24,25]. Improvement of the chiral recognition power of these phases by rationally-designed structural modifications of the CSP have led to exceptionally high enantioselectivity which, of course, are of great interest for preparative applications [25].

Table 1 summarizes the currently most used CPSs for preparative separations and Fig. 3 shows the chemical structure of these CSPs. Although most of these phases are available in bulk quantities and have a sufficient loading capacity, in our hand, it appears that up to 90% of the racemic compounds can be resolved with not more than four different polysaccharide-based phases.

2.2. Loading capacity

The loading capacity of the stationary phase is an important parameter when preparative separations have to be performed. For the same reason, only a limited number of materials have been usually applied among the broad range of available chiral stationary phases. The estimated loading capacity of the most commonly used phases has already been previously discussed [10,11], and is in accordance with other evaluations recently reported for different phases [26,27]. However it must be emphasized that the optimal phase in terms of loading capacity may vary from one racemate to another depending on the structure of the racemic compound to be resolved, as it is a function of the number of accessible interaction sites per mass unit of phase. The usual range of loading capacity of the most common CSPs is shown in Fig. 4.

3. Throughput

In preparative chromatography, throughput is defined as the amount of purified material per unit of time and per unit of mass of stationary phase. It is affected by different factors including, loading capacity of the CSP, column efficiency, selectivity, temperature, column size, flow-rate, cycle time, and feed concentration which itself depends on the solubility of the solute [28,29]. Some factors are related to the properties of the CSP, other are related

Table 1
Currently most used chiral stationary phases for preparative separations

Name	Trade Name
Cellulose triacetate	CTA I
Cellulose tris(3,5-dimethylphenylcarbamate)	Chiralcel OD
Cellulose tris(4-methylbenzoate)	Chiralcel OJ
Cellulose tribenzoate	Chiralcel OB
Amylose tris(3,5-dimethylphenylcarbamate)	Chiralpak AD
Amylose tris[(*S*)-phenylethylcarbamate]	Chiralpak AS
Poly[(*S*)-*N*-acryloylphenylalanine ethyl ester]	Chiraspher
3,5-Dinitrobenzoylphenylglycine	DNBPG
Crosslinked di-(3,5-dimethylbenzoyl)-L diallyltartramide	Kromasil CHI-DMB
Crosslinked di-(4-*tert*.-butylbenzoyl)-L diallyltartramide	Kromasil CHI-TTB
Tetrahydro-aminophenanthrene 3,5-dinitrobenzamide	WHELK-O 1

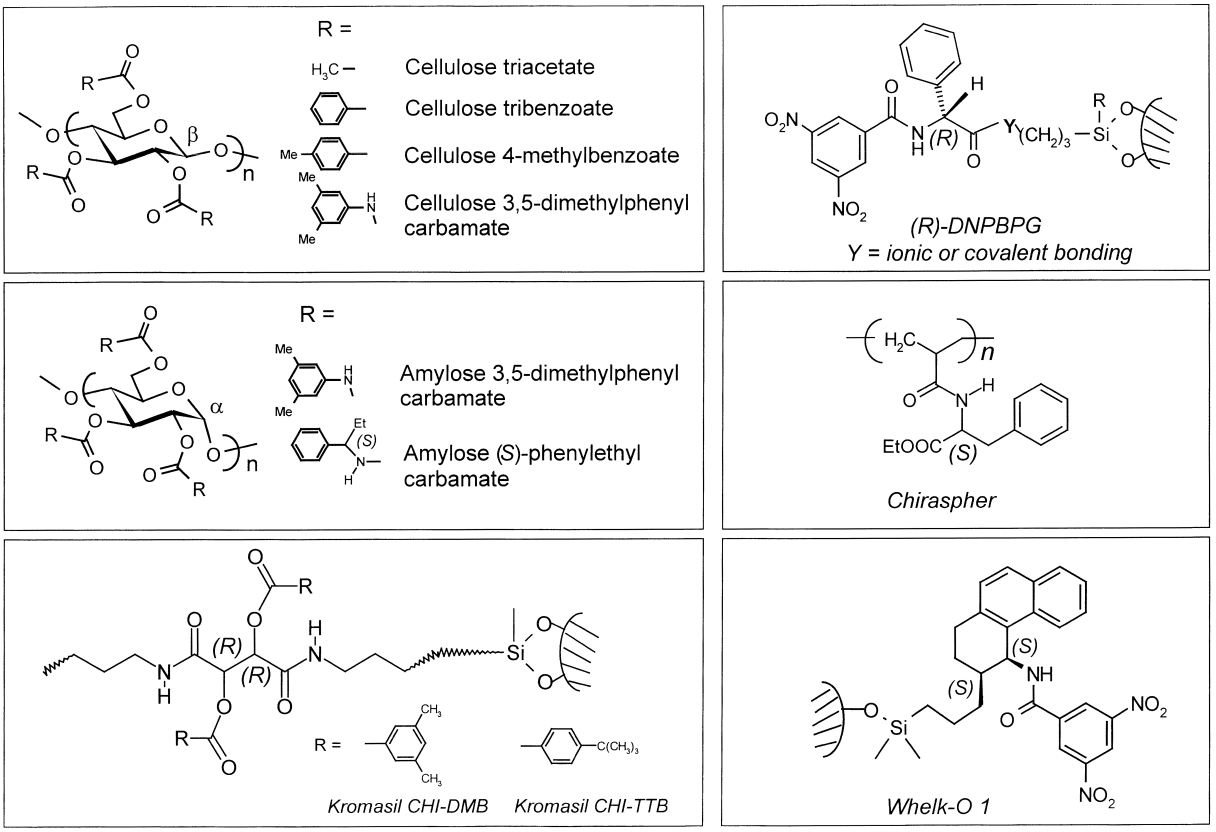

Fig. 3. Chemical structure of the most used commercially available CSPs for preparative separations.

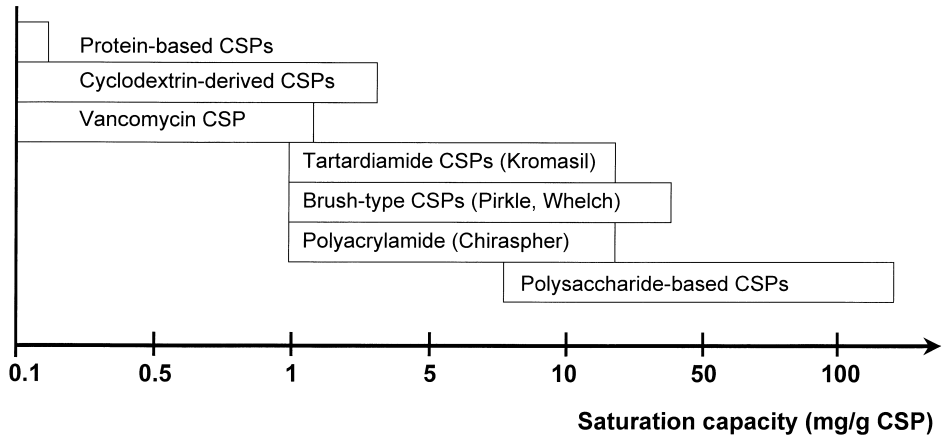

Fig. 4. Typical saturation capacity of the most used commercially available CSPs.

to the properties of the racemate, or to the technical capability of the system.

A general rule for estimating optimal throughput cannot be given and it must be determined for each case. It becomes even more complicated if one considers the different technical options available for the preparative separation of enantiomers. So far, high-performance liquid chromatography, gas chromatography, supercritical fluid chromatography, and simulated moving bed chromatography have been applied.

In HPLC, various options have also been used to improve throughput, including peak shaving and recycling [9,10,30–32].

All the considerations above show that optimal throughput is only reached when many factors are taken into account. In enantioselective preparative chromatography, special attention is given in particular to the loading capacity which is a characteristic of the stationary phase, flow-rate which is limited by the mechanical resistance of the material to pressure and its granulometry, and feed concentration which is often related to the solubility of the solute in the applied mobile phase.

It may occur that some factors are interdependent and influence throughput in opposite manner. For example, a high loading capacity associated with a low flow-rate because of limited pressure resistance of the CSP may result in a lower throughput compared to a medium loading capacity associated to a high flow-rate (short cycle time).

4. Mobile phase

The choice of the mobile phase is critical for at least three reasons, which directly influence throughput in preparative separations of enantiomers. Indeed, selectivity of the separation, retention time and solubility of the racemate often are very sensitive to changes of the composition of the mobile phase. Other parameters such as resolution (efficiency) can also be affected by the mobile phase. This influence has been shown for analytical separations, but no systematic investigations of the effect of the mobile phases have been reported on these parameters for preparative enantioselective separations. Of course, viscosity, which may limit the flow-rate, safety, costs, and solvent handling after the separation (evaporation, recycling) are other factors which are directly related to the choice of the mobile phase.

4.1. Mobile phase and retention

It is quite well known that mobile phase composition considerably influences retention time in chromatography. This effect is also observed for enantioselective separations on CSPs and has been exploited on an empirical basis for hundreds of analytical and preparative separations. Under normal-phase chromatographic conditions, reduction of retention time is usually achieved by increasing the content of the polar modifier. Alcohols like methanol, ethanol and isopropanol are the most commonly used polar modifiers, especially with the polysaccharide-based phases, but dichlomethane, ethyl acetate, dioxane have also been employed with the CSPs which tolerate these solvents. However, it cannot be predicted how this change affects the resolution and the selectivity. This field should clearly receive more attention and would benefit from the application of chemometric approaches and from the development of expert systems [33].

The possibility of managing retention time has become especially important in connection with the introduction of the simulated moving bed technology which is more effective in terms of throughput when shorter cycle times are applied.

4.2. Mobile phase and stereoselectivity

The mobile phase, which is often considered as an inert component, plays an essential role in the interaction process. It may not only influence the retention time but it can also considerably affect selectivity. This is particularly the case for the polysaccharide-based phases. There are examples of enantiomers which are not separated with a mixture of hexane/ethanol 9/1 and well separated on the same CSP with a mixture of hexane/2-propanol 9/1 [34].

Unfortunately, even if numerous reports mention such effects [34–49], practically no systematic investigation of this parameter has been carried out, intending to deliberately use the mobile phase as a practical optimization parameter. Only very recently,

Blackwell et al. reported on the investigation of the possible rationalization of such effects for a few chiral stationary phases [50], but the prediction of the influence of the mobile phase on the chiral recognition process clearly remains a challenge. Currently, automated screening devices are usually applied to help to find the optimal mobile phase composition.

In some instances, the impact of the mobile phase is so strong that changing its composition causes an inversion of the elution order. Therefore, before performing a preparative separation it is recommended to clearly establish the elution order of both enantiomers under the same conditions as those applied for the preparative separation. This can be done by polarimetric detection or by injection of the individual enantiomers. However, this must be carefully interpreted because for some chiral compounds, the signal of the optical rotation can invert when changing the solvent (Fig. 5).

4.3. Solubility of the chiral solute

Another critical aspect related to the mobile phase is the solubility of the chiral solute (racemate) to be resolved in the used mobile phase. In fact, the solubility of the solute is often the limiting factor in terms of throughput. For the CSPs which are constituted of chiral selectors chemically bonded to an insoluble carrier (mostly silica gel), there is practically no limitation regarding the choice of mobile phase and selectivity is the factor governing the choice. Ideally, the best selectivity under high solubility conditions should be applied. However, it is noteworthy that in many instances the highest selectivity is obtained with poorly solvating mobile phases while good solvents give lower selectivity. In practice a good compromise between selectivity and solubility has to be found. Among the most used preparative CSPs (Table 1), those derived from the polysaccharides cellulose and amylose are extremely popular and have now been used for large scale separations up to tones of racemates. Nevertheless, these phases have one major drawback, they are more or less soluble in many organic solvents such as tetrahydrofurane, dioxane, toluene, chorinated solvents, ethyl acetate, etc... This property considerably reduces the choice of mobile phase, thus limiting the possibility of increasing selectivity, of varying retention time, and of improving the solubility of the racemate. However, we recently designed a process to make the polysaccharide-based phase insoluble [51–53]. The process basically consists in a photochemical or thermal treatment of the usual coated polysaccharide stationary phases (Fig. 6).

By simple application of one of these processes, immobilization occurs by presumably crosslinking of the polysaccharide chains. Depending on the derivatizing group and on the polysaccharide type, the

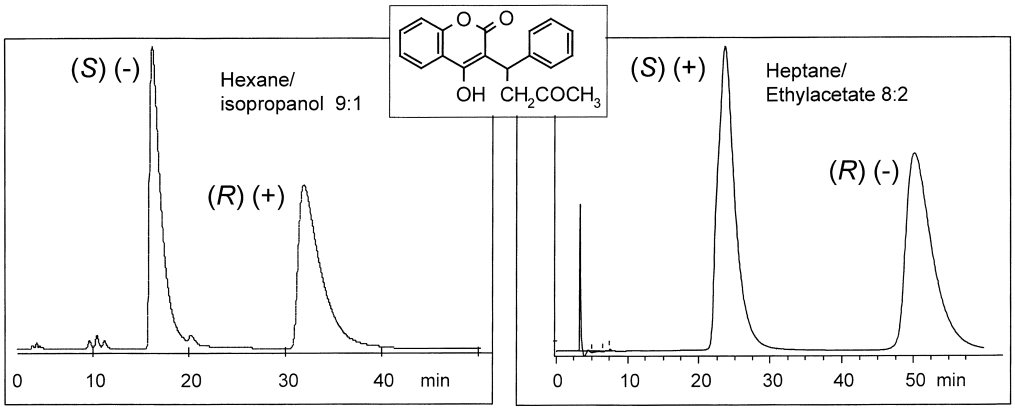

Fig. 5. Elution order and polarimetric detection (polarimeter Jasco OR-990, λ 350–900 nm); separation of the enantiomers of warfarin on immobilized cellulose tris(3,5-dimethylphenylcarbamate) coated on silica gel (column, 4×250 mm). Mobile phase: (a) hexane/2-propanol 90/10; (b) heptane/ethyl acetate 80/20.

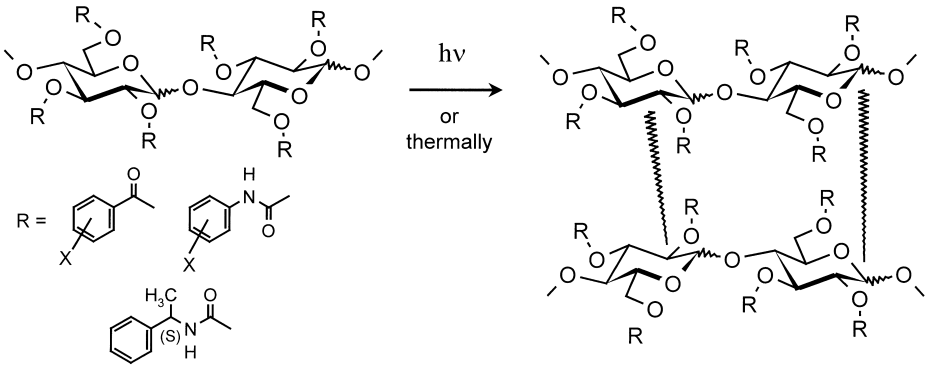

Fig. 6. Process of immobilization of polysaccharide-based CSPs according to [51,52].

thermal or photochemical process is the most effective one. However, the exact reaction mechanism which leads to immobilization is still not yet elucidated. A broad range of immobilized polysaccharide derivatives have been prepared according to the new processes and their chiral recognition power have been investigated. Fig. 7 demonstrates the advantages of the new immobilized polysaccharide-based phases.

Both racemates can be more or less successfully resolved by chromatography on cellulose tris(4-methylbenzoate) before of after immobilization using the conventional mobile phase consisting of a mixture of hexane/2-propanol 90/10 (v/v). However, using the immobilized stationary phase with chloroform in the mobile phase, an increase in selectivity is observed, the separation is much faster, and the solubility of the sample can be increased by a factor of 300–500. Similar improvements have been obtained for other racemates and with other immobilized polysaccharide-based CSPs [51–53]. Other approaches aiming to immobilize polysaccharide de-

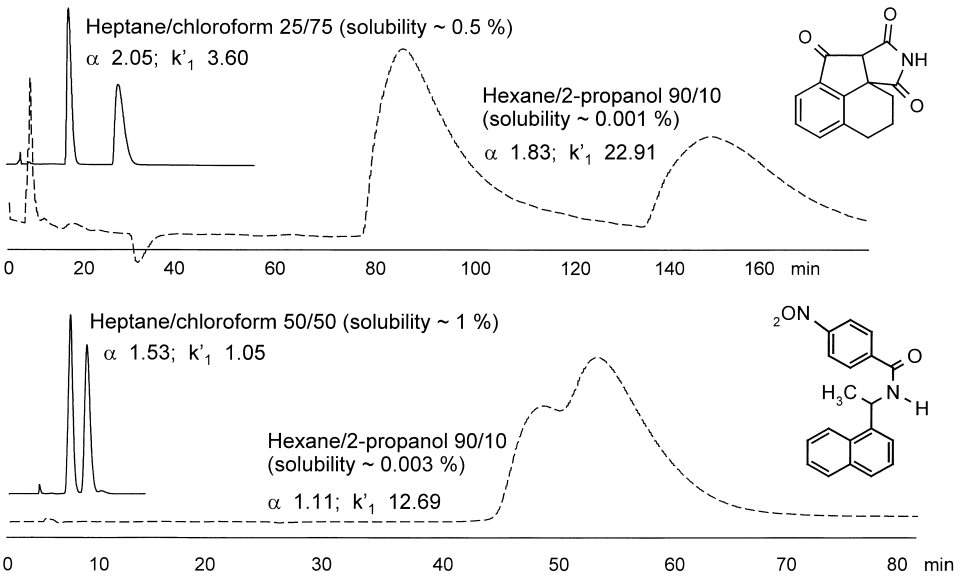

Fig. 7. Chromatographic separation of enantiomers on cellulose tris(3,5-dimethylphenylcarbamate) coated on silica gel (7 μm, 4000 Å) before (---) and after (—) immobilization (column, 4×250 mm). a) separation of the enantiomers a tetracyclic imide derivative. b) separation of the enantiomers of 1-naphthylethylamine para-nitrophenylamide.

rivatives in order to enlarge the choice of mobile phase, have been also developed. Okamoto and his group reported on attempts to immobilize cellulose on silica gel through a dicarbamate linkage using diphenyl diisocyanate as a crosslinking agent [54,55]. However, this approach necessitates additional synthetic steps and appeared to negatively affect selectivity as the number of linkage increases. More recently, the groups of Oliveros and Minguillon prepared a series of immobilized polysaccharide CSPs by reacting allyl silica gel with the undecenoyl side chains introduced onto the polysaccharide [56,57]. However, this process also necessitates additional synthetic steps and has the disadvantage of introducing a structural disturbing factor due to the presence of the undecenoyl ester moieties. In the latter work [57], it has been observed for one immobilized polysaccharide-based CSP, that the loading capacity of the CSP may be influenced by the type of mobile phase used. One preparative separation has been reported on a phase prepared according to this process [58].

5. Strategies for enantioselective separations

For the direct separation of enantiomers by chromatography on chiral stationary phases, two strategies are essentially applicable (Fig. 8). The first strategy consists in selecting the best available CSP for the racemic compound of interest, while the second option consists in modifying (derivatizing) the racemic solute to accommodate it to a defined CSP until it separates on this particular CSP.

5.1. Selecting the right CSP

The strategy 1 is generally applied first, pursuing the goal of identifying the CSP providing the best selectivity. Numerous preparative applications corresponding to this option have been reported. Some tools, such as electronic data base [59,60] and user guides are available for helping the choice of the appropriate CSP, but the screening of a few CSPs is still the more used approach in the practice. There are only rare cases for which the chromatographic separation could be predicted [61].

For chiral compounds of biological interest and especially at an early stage of development, it is generally preferred to separate the final active compound, even though a particular intermediate is much better separated. At larger separation scales, the situation may be different for cost reasons and it can be more appropriate to resolve a synthetic intermediate. In this instance, it is usually preferable to achieve the separation early in the synthetic process to reduce the amount of chemicals to be processed. However, a separation at an early stage of the

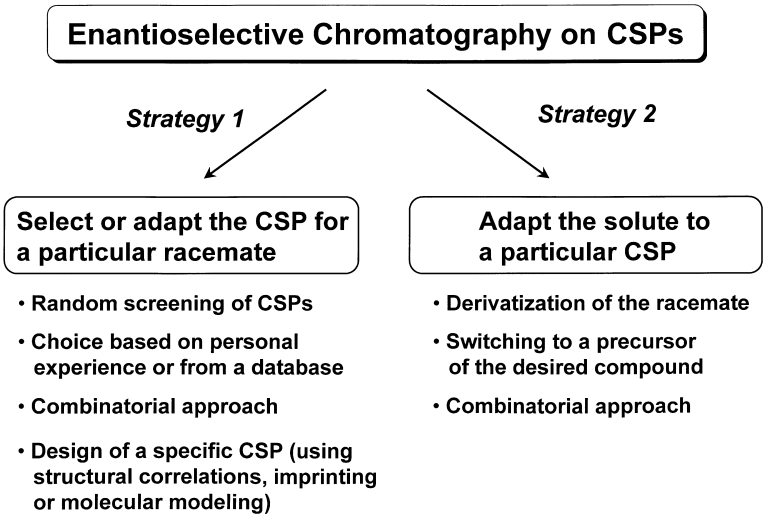

Fig. 8. Strategy for enantioselective separations by chromatography.

synthesis can also be disadvantageous because of the risk of racemisation in a later step. All these questions must carefully be answered before choosing the appropriate racemic structure, in particular when a large scale separation is planned.

In a few cases, the option of preparing tailor-made CSPs for a particular racemic structure has been applied. For example, we prepared on an empirical basis a particular polysaccharide-based CSP for the separation of the enantiomers of the enantiomers of the LTD4 antagonist iralukast and of the antimalaria agent benflumethol [62,63]. These two racemic drugs were only poorly resolved on the commercially available polysacharide-based phases whereas an excellent separation was obtained on the carbamate derivative of cellulose obtained from cellulose and 3-chloro-4-methylphenylisocyanate (Fig. 9). The prepared CSP was used for performing pharmacokinetic studies.

These examples shown how it is possible to improve enantioselective separations by slight modification of the CSP. The usefulness of the CSP shown on Fig. 9 was also discussed in several publications in which its preparation has been described in details [64–66].

On a more rational basis (concept of reciprocity), Pirkle and Welch developed also a particular CSP for the separation of the enantiomers of the analgesic agent naproxen and other non-steroidal anti-inflammatory drugs (NSAID) [67]. It appeared later that this CSP had a relatively broad application range [68].

For the preparation of 'optimal' CSPs, a new approach based on the combinatorial concept has recently been evaluated by different groups [69–74]. Although this approach is probably not very attractive for the development of specific CSPs for analytical purposes, it could be very valuable for preparative applications as optimized separation conditions may have a determining impact on cost. The approach consists in screening a great number of CSPs on a microscale in order to identify the CSP providing the best separation for a particular racemate. In different model experiments, this approach has been shown to be useful for the rapid development of highly selective CSPs, even on a preparative

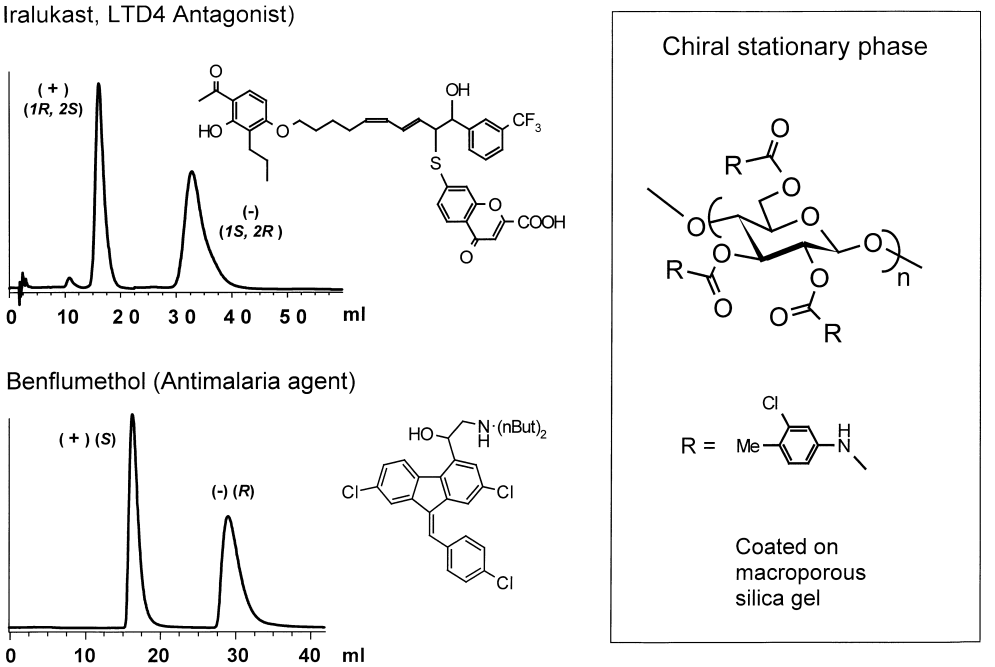

Fig. 9. Separation of the enantiomers of a) iralukast and b) benflumethol on a tailor-made cellulose-based CSP (cellulose tris[3-chloro, 4-methylphenyl-carbamate] of cellulose coated on silica gel). Column, 4.6×25 mm. Mobile phase, hexane/2-propanol 90/10 (1 ml/min).

scale [71]. However no practical preparative application of pharmaceutical relevance has been reported so far. The combinatorial approach has also been combined with the reciprocal concept of chromatographic separation [73,74], leading to the identification of improved CSPs for prostaglandin precursors, aryldihydropyrimidines and profen derivatives.

5.2. Selecting the racemic solute

The second strategy (Strategy 2 in Fig. 8) is also extremely valuable when the separation of the enantiomers of the racemic substance of interest cannot be achieved on available preparative CSPs. This approach consists in selecting a particular CSP and adapting the solute by altering its structure (e.g. derivatization). It can be particularly useful when a limited number of CSPs are available in the environment or when changing the CSP requires extensive and/or expensive modifications of the infrastructure. We frequently applied this strategy which led to successful resolutions of racemates that otherwise could not be separated on a defined CSP [63].

Using the cheep stationary phase cellulose triacetate, for example, we found empirically that the introduction of the para-nitrobenzoyl or para-chlorobenzyl group into the racemic structure could considerably enhance the separation. This strategy of derivatisation was applied to the separation of the enantiomers of various racemic compounds. Two examples of structures are shown in Fig. 10. The first example was elaborated in connection with the need

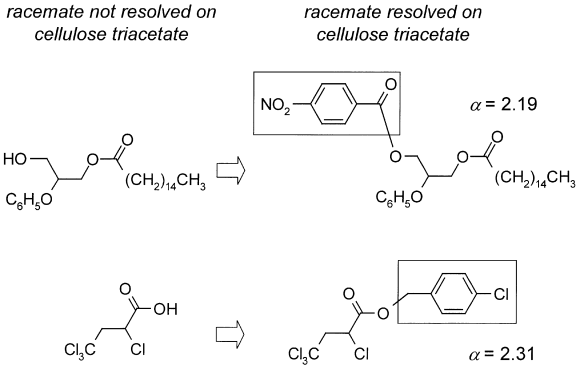

Fig. 10. Examples of achiral derivatisation for chromatographic separations on cellulose triacetate (CTA I). Column, 1.25×30 cm. Mobile phase, ethanol/water 95/5 [63].

for an enantioselective method to determine the optical purity of an intermediate used for the synthesis of lecithin. In the second example we applied the strategy to isolate the enantiomers of 1-chlorobutyric acid needed for an enantioselective synthesis.

We recently found that the same strategy of derivatisation can be generally applied to aliphatic and aromatic alcohols when tribenzoylcellulose is used as a CSP (Fig. 11) [75]. In this case, the first choice derivative is the para-methoxy benzoate, but for some alcohols, the best selectivity was obtained with the para-methyl or orho-methoxy ester [75]. These results are still difficult to explain, but they can nevertheless be exploited to solve practical problems, as shown for the enantiomers of an intermediate used for the synthesis of the chiral anticancer agent edatrexate [9,63].

6. Preparative enantioselective separations

The environment and the requirements associated with the development of new drugs strongly depends on the development stage of the compound of interest (Fig. 2).

6.1. Laboratory scale separations

At the discovery stage, the most important factor is time. The required amount usually ranges between a few milligrams to 50 g and the cost factor as well the scale-up feasibility are negligible. However, it is important to be able to isolate the single enantiomers in a short period of time in order to rapidly perform the biological test. Considering that most of the enantiomers of chiral molecules can now be separated on at least one of the few CSPs listed in Table 1 and in view of the requirements shown in Fig. 2, at this stage, chromatography is the most general, the most rapid and the most efficient method. In addition and as mentioned previously, it generally furnishes both enantiomers in one operation. At this stage, the development of an enantioselective synthesis for each new chiral entity would be much more time consuming, as it is common that the development of such a synthesis requires weeks or months. With the chiral stationary phases currently available for preparative separations, especially those derived from

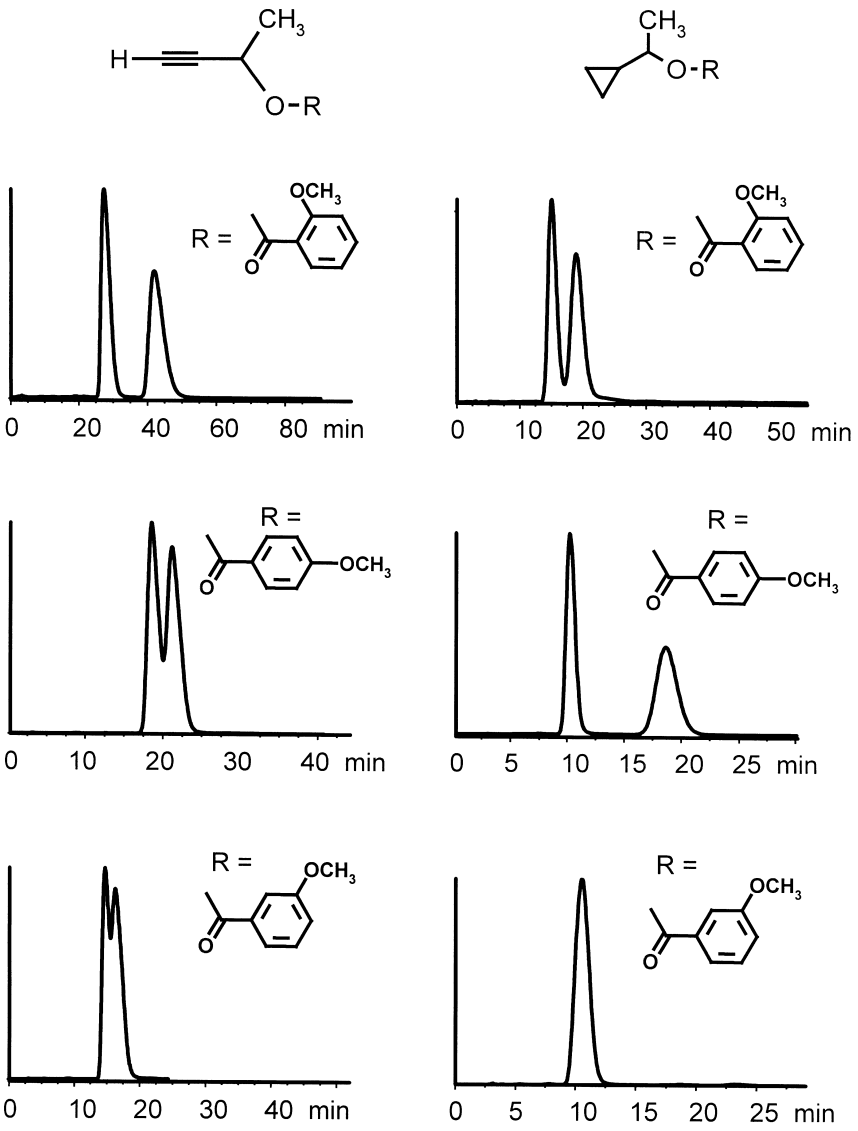

Fig. 11. Improvement of enantioselective separations on cellulose tribenzoate (beads) by means of achiral derivatisation [71].

polysaccharides, it is now possible to resolve almost all racemic compounds by chromatography at a scale of several grams just by scaling-up the analytical separation. This approach has now become an established procedure in many specialized laboratories and does not present any major difficulty. Figs. 12 and 13 show recent applications from our laboratory for the separation of drug candidates or drug intermediates which have been performed on respectively Chiralpak AD and on Chiralcel OJ [76]. Both the analytical and the corresponding preparative separations are shown.

Almost all small scale separations published till 1994 have been discussed in a comprehensive review collecting more than 85 pharmaceutical applications listed according to the major therapeutic indications [9]. In the mean time, some new applications based on the discussed strategy have been reported on polysaccharide-based CSPs [32,49,58,63,77–88], on Pirkle and Whelk-O CSPs [32,87,89,90], and on

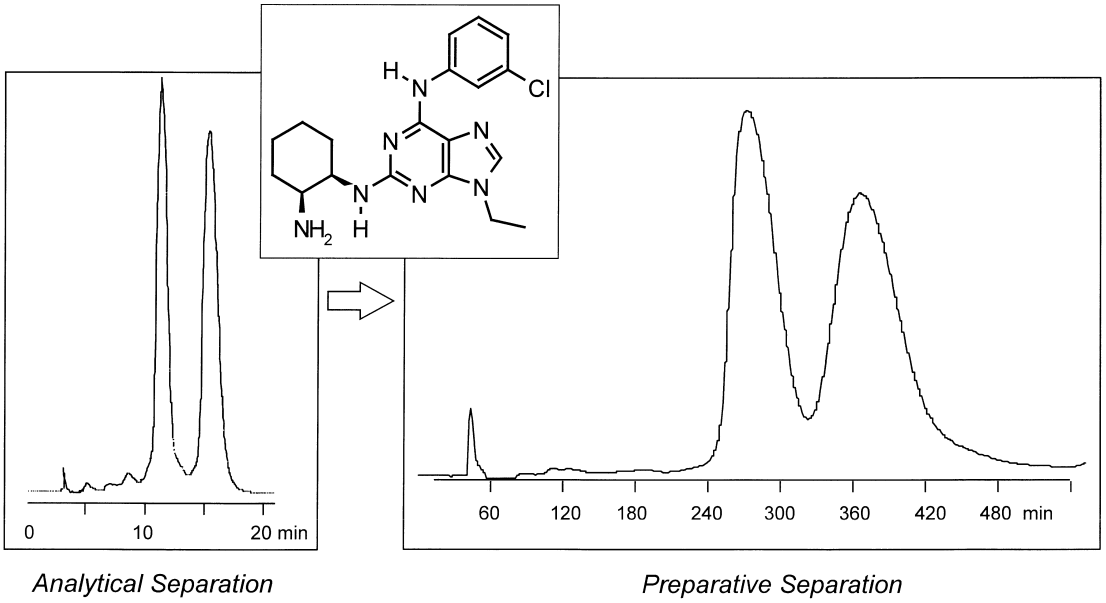

Fig. 12. Separation of the enantiomers of a cis-diaminocyclohexane derivative on amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD); mobile phase, heptane/2-propanol 90/10+0.1% diethylamine. a) analytical separation; column 4.6×250 mm, injection 20 μg, flow-rate 1 ml/min. b) preparative separation; column 50×500 mm, injection 500 mg, flow-rate 150 ml/min.

Kromasil CHI [17,18,87,91], but the number of published examples does probably not reflect the real degree of utilization of the method which is now routinely applied in many laboratories, especially in the pharmaceutical industry. This is particularly true if one considers that almost all analytical separations can potentially be up-scaled to a preparative separation. The mostly used technique is the classical

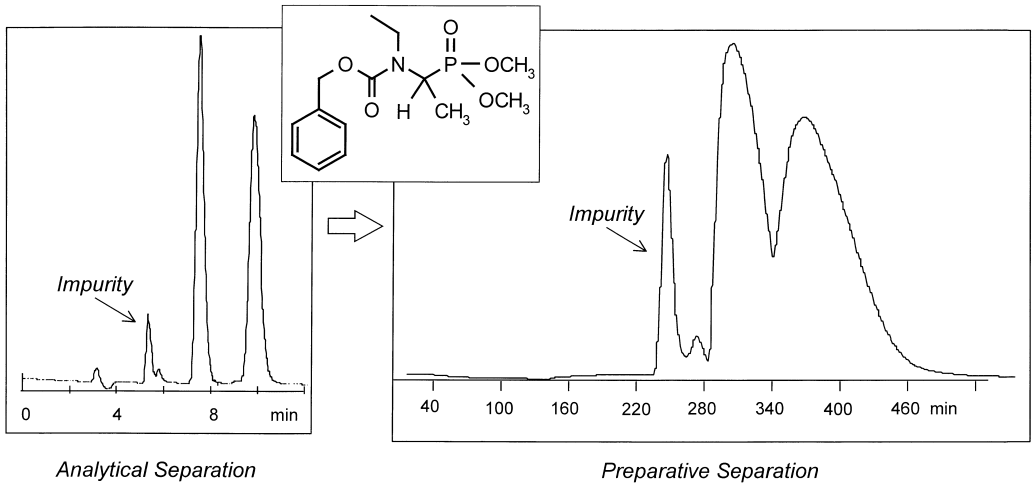

Fig. 13. Separation of the enantiomers of O,O-dimethyl-2-benzyloxycarbonyl-N-ehtylamino-phosphate on cellulose tris(4-methylbenzoate) (Chiralcel OJ): a) analytical separation; column 4.6×250 mm, mobile phase hexane/2-propanol 85/15, injection 20 μg, flow-rate 1 ml/min. b) preparative separation; column 100×500 mm, mobile phase heptane/ethanol 40/60, injection 4000 mg, flow-rate 140 ml/min.

elution batch chromatography consisting in introducing the solution containing the dissolved racemate on the top of the column and eluting with an appropriate mobile phase. The size of the column is variable and depends on the amount needed. Usually, enantioselective separations are performed under isocratic conditions, but when larger separation factors are observed, it may be appropriate to work under gradient conditions. On the other side, for more difficult separations or to improve throughput, the peak shaving and recycling techniques are often used. We systematically apply this technique in our laboratory for difficult separations and its usefulness has been generally recognized [10,63,30–32,84,89,91] and discussed in details [32].

Apolar normal-phase conditions have been normally applied, including supercritical fluid chromatography [10,92], but the option of using polar mobile phase conditions have also been successfully demonstrated [88]

6.2. Pilot scale separations

In early development where larger amounts of material are needed, the requirements change from those relevant to drug discovery (Fig. 2). Time frame is several weeks, cost importance is still moderate, but the scale-up feasibility becomes more important. Indeed, the process elaborated at this stage generally constitutes the basis for the development of the approach applied in process development.

At this stage, chromatography also constitutes an alternative to the classical approaches of producing optically active compounds, in particular since the introduction of the simulated moving bed technology in this application field. Hundred gram to several kilogram separations are now routinely performed in many specialized laboratories but relatively little is published because most of these applications have been carried in the industry. Two technological options are generally applied, the batch chromatography and the simulated moving bed chromatography (Fig. 14).

Various separations of enantiomers at a scale of 100 g and more have been reported in the batch chromatographic mode [10,32]. For this purpose, larger columns with an internal diameter ranging between 5 and 30 cm have generally been used. The desired amount of enantiomer has been obtained by repetitive injections of small quantities of racemate, most under overload conditions. To improve throughput, techniques such as overlapping injections and peak shaving/recycling have been applied. Recently published applications at this scale include the separation of the enantiomers of the benzylester of the analgesic agent oxindanac [9], of hetrazepine, of benztriazole and imidazole derivatives [32], and of glycine derivatives [84].

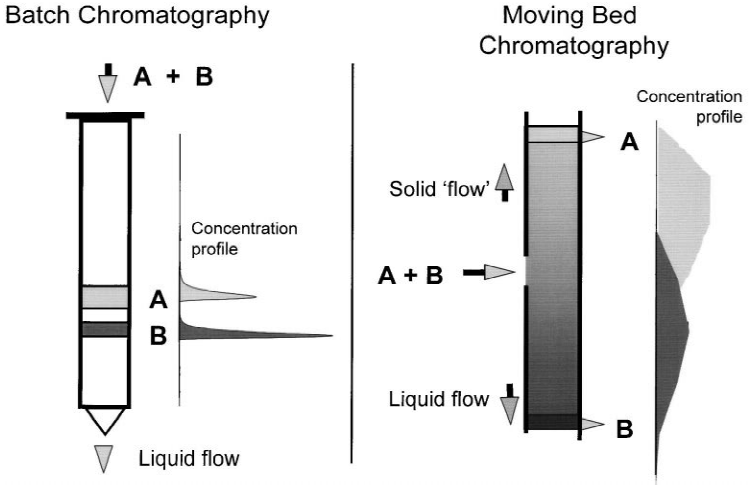

Fig. 14. Schematic representation of the technological options applied for enantioselective preparative separations by liquid chromatography.

For pilot scale separations the second option called 'simulated moving bed chromatography' (SMBC) is gaining increasing interest [91,93–110]. This technology is reviewed in another chapter of this volume and therefore it will not be discussed in details in this section. However, it has to be mentioned in this chapter, because it has largely contributed to radically change the level of acceptance of chromatography as a real and powerful alternative for the preparative isolation of enantiomerically pure compounds. The technical feasibility of scaling-up the technology and its relatively low running costs have rendered this technique very attractive to the pharmaceutical industry. SMBC typically operates under overload conditions, leading to nonlinear competitive adsorption behavior. Compared to the batch preparative chromatography, SMB technology shows several advantages. In SMBC the whole stationary phase is used for the separation while in batch chromatography only a small section of the column is involved in the separation (Fig. 14); this allows the productivity with respect to the stationary phase to be significantly increased. Moreover, the process is continuous and the solvent consumption is considerably reduced. In particular this latter characteristic makes the use of SMBC very useful even at relatively low separation scale.

The increasing interest of the pharmaceutical sector in this technology is emphasized by several examples recently reported in the literature [84,91,93–110]. These examples include the separation of the enantiomers of rolipram [91], of propranolol [98], of the antiasmathic agent formoterol [95,107], of the hypolipidaemic agent CGS 26214 [76], of various chiral building blocks [84], of the antitussive agent guaifenesine [107,108], of the anticancer agent orimeten [107], of the analgesic drug tramadol [106], and of various drug intermediates [26,94,97–99,109–111] at a scale of 50 to several hundred grams. In most of these investigations it was concluded that SMBC is clearly more efficient in terms of productivity and of mobile phase consumption than batch chromatography [84,91,105,107,111].

Recently, Grill reported on another elaborated technique for the separation of binary mixtures [112]. The method is called Closed-loop Steady State Recycling (CLSSR) and is similar to the simulated moving bed chromatography, although it mainly differs from it in that CLSSR is not continuous. However, this technology is considered to offer several advantages over SMB for small scale separations [113].

Most of the chiral SMBC separations reported so far have been achieved using polysaccharide-based stationary phases. In particular the high loading capacity of this kind of CSP has been recognized to be a major asset since productivities of 500 g to 1.5 kg of racemate per kilogram of CSP per day have been obtained in several instances.

6.3. Process scale separations

For full development, time frame can be longer, but cost and scale-up feasibility are determining factors. At this stage, it should actually be decided what the production process will be. Therefore, the available options to prepare the chiral compound of interest with the desired optical purity must be compared. As scale-up is technically no longer an issue for chromatography since the introduction of the simulated moving bed technology, this approach constitutes a new alternative for process scale and for production. The choice regarding what option should be applied at the time where the decision has to be made, will mainly depend on the cost balance of each process. In any case, it will constitute a case by case decision. In the cost calculation, many factors are involved including equipment and packing investment, running costs (solvent, energy), recycling costs, man power and environmental impact. Regarding the chromatographic process, it is clear that recycling the mobile phase and the possibility of recycling the 'wrong' enantiomer (after racemisation) will have a favorable impact on the cost calculation. Compared to an enantioselective synthetic approach, chromatography will be all the more attractive since the number or reaction steps for preparing the racemate (for chromatography) is considerably reduced compared to the number of steps needed for the asymmetric synthetic route.

There are currently only a very limited number of reported applications of chromatographic enantioselective separations at this scale and these few examples were probably only achievable thanks to the emergence of the simulated moving bed technol-

ogy in the pharmaceutical industry. The applications include the isolation of intermediates for the synthesis of drugs [114] and drug precursors [110,115].

6.4. Production scale separations

At a production scale, only one application has been reported so far using a polysaccharide-based stationary phase that was combined with the SMB technology. The annual capacity of the system, which is constituted of 6 columns (450 mm internal diameter) is about 12 tones of the particular racemate and the desired enantiomer is obtained with a purity of 98.5% with a recovery of 90% [114]. Another larger unit capable of processing annually several hundred tones of the same compound is currently implemented at Aerojet Fine Chemicals (Sacramento, CA, USA). It is also speculated that further companies are now building large production plans based on the SMB technique.

These different examples and the willingness shown by several companies to considerably invest into this new approach for preparing the enantiomers of chiral drugs clearly demonstrates the strong potential of the chromatographic method.

7. Other enantioselective chromatographic techniques

Further techniques have been investigated as potential methods for the preparation of optically pure compounds. Among these techniques, gas chromatography and supercritical fluid chromatography have already shown some capability in particular cases. They have been discussed in a recent review [10] and not very much has been newly published about applications using these technical approaches. This section will focus only on new applications of these techniques and on more speculative approaches.

7.1. Gas chromatography

Although gas chromatography (GC) is a well established method for the analytical determination of enantiomeric purity, the number of preparative applications is quite limited. Most of these preparative applications by gas chromatography have been recently reviewed [10] and were performed on a relatively small scale. The method is particularly suited for volatile compounds such as the inhalation anesthetic agents enflurane, isoflurane and desflurane [116] and it has also been recently applied to the resolution of racemic α-ionone [117]. The feasibility of separating the enantiomers by gas phase simulated moving bed chromatography has also been demonstrated for the first time and was applied to the anesthetic enflurane [118]. However, the productivity of the system was relatively low.

7.2. Electrophoretic methods

Electrophoretic methods are widely used alternatives for the analytical determination of enantiomeric purity of chiral compounds [119]. Due to the high efficiency of capillary electrophoresis, separations can be achieved even when very low selectivity are observed. At a preparative scale, these methods are well established for the purification of proteins and cells [120] but there is very little published on enantioselective separations. Only recently, some interest for chiral preparative applications has been manifested. Separation of the enantiomers of terbutaline [121] and piperoxan [122] have been reported by classical gel electrophoresis using sulfated cyclodextrin as a chiral additive, while the separation of the enantiomers of methadone could be successfully achieved by using free-fluid isotachophoresis [123] and by applying a process called interval-flow electrophoresis [124]. In a model experiment, it was also demonstrated that the enantiomers of dansylphenylanine can be separated by isoelectric focusing with hydroxypropyl-β-cyclodextrin as the chiral resolving agent [125]. The feasibility of separating the enantiomers of chlopheniramine on a micropreparative scale was also recently shown by applying flow-counterbalanced CE using carboxymethyl β-cyclodextrin as a chiral selector [126]. All these applications were performed at a mg scale or even less and cannot currently compete with the liquid chromatographic methods discussed previously. However, the scope and limitations of the electrophoretic approach still need to be challenged.

7.3. Membranes

Several attempts to perform enantioselective separations using membranes constituted of a chiral mobile carrier have been reported and have been extensively discussed in a recent review [127]. Various chiral carriers, mainly crown ethers, were used for this purpose but poor enantioselectivity have been usually obtained and no preparative application has been described.

Further investigations have led to the development of more efficient systems based on the concept of hollow-fiber membranes. The first applications of this type were reported by Pirkle and Bowen for the separation of the enantiomers of amino acid derivatives using the dioctylamide of (*S*)-*N*-(1-naphthyl)leucine as a chiral selector [128,129], and by Ding et al. for the enantiomers of leucine using *N*-dodecylhydroxyproline as the chiral carrier [130]. More recently, Nakamura et al. shown the resolution of racemic tryptophan using a bovine serum albumin-multilayered porous hollow-fiber membrane [131]. Okamoto and co-workers also demonstrated the feasibility of separating enantiomers using a cellulose-based membrane for the resolution of racemic oxprenolol [132].

As a continuous process, the hollow-fiber membrane technology shows some potential for preparative use in terms of throughput and cost. However, its relatively poor efficiency currently restricts its applicability to separations exhibiting high enantioselectivity.

8. Conclusion

There is no doubt that chromatography can be considered as a powerful alternative for the preparation of optically pure compounds. At the laboratory scale it is the method of choice as it is rapid, easily and generally applicable, and it furnishes both enantiomers. At a pilot and process scale, the chromatographic approach permits to ensure a continuous supply of optically pure substances in quantities required to perform the desired biological investigations while other preparative approaches are evaluated. Even at a production scale and especially since the introduction of the simulated moving bed technology, chromatography must now be considered as one of the possible approaches for obtaining single enantiomers. However, as cost is a major factor at production scale, it will be determining for deciding which approach should be applied, and the choice will remain a case by case decision. Nevertheless, the implementation of the SMB technique at a scale of several tons per year already demonstrates the real potential of this approach.

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