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Review

Contribution of preparative chromatographic resolution to the investigation of chiral phenomena

Eric Francotte

Pharmaceuticals Division, Research Department, K-122.P.25, Ciba-Geigy Limited, CH-4002 Basle (Switzerland)

ABSTRACT

In the context of the investigation of the consequences of chirality on physical and biological phenomena, the demand for new general methods for preparing optically pure compounds in growing rapidly. In this respect, preparative chromatographic resolution on chiral stationary phases has been recognized as a powerful tool and its usefulness is demonstrated in this review with applications covering different fields and different kinds of molecular chirality. The applications are arranged in tables according to their use such as for drugs, pesticides, chiral agents and auxiliaries, or to their stereogenic unit.

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1. INTRODUCTION

The chirality principle is the source of diverse phenomena on both the macromolecular and the molecular levels, governing the environment and the existence of living organisms. Although the origin of chirality in life is still obscure, it has a profound influence on most biological mechanisms and the significance of this principle has now been generally accepted. Because of the interest in the consequences of chirality on the physical and biological properties of molecules, the preparation of enantiomerically pure compounds is a topic of increasing importance and methods of supplying optically pure isomers are being intensively pursued. Among the different methodologies developed for this purpose, chromatographic resolution on chiral stationary phases (CSPs) has been recognized as a useful approach.

There are actually two possible approaches for preparing enantiomerically pure compounds (Fig. 1). The first is the design and elaboration of a stereoselective synthesis leading to the production of one enantiomer. The second consists in the preparation of the racemic compound, which is subsequently resolved into its antipodes, usually leading to the production of both enantiomers. Each approach has its advantages and disadvantages.

Enantioselective synthesis usually needs a chiral starting material as a building block or as an auxiliary. Catalytic procedures where the chiral information is transferred from the chiral catalyst to the prochiral substrate or where one enantiomer is preferably transformed (*e.g.*, enzymatic resolutions) are also often applied in the enantioselective approach. The degree of difficulty is generally related to the desired degree of optical purity. In contrast, in the first step of the "racemic approach" the substrate is prepared as the racemate by a reaction sequence which

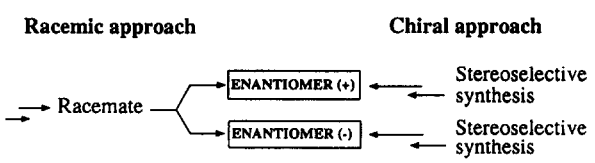


Fig. 1. Preparation of enantiomers.

generally presents a much lower degree of difficulty than for the corresponding optically active forms. In the second step, the enantiomers of the racemate are separated via the formation of diastereoisomers or by chromatography on chiral stationary phases. Although the separation using the formation of diastereoisomers is still often used, especially for compounds bearing acid or base functions, the contribution of the direct separation of enantiomers by chromatography on chiral stationary phases is rapidly increasing. The main advantages of this last technique are the following: application to a broad variety of racemic structures; both enantiomers are usually obtained; high degree of optical purity of the isolated enantiomers; rapid and easy achievement; and separation of the enantiomers of racemates with special features such as compounds which cannot be derivatized (hydrocarbons), which easily racemize or have an unusual kind of chirality (*e.g.*, helical or propeller-type chirality).

However, like all methods, the chromatographic approach suffers from certain drawbacks, such as the high cost of the stationary phases, the high dilution conditions, the consumption of large amounts of mobile phase and the difficulties associated with recycling of the mobile phase. These are obstacles to scale-up, but ones that could to a large extent be overcome thanks to recent improvements in chromatographic techniques and the development of new, relatively cheap chiral stationary phases for preparative purposes. Although chromatography is generally considered an expensive technique, it is coming to be regarded as both technically and even economically attractive for the preparation of high-value compounds, or of optical isomers that are otherwise accessible only with difficulty. Amounts of up to several kilograms of optically pure isomers are already being produced using this technology, and it seems likely to afford a useful approach to the manufacture of chiral drugs that are very potent (*i.e.*, produced in small amounts) and cannot easily be prepared by other methods. The combination of this application to chiral compounds with the simulated moving-bed chromatographic process, in particular, could considerably reduce produc-

tion costs and save large amounts of mobile phase.

The utilization of chromatography on CSPs has permitted the preparation of optically active isomers needed for various applications covering the investigation of biological activities, the investigation of interaction mechanisms, the determination of chiroptical properties, the preparation of chiral synthons and more generally the investigation of chiral phenomena. This review surveys the contribution of chromatography on CSPs to these different applications, emphasizing the versatility of the method but without discussing the interaction mechanism between CSPs and solutes, as this aspect has already been discussed in different original publications and surveys [1–3].

2. CHIRAL STATIONARY PHASES

The chromatographic separation of enantiomers on chiral stationary phases (CSPs) has undergone a spectacular development owing to the concomitant evolution of the chromatographic techniques and the design of numerous new chiral phases. Numerous analytical columns have become available and are now routinely used for the determination of the enantiomeric composition of mixtures of optical isomers from enantioselective syntheses, from biological investigations or from pharmacokinetic or toxicological studies [1–3]. The preparative potential of the method has also been recognized [4] and the number of applications is rapidly growing mainly because of the necessity of isolating the optically pure enantiomeric forms of new chiral biologically active compounds.

One can fundamentally distinguish two kinds of CSPs, chiral polymers (Type I) and achiral matrices (*e.g.*, silica gel) modified with chiral moieties (Type II) (Fig. 2).

In the first class (Type I), owing to the nature of the polymeric structure, the density of chiral information is generally high and the simultaneous participation of several chiral interaction sites or several polymeric chains is conceivable. This is the "multimolecular interaction" approach. The polymer can be in pure form or in a diluted form when coated or grafted. Nevertheless, even in the "diluted" form, the possibility for a multimolecular interaction is maintained. Oligo- and polysaccharides and their derivatives, polyacrylamides, polyacrylic esters and protein-based phases belong to this type of CSP.

The second most often used approach to preparing chiral sorbents consists in attaching optically active units to achiral carriers (mainly silica gel) by means of ionic or covalent bonds. A wide range of optically active moieties have already been applied, including amino acid derivatives, crown ethers, cinchona alkaloids, carbohydrates, amines, tartaric acid derivatives, cyclodextrins and binaphthol. Although the silica carrier is also a polymer, in this class of CSPs the chiral interaction sites distributed at the surface or in the network of the achiral support are

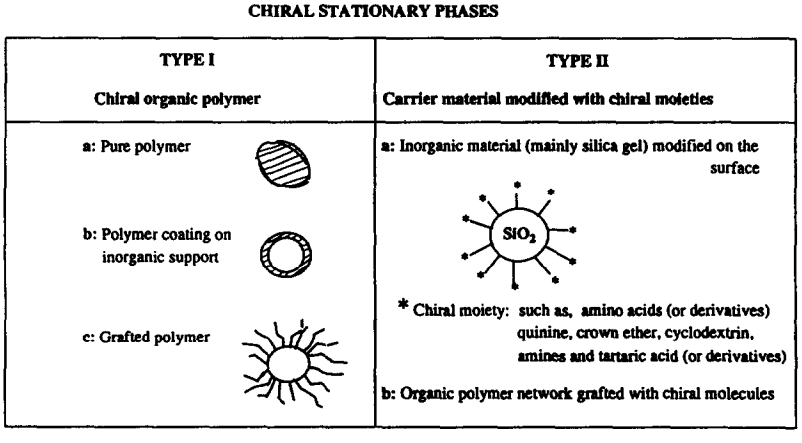


Fig. 2. Classification of chiral stationary phases.

relatively far away from each other and virtually only a "bimolecular" stereoselective interaction is possible between the chiral solute and the chiral selector. However, by using a dimeric solute bonded via a long spacer, Pirkle was able to induce a double bimolecular interaction involving two chiral selectors simultaneously [5]. Cyclodextrins as chiral selectors constitute an intermediate case because the inclusion complexation with this macromolecule involves the interaction with several glucose residues but nevertheless, a simultaneous interaction with two or more cyclodextrin molecules is very unlikely. In both classes of CSPs the classical interaction forces such as ionic, hydrophobic, dipolar and $\pi-\pi$ interactions, hydrogen bonding can be involved.

The properties of the CSPs developed for preparative purposes have recently been reviewed, including a table summarizing the commercially available CSPs [4]. The CSPs used for practical preparative applications are briefly presented below and more information concerning these CSPs is available in a previous paper [4]. A list summarizing the different CSPs mentioned in this review in relation to preparative applications is given in Table 1.

2.1. Cellulose and amylose derivatives

A wide range of cellulose-based stationary phases (Fig. 3) have been developed during the last 10 years [6]. These polymeric materials are used as pure polymers in a form suitable for chromatographic purposes or as a coating on an inert achiral support conferring mechanical stability.

So far, the most widely used derivative for separations on a preparative scale is cellulose triacetate (CTA-I), introduced in its fully

TABLE 1

CHIRAL STATIONARY PHASES REFERRED TO IN TABLES 2–14

Abbreviation	Chiral selector	CSP Type
CD-Poly	Cross-linked β-cyclodextrin polymer	IIb
ChiraDex	β-Cyclodextrin	IIa
Chiral-AGP	α-Acid glycoprotein	Ic
Chiralcel OB	Cellulose tribenzoate	Ib
Chiralcel OC	Cellulose phenylcarbamate	Ib
Chiralcel OD	Cellulose 3,5-dimethylphenylcarbamate	Ib
Chiralcel OJ	Cellulose *p*-methylbenzoate	Ib
ChiralPak AD	Amylose 3,5-dimethylphenylcarbamate	Ib
Chiraspher	Poly[(*S*)-N-acryloylphenylalanine ethyl ester]	Ic
CTA-I	Cellulose triacetate	Ia
Cyclobond I	β-Cyclodextrin	IIa
DACH-DNB	*trans*-Cyclohexyldiamine 3,5-dinitrobenzamide	IIa
DNBLeu	3,5-Dinitrobenzoylleucine	IIa
DNGPG-co	3,5-Dinitrobenzoylphenylglycine (covalent bonding)	IIa
DNBPG-io	3,5-Dinitrobenzoylphenylglycine (ionic bonding)	IIa
DNB-Tyr-A	3,5-Dinitrobenzoyltyrosine butylamide	IIa
DNB-Tyr-E	3,5-Dinitrobenzoyltyrosine methyl ester	IIa
MMBC	Cellulose *m*-methylbenzoate beads	Ia
NAP-Al	Naphthylalanine	IIa
PMBC	Cellulose *p*-methylbenzoate beads	Ia
Poly-CHMA	Poly[(*S*)-N-acryloylcyclohexylethyl amine]	Ia
Poly-MA	Poly(N-acryloylmenthylamine)	Ia
Poly-PEA	Poly[(*S*)-N-acryloylphenylalanine ethyl ester]	Ia
PTrMA	Poly(triphenylmethyl methacrylate)	Ic
TBC	Cellulose tribenzoate beads	Ia
Ultron ES-OVM	Ovomucoid protein	Ic

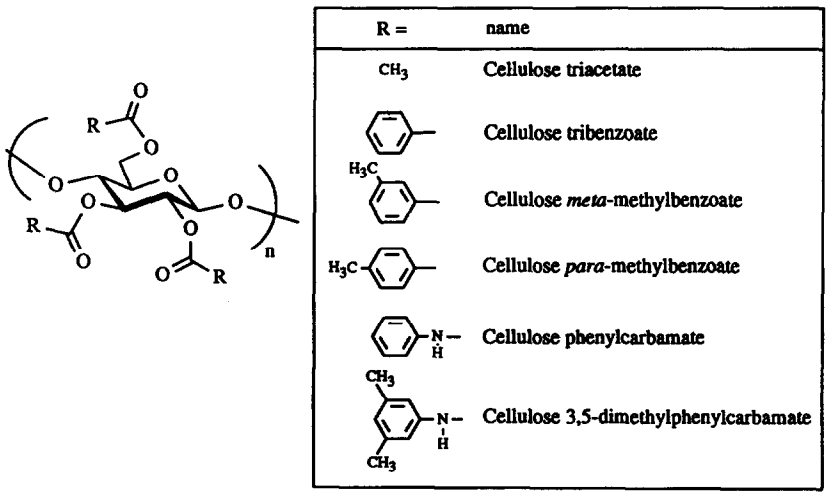


Fig. 3. Structures of cellulose derivatives.

acetylated form by Hesse and Hagel in 1973 [7]. This CSP is able to resolve a broad range of structurally different racemates [4]. The great versatility, the high loading capacity and the low preparation costs have certainly contributed to the extended use of this sorbent, even if they are some practical limitations [4]. Various benzoylcellulose derivatives have also been developed in the pure polymeric form [8–10] or as a coating on silica gel [11]. A wide range of racemic structures have also been resolved on phenylcarbamate derivatives of cellulose [12] on an analytical scale but the number of preparative applications is still limited, probably because of the high costs of these CSPs.

Some amylose derivatives have also been developed as a coating on silica and investigated as CSPs [13,14]. The 3,5-dimethylphenyl carbamate derivative seems to be particularly useful, and preparative resolutions have already been reported.

Except for microcrystalline cellulose triacetate (CTA-I), which has mostly been used in the reversed-phase mode (usually with methanol– or ethanol–water mixtures), the cellulose- and amylose-based CSPs have been used under both normal- and reversed-phase conditions. Improvement of the selectivity and/or resolution is generally achieved by varying the composition of the mobile phase.

2.2. Cyclodextrin-based phases

Cyclodextrins (CDs) are cyclic oligosaccharides (Fig. 4) exhibiting the property of forming stable inclusion complexes in their highly hydrophobic cavity with a wide variety of molecules [15]. The size of the cavity, which differs for α-, β- and γ-cyclodextrins, and the substituent on cyclodextrin play a determining role in the ability for complexing a defined molecule. Cyclodextrin CSPs were prepared by immobilizing CD in polymeric structures [16,17] or on silica gel [18,19], the latter CSPs showing good performance on an analytical scale. Preparative applications using cyclodextrins as chiral hosts were

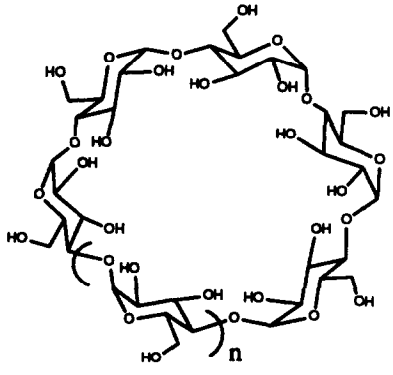


Fig. 4. Structure of cyclodextrin.

reported on polymers obtained by cross-linking of cyclodextrin with bis(epoxypropyl) ethylene glycol [17] and on the silica-modified materials Cyclobond I [20] and ChiraDex [21].

Optimization of the enantioselectivity can be achieved by modifying different factors, such as the concentration and nature of organic modifiers, pH, temperature and buffer concentration. Although reversed-phase conditions are usually applied with the cyclodextrin-based CSPs, analytical applications in the normal mode have also been reported [22].

2.3. Poly(meth)acrylamides

Cross-linked, optically active polyacrylamides and polymethacrylamides constitute another class of polymeric CSPs. These CSPs were introduced some years ago by Blaschke and co-workers, who rapidly demonstrated their usefulness for preparative applications [23]. However, the gel structure of these cross-linked polymers prevents their utilization at high pressure. Improvement of the mechanical performances of these CSPs was achieved by the same group by polymerization of the acrylic monomer on the surface on silica gel, giving a grafted polymer [24,25].

The preparative separations reported in the literature have been carried out using (*S*)-phenylalanine ethyl ester, (*S*)-1-cyclohexylethylamine and menthylamine [26,27] as the chiral selector (Fig. 5).

Although these materials are polymers, they probably interact in a "bimolecular" way like Type II CSPs (Fig. 2), the polyacrylamide backbone playing the role of an achiral matrix and the amide part constituting the chiral unit. Indeed, differential scanning calorimetric measurements and X-ray diffraction analysis suggest that these polymers are amorphous [28]. This lack of regularity of the polymeric structure prevents an amplification of the chiral recognition, owing to the participation of several chiral moieties in a concerted way, as has been speculated for most of the polysaccharide-based phases. Both the normal- and reversed-phase modes can be used with these CSPs.

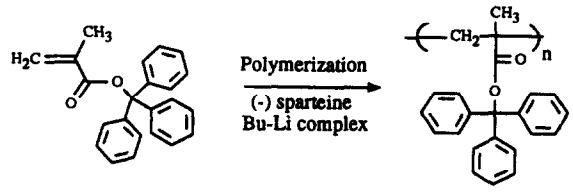
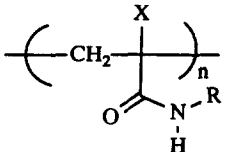


Fig. 6. Structure of poly(triphenylmethyl methacrylate).

2.4. Poly(triphenylmethyl methacrylate)

By initiating the polymerization of triphenylmethyl methacrylate with a chiral anionic catalyst such as (−)-sparteine–butyllithium complex, Yuki *et al.* [29] were able for the first time to prepare a fully synthetic optically active polymer whose chirality is caused only by helicity (Fig. 6). This polymer was developed, originally in its pure form and later as a coating on silica gel, as a new CSP for the separation of enantiomers. Although the large-scale resolution of racemic compounds is probably not feasible because of the limited chemical stability of the polymer, some interesting semi-preparative resolutions could be achieved on this CSP. Poly(triphenylmethyl) methacrylate (PTrMA) seems to



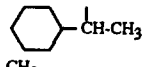
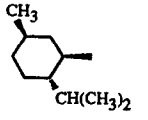
X =	R =	Abbreviation (Table 1)
H	$H_5C_6CH_2\underset{}{C}HCOOEt$	Poly-PEA
CH_3		Poly-CHMA
H		Poly-MA

Fig. 5. Structures of the poly(meth)acrylamide CSPs.

be particularly adapted to resolve compounds having an axial (C_2 symmetry), planar or helical chirality. Most of the reported resolutions were performed with methanol or methanol–hexane mixtures as the mobile phase.

2.5. Protein-based phases

A number of CSPs have been developed by immobilizing proteins or enzymes such as bovine [30] or human [31] serum albumin, α_1-acid glycoprotein [32], α-chymotrypsin [33] and ovomucoid [34,35] on silica gel. These CSPs usually operate under reversed-phase conditions (phosphate buffers with addition of organic modifiers) and exhibit a very high chiral recognition ability towards various classes of racemates. Enantioselectivity and retention can be regulated by changing the pH, the concentration or nature of the modifier and the mobile phase composition [35–39]. The presence of different types of highly stereoselective interaction sites renders these CSPs very versatile, but because the number of interaction sites is very limited, the loading capacity is low. This feature and the high costs of these CSPs are an obstacle to their use on a preparative scale. Nevertheless, a few semi-preparative (0.1–3-mg scale) applications have been reported on the α_1-acid glycoprotein phase, demonstrating the feasibility of scale-up [35,40]. Although this class of CSP does not exhibit the features required for large-scale separations, it could be useful for the resolution of racemic compounds for which only small amounts are required (reference, labelled compounds).

2.6. π-Acid and π-base phases

The best known CSPs of the π-acid or π-base type are the "Pirkle phases", classified into π-acceptor and π-donor phases [41]. The most frequently used π-acceptor phases are derived

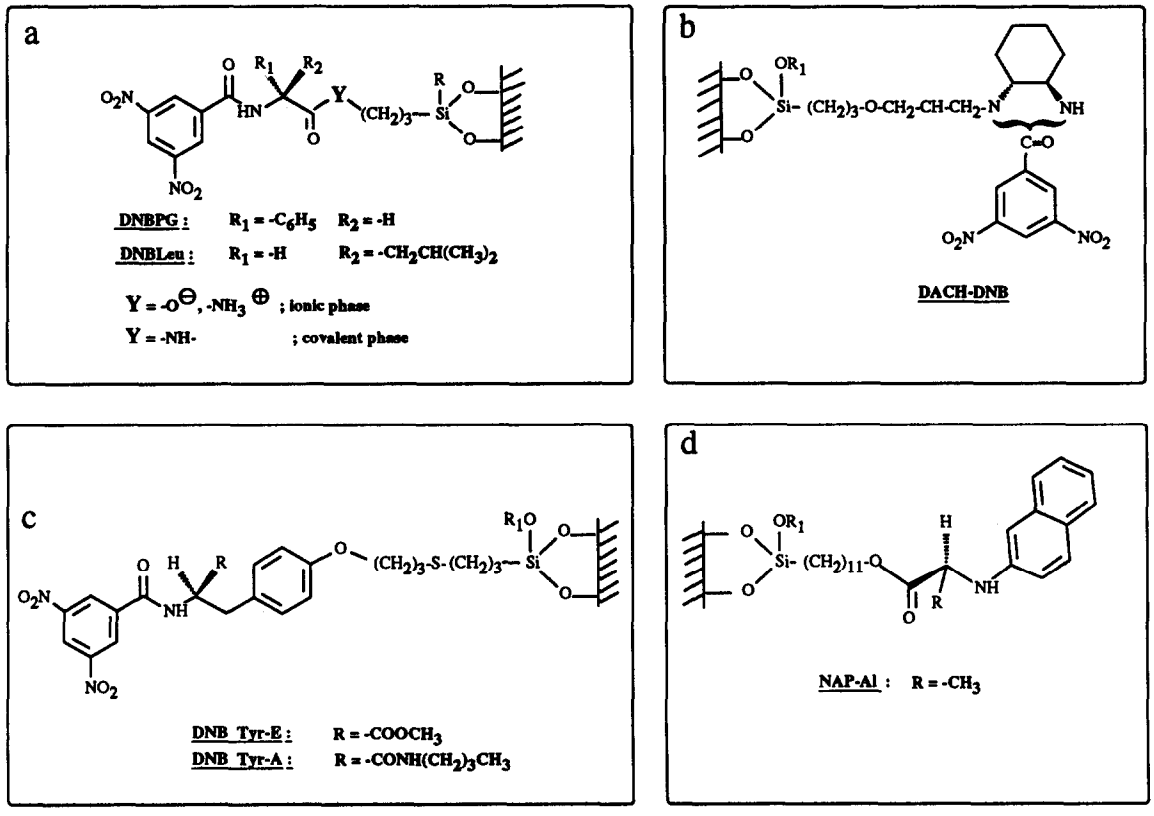


Fig. 7. Structures of π-acid and π-base phases.

from the amino acids phenylglycine (DNBPG) or leucine (DNBLeu) covalently or ionically bonded to 3-aminopropylsilica gel (Fig. 7a) [42,43]. These CSPs are commercially available for the analytical or preparative separation of enantiomers. Further CSPs based on other conventional chiral selectors such as valine, phenylalanine, tyrosine [44] and 1,2-*trans*-diaminocyclohexane have also been developed (Fig. 7b and c) [4].

The application of the reciprocality concept has led to the design of various phases of the π-donor type [45,46]. Only naphthylalanine (Fig. 7d) and naphthylethylamine phases are currently available commercially. Further CSPs based on amino acid derivatives have been developed by Oi and Kitahara [47] following the same principle and are available for preparative separations [4], but to our knowledge no preparative application has been reported.

These chemically modified silica gels are stable at high pressures and exhibit good chromatographic performances. Usually, these CSPs are used under normal-phase conditions, but Pirkle and co-workers also demonstrated the possibility of achieving chiral resolutions in the reversed-phase mode [48–50].

2.7. Chiral phases for ligand-exchange chromatography

Preparative separations of enantiomers on CSPs designed on the principle of ligand-exchange chromatography have been reported and these applications have been summarized in a recent review [4]. As no practically relevant examples related to the application fields covered by this survey have been presented, no further consideration need be given here to this chromatographic mode.

3. TECHNICAL AND PRACTICAL ASPECTS OF ENANTIOMERIC SEPARATIONS

3.1. Chromatographic mode

Various technologies have been applied for the preparative chromatographic separation of enantiomers. Although most separations have been performed in the conventional batch-mode process, there is growing interest in simulated moving-bed technology, because it permits large amounts of mobile phase to be saved and productivity increased, thus decreasing production costs [51–54]. Nevertheless, as relatively large investments are needed to set up this technology, which is also fairly difficult to apply successfully, its practical uses will probably be limited to costly compounds that are produced on a large scale. At present, the batch-mode process has the advantage of being a well established and a relatively simple technology that is applicable on any scale according to the needs of the user.

Some preparative separations using supercritical or subcritical fluid chromatography (SFC or SubFC) have also been reported [55–58] up to pilot scale with 6 cm I.D. axial compression columns. Because of the low viscosity of the mobile phase (usually consisting mainly of carbon dioxide), SFC can be performed at high flow-rates and is generally considered to be faster than HPLC [59], thus permitting greater throughputs per unit time. However, the retention time can also be shortened in HPLC, *e.g.*, by increasing the content of polar component in the mobile phase, without seriously detracting from the selectivity or the resolution [60]. This possibility diminishes the superiority of SFC in terms of speed, but SFC still has the advantage of operating with safer (non-flammable) and considerably cheaper mobile phases, two factors that are very important for large-scale separations. A difficulty encountered in preparative SFC is the collection of the solutes, because of the technical problems associated with pressure reduction and the formation of considerable amounts of carbon dioxide. This CO_2 formation has serious consequences (freezing and blocking of the outlet, loss of volatile substances and low recovery) and this is a technical aspect that still has to be properly rectified, even though different devices have already been proposed [57].

3.2. Method development

The general strategy to be adopted for the performance of preparative chiral separations has been presented in detail in a previous review

[4]; the different steps can be summed up as follows: (a) preselection of analytical columns filled with chiral stationary phases also available in preparative amounts; (b) screening on the selected analytical columns and optimization of the various chromatographic parameters; (c) transfer of the analytical separation to the preparative column, with final adjustment of the chromatographic conditions; (d) performance of the preparative separation and if necessary automation of the process. When no separation is obtained on the available chiral columns, it can be useful to modify the structure of the solute (*e.g.*, achiral derivatization) in order to improve the selectivity [61–63].

Throughput improvement can be achieved by optimization of the parameters affecting the chromatographic separation (mobile phase composition, flow-rate, temperature) and determination of the optimum loadability. This strategy and the choice of column hardware have been discussed previously [4].

UV detection is usually applied, but in addition it is recommended to use polarimetric detection at least during the optimization phase on the analytical column and the loading experiments. Indeed, in some instances, it has been observed that an inversion of the elution order could occur when the amount of injected racemate is increased from the analytical scale to the preparative scale. This unusual concentration dependence of elution order has been pointed out in at least two cases where CTA-I was used as a CSP [64,65], suggesting the presence of different types of interaction sites [61,64,66] and reflecting the complexity of the interaction mechanism with this stationary phase. Inversion of the elution order can also occur on changing the composition of the mobile phase and/or the pH. Such a phenomenon has been observed with the cellulose-based CSPs [67–69] and with the protein-based CSPs [70–72].

3.3. Isolation of solutes

Isolation of the resolved compounds is obviously a crucial step in a preparative chromatographic separation. As already mentioned, technical difficulties such as those encountered in the SFC mode can strongly affect the isolation step. In the conventional batch-elution process, however, this step generally does not constitute a difficulty. Fractionation is usually achieved in a conventional way, *i.e.*, the fractions containing the solute at the desired optical purity are collected. To improve the separation and/or the throughput, recourse is increasingly being made to such techniques as recycling and peak shaving. Recycling is especially useful for racemates that are poorly resolved and consists in passing the solute through the column several times [21,73–76]. Peak shaving is usually combined with recycling, allowing work to be performed under overload conditions and the throughput improved [75]. In this case, the first and the last fractions containing the enantiomers at the desired optical purity are collected at each passage through the column, while the eluate containing a mixture is recycled. Some practical examples illustrating this technique are presented later (Figs. 11, 13 and 18).

4. ENANTIOMERS OF CHIRAL DRUGS

The pharmaceutical field has shown a rapidly expanding number of applications of chiral chromatographic separations. The utilization of CSPs as an analytical tool for the determination of the composition of enantiomeric mixtures in biological and pharmacokinetic studies is now a well established technique [1–3]. However, the application of the method on a preparative scale for the production of optically active materials in amounts suitable for biological testing, toxicological studies and even, in a later stage, clinical testing is gaining increasingly wide acceptance. In this respect, the chromatographic method offers the advantage of furnishing both enantiomers obviously required for comparative biological testing and this approach is widely applicable also to elaborated drug structures which would be not easily accessible by a synthetic route. At least during the preliminary testing phase of new chiral drugs, chromatography allows rapid access to the pure enantiomers and can advantageously replace the often lengthy elaboration of an enantioselective synthesis. A broad variety of racemic drugs have

TABLE 2

PREPARATIVE RESOLUTION OF RACEMIC DRUGS AND DRUGS INTERMEDIATES

nm. = Not mentioned.

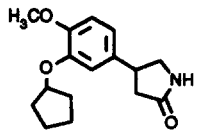
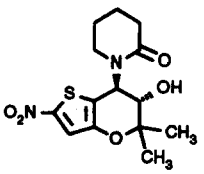
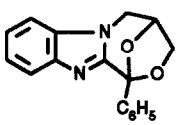
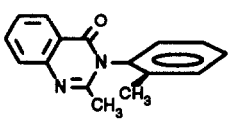
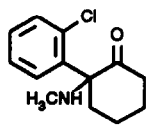
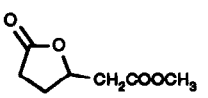
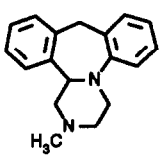
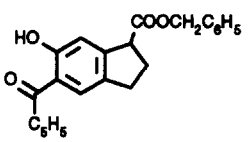
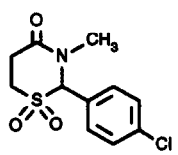
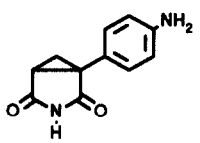
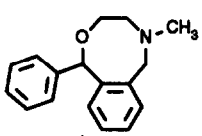
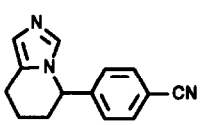
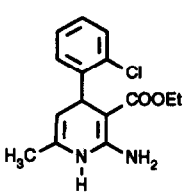
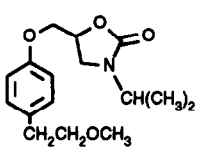
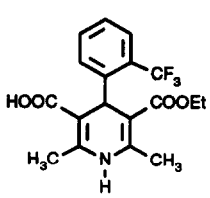
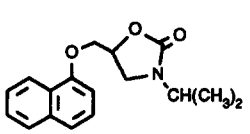
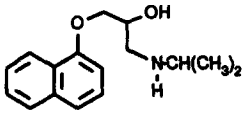
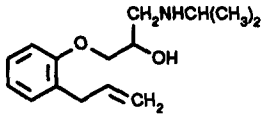
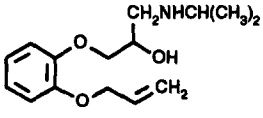
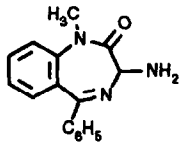
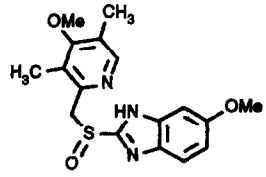
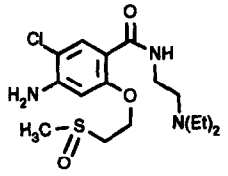
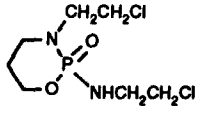
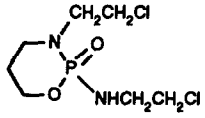
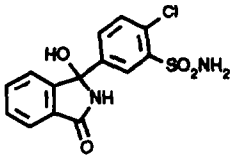
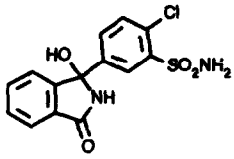
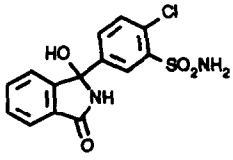
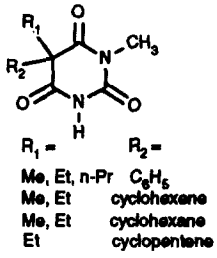
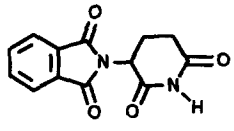
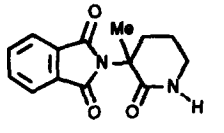
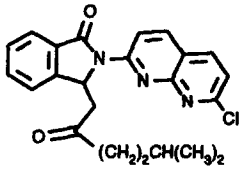
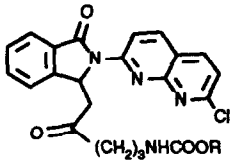
	***Drug:*** Rolipram ***Sample size:*** 205 mg ***CSP:*** CTA I [81] ***Column (cm):*** 2.4 x 85		***Drug:*** Thienopyran ***Sample size:*** 30 mg ***CSP:*** Cyclobond I [20] ***Column (cm):*** 1 x 25
	***Drug:*** Oxapadol ***Sample size:*** 2.1 g ***CSP:*** CTA I [81] ***Column (cm):*** 3.8 x 70		***Drug:*** Methaqualone ***Sample size:*** 50-300 mg ***CSP:*** CTA I [77, 78] ***Column (cm):*** 4 x 24
	***Drug:*** Ketamine ***Sample size:*** 450 mg ***CSP:*** CTA I [81] ***Column (cm):*** 3.8 x 70		***Drug:*** antibiotic intermed. ***Sample size:*** 4.2 g ***CSP:*** CTA I [95] ***Column (cm):*** 5 x 60
	***Drug:*** Mianserin ***Sample size:*** 8 mg ***CSP:*** CTA I [81] ***Column (cm):*** 1.5 x 20		***Drug:*** Oxindanac ***Sample size:*** 200 mg-20 g ***CSP:*** CTA I [80, 96] ***Column (cm):*** 20 x 100
	***Drug:*** Chlormezanone ***Sample size:*** 250-700 mg ***CSP:*** CTA I [84, 85] ***Column (cm):*** 3.8 x 70		***Drug:*** CGP 27216 ***Sample size:*** 300 mg ***CSP:*** CTA I [28, 97] ***Column (cm):*** 5 x 60
	***Drug:*** Nefopam ***Sample size:*** 100 mg ***CSP:*** CTA I [93] ***Column (cm):*** 5 x 75		***Drug:*** Fadrozole ***Sample size:*** 1 g ***CSP:*** TBC [79, 91] ***Column (cm):*** 5 x 75
	***Drug:*** Dihydropyridine ***Sample size:*** nm. ***CSP:*** CTA I [94] ***Column (cm):*** 2 (1 x 60)		***Drug:*** Metoprolol deriv. ***Sample size:*** 10 mg ***CSP:*** CTA I [98] ***Column (cm):*** 2 (1 x 60)
	***Drug:*** Dihydropyridine ***Sample size:*** 50 g ***CSP:*** Poly-MA [26] ***Column (cm):*** nm.		***Drug:*** Propranolol deriv. ***Sample size:*** 10 mg ***CSP:*** CTA I [98] ***Column (cm):*** 2 (1 x 60)

TABLE 2 *(continued)*

	***Drug:*** Propranolol ***Sample size:*** 100 mg ***CSP:*** Chiralcel OD [111] ***Column (cm):*** 2 x 50
	***Drug:*** Alprenolol ***Sample size:*** 150 mg ***CSP:*** Chiralcel OD [92, 111] ***Column (cm):*** 2 x 50
	***Drug:*** Oxyprenolol ***Sample size:*** 400 mg ***CSP:*** Chiralcel OD [111] ***Column (cm):*** 2 x 50
	***Drug:*** L-365.260 ***Sample size:*** 2 g ***CSP:*** Chiralcel OC [99] ***Column (cm):*** 4 x 20
	***Drug:*** Omeprazole ***Sample size:*** 5 mg ***CSP:*** Chiralcel OC [100] ***Column (cm):*** 2 (1 x 30)
	***Drug:*** ML 1035 ***Sample size:*** 0.5 mg ***CSP:*** Chiralcel OD [101] ***Column (cm):*** 0.46 x 25
	***Drug:*** Ifosfamide ***Sample size:*** 8 mg ***CSP:*** Chiralcel OD [103] ***Column (cm):*** 1 x 25
	***Drug:*** Ifosfamide ***Sample size:*** 1.55 g ***CSP:*** Poly-PEA [104, 105] ***Column (cm):*** 5 x 155
	***Drug:*** Chlorthalidone ***Sample size:*** 530 mg ***CSP:*** Poly-PEA [81, 82] ***Column (cm):*** 3.2 x 36
	***Drug:*** Chlorthalidone ***Sample size:*** 250 mg ***CSP:*** ChiraSpher [106] ***Column (cm):*** 2.6 x 46
	***Drug:*** Chlorthalidone ***Sample size:*** 400 mg ***CSP:*** Poly-MA [27] ***Column (cm):*** nm.
 R_1 = Me, Et, n-Pr; R_2 = C_6H_5 Me, Et; cyclohexane Me, Et; cyclohexane Et; cyclopentane	***Drug:*** Barbiturates ***Sample size:*** 140-257 mg ***CSP:*** CTA I [81, 107] ***Column (cm):*** 2.5 x 85
	***Drug:*** Thalidomide ***Sample size:*** 505 mg ***CSP:*** Poly-CHMA [108,109] ***Column (cm):*** 2.3 x 62
	***Drug:*** Thalidomide analog ***Sample size:*** 30 mg ***CSP:*** Poly-CHMA [81,110] ***Column (cm):*** 4 x 20
	***Drug:*** RP 59037 ***Sample size:*** 500 mg ***CSP:*** Chiralcel OC [112] ***Column (cm):*** 7 x 22
 R = Me, Et	***Drug:*** RP 60603/60977 ***Sample size:*** 2-4 g ***CSP:*** Chiralcel OC [112] ***Column (cm):*** 7 x 22

(Continued on p. 576)

TABLE 2 *(continued)*

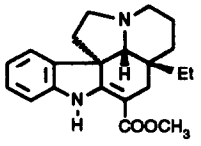
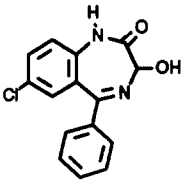
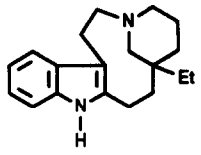
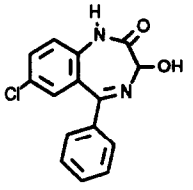
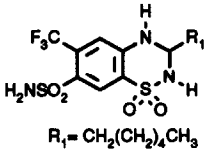
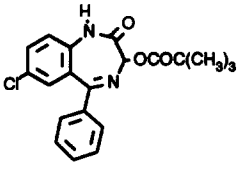
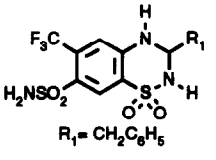
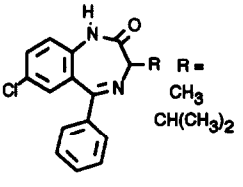
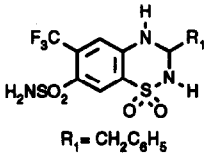
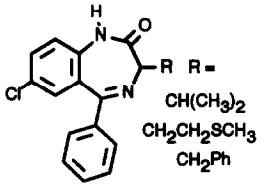
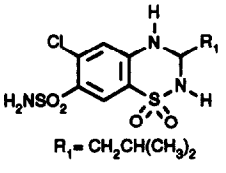
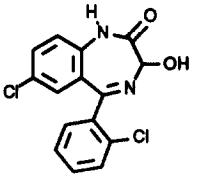
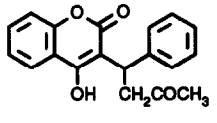
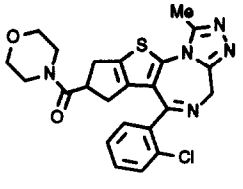
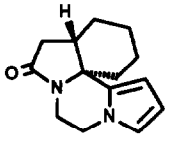
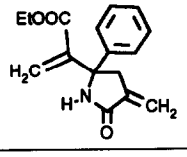
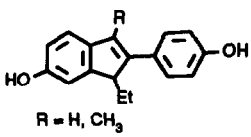
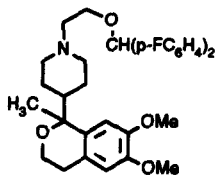
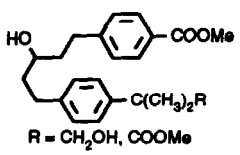
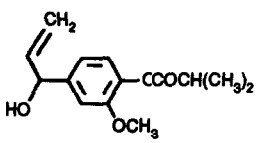
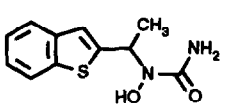
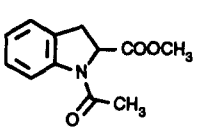
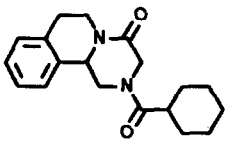
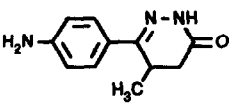
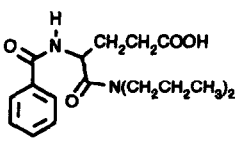
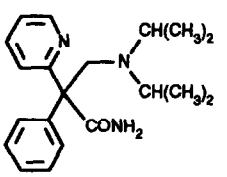
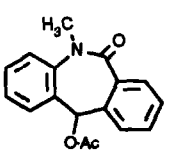
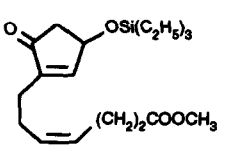
	***Drug:*** Vincadimorfine ***Sample size:*** 500 mg ***CSP:*** CD-Poly [17] ***Column (cm):*** 5 x 95		***Drug:*** Oxazepam ***Sample size:*** 46 mg ***CSP:*** Poly-PEA [82] ***Column (cm):*** 3.2 x 76
	***Drug:*** Quebrachine ***Sample size:*** 200 mg ***CSP:*** CD-Poly [17] ***Column (cm):*** 5 x 95		***Drug:*** Oxazepam ***Sample size:*** 5 mg ***CSP:*** DNBPG-io [114] ***Column (cm):*** 2 x 25
	***Drug:*** Penflutizide ***Sample size:*** 210 mg ***CSP:*** Poly-PEA [83] ***Column (cm):*** 3.8 x 79		***Drug:*** Oxazepam pivalate ***Sample size:*** 200 mg ***CSP:*** DNBPG-co [115] ***Column (cm):*** 4 x 15
	***Drug:*** Bendroflumethiazide ***Sample size:*** 210 mg ***CSP:*** Poly-PEA [83] ***Column (cm):*** 3.8 x 79		***Drug:*** Benzodiazepinones ***Sample size:*** 40 mg-1 g ***CSP:*** DNBPG-co [115-116] ***Column (cm):*** 1 x 25
	***Drug:*** Bendroflumethiazide ***Sample size:*** 2.32 mg ***CSP:*** Chiral-AGP [40] ***Column (cm):*** 1 x 15		***Drug:*** Benzodiazepinones ***Sample size:*** 95-104 mg ***CSP:*** DNBLeu [116-118] ***Column (cm):*** 1 x 25
	***Drug:*** Buthiazide ***Sample size:*** 253 mg ***CSP:*** Poly-PEA [83] ***Column (cm):*** 3.8 x 79		***Drug:*** Lorazepam ***Sample size:*** 5 mg ***CSP:*** DNBPG-io [114] ***Column (cm):*** 2 x 25
	***Drug:*** [^{14}C]-Warfarin ***Sample size:*** nm. ***CSP:*** Chiralcel OD [90] ***Column (cm):*** 0.46 x 25		***Drug:*** WEB 2170 ***Sample size:*** 1.3 Kg ***CSP:*** CTA I [88] ***Column (cm):*** 45 x 50
	***Drug:*** CGS 16920 ***Sample size:*** 2 g ***CSP:*** TBC [28, 87] ***Column (cm):*** 5 x 45		***Drug:*** β-lactam ***Sample size:*** 120 mg ***CSP:*** DNB-Tyr-A [119] ***Column (cm):*** 2.6 x 40

TABLE 2 *(continued)*

	***Drug:*** Indenestrol ***Sample size:*** nm. ***CSP:*** PTrMA [120] ***Column (cm):*** 0.46 x 25	
	***Drug:*** ORG 12983 ***Sample size:*** 40 mg ***CSP:*** Chiralcel OJ [121] ***Column (cm):*** 2 x 50	
	***Drug:*** Lifibrol metabolite ***Sample size:*** 100-250 mg ***CSP:*** Chiralcel OD [122] ***Column (cm):*** 2 x 50	
	***Drug:*** Hydroxyeugenol ***Sample size:*** 20 mg ***CSP:*** CTA I [123] ***Column (cm):*** 2 x 50	
	***Drug:*** [^{14}C]-Zileuton ***Sample size:*** 7 mg ***CSP:*** Chiralpak AD [89] ***Column (cm):*** 2 x 25	
	***Drug:*** ACE inhibitor interm. ***Sample size:*** 45 g ***CSP:*** CTA I [86] ***Column (cm):*** 20 x 100	
		 ***Drug:*** Praziquantel ***Sample size:*** 1 g/h ***CSP:*** CTA I [53] ***Column (cm):*** 4 (1.25 x 44)
		 ***Drug:*** — ***Sample size:*** 1 g ***CSP:*** DNBPG-io [113] ***Column (cm):*** nm.
		 ***Drug:*** Proglumide ***Sample size:*** 0.84 mg ***CSP:*** Chiral-AGP [40] ***Column (cm):*** 1 x 15
		 ***Drug:*** Disopyramide ***Sample size:*** 0.8 mg ***CSP:*** Chiral-AGP [40] ***Column (cm):*** 1 x 15
		 ***Drug:*** Etazepine acetate ***Sample size:*** 300 mg ***CSP:*** CTA I [28] ***Column (cm):*** 5 x 100
		 ***Drug:*** Prostaglandine interm. ***Sample size:*** 500 mg ***CSP:*** Chiralcel OC [102] ***Column (cm):*** 2 x 50

already been resolved on CSPs, covering different therapeutic classes of compounds such as analgesics, β-blockers, tranquillizers, calcium antagonists, antiaromatases, diuretics, anticonvulsive drugs and nootropics. The structural variations are broad and Table 2 summarizes most the preparative separations achieved up to now in this domain. Most of the applications have been performed on CTA-I but an increasing number of racemic drugs or drug intermediates were resolved on other cellulose-based CSPs such as cellulose tribenzoate, cellulose phenyl carbamate (Chiralcel OC) and cellulose 3,5-dimethylphenyl carbamate (Chiralcel OD). Polyacrylamide-type CSPs were also found to be useful for some drugs such as ifosfamide (chiral phosphorus atom), phthalimides (thalidomide, chlorthalidone) and benzothiadiazines. The preparative resolution of a series of racemic benzodiazepinones could be achieved on various π-

acid CSPs derived from phenylglycine and leucine.

For several drugs such as the hypnotic and anticonvulsive drug methaqualone [77,78], the antiaromatase fadrozole [79], the anti-inflammatory oxindanac [80], the diuretic chlorthalidone [81,82], the diuretic benzothiadiazines [83], the benzodiazepinone oxazepam [81], the muscle relaxant chlormezanone [84,85], and the other new chiral drugs or drug intermediates, the enantiomers were obtained for the first time by chromatography on CSPs (Table 2). The preparative separation of the enantiomers of 2.1 g of oxapadol [81], 45 g of indolinecarboxylic acid methyl ester (ACE inhibitor intermediate) [86], 300 mg of etazepine acetate [28] and 2 g of the nootropic drug CGS 16920 [28,87] are shown in Figs. 8–11. The chromatographic method is also applicable to the resolution of larger amounts of racemate, as demonstrated by the enantiomeric separation of WEB 2170 [88], of which not less than 1.3 kg of racemate have been chromatographed per run on a CTA-I column.

This approach is particularly suitable for the amounts usually required when the enantiomers of labelled compounds are desired. This strategy has been applied, for instance, for [^{14}C]zileuton [89] and [^{14}C]warfarin [90] on a milligram scale and for the carbon-14- or deuterium-labelled anticancer drug fadrozole on a gram scale (Fig. 12) [19].

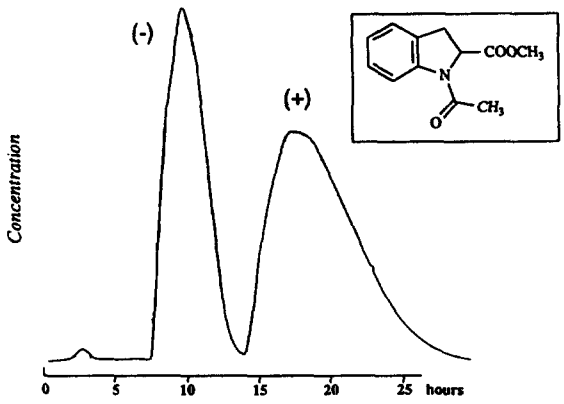


Fig. 9. Preparative separation of the enantiomers of 45 g of racemic N-acetylindoline-2-carboxylic acid methyl ester on cellulose triacetate (CTA-I) [86]. Column, 100 cm × 20 cm I.D. (12 kg CTA-I); mobile phase, ethanol–water (94:6); flow-rate, 5 l/h.

The growing number of applications in the pharmaceutical field attests to the usefulness of the method which in many cases allows access to several grams of the pure enantiomers of chiral

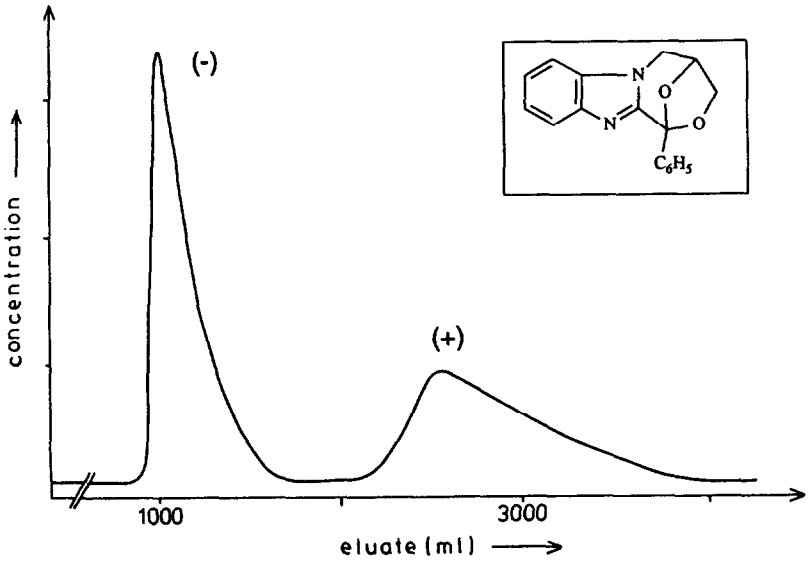


Fig. 8. Chromatographic separation of the enantiomers of oxapadol (2.1 g) on cellulose triacetate (CTA-I). Column, 70 cm × 3.8 cm I.D.; mobile phase, ethanol–water (95:5). From ref. 81.

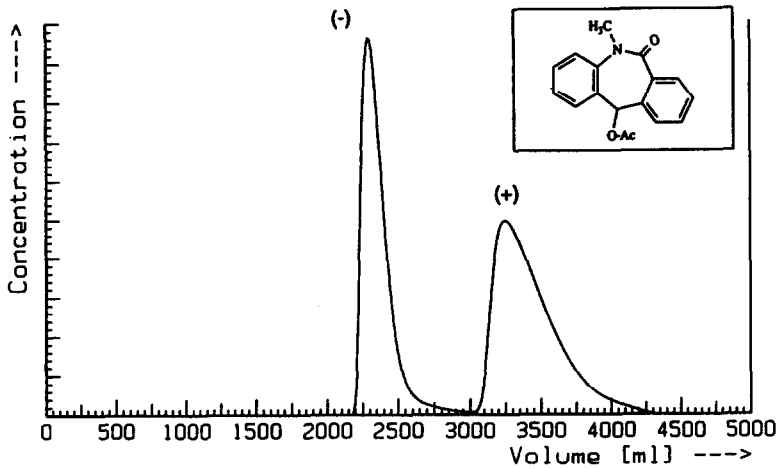


Fig. 10. Preparative separation of the enantiomers of the acetate derivative of etazepine (300 mg) on cellulose triacetate (CTA-I) [28]. Column 100 cm × 5 cm I.D.; mobile phase, ethanol–water (94:6); flow-rate, 300 ml/h.

drugs in a simple way and in a relatively short time compared with other approaches. Chromatographic resolution on CSPs will probably become the method of choice for the preparation of enantiomeric drugs, at least in the first stage of development.

5. ENANTIOMERS OF CHIRAL PESTICIDES AND PHEROMONES

Animals and plants also exhibit different biological responses depending on the absolute configuration of chiral pesticides [124] or sex

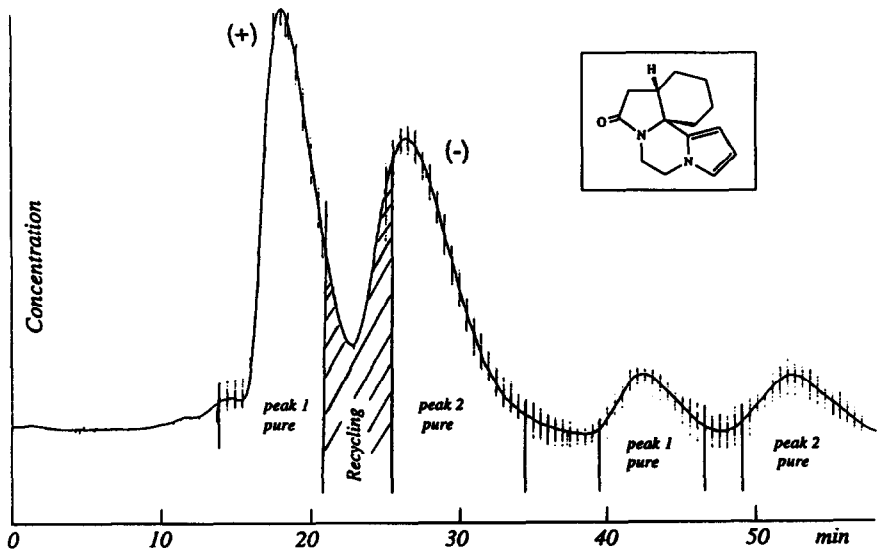


Fig. 11. Chromatogram of the resolution of the racemic nootropic drug CGS 16920 (2 g) on tribenzoylcellulose beads (TBC) with recycling and peak shaving [91]. Column, 45 cm × 5 cm I.D.; mobile phase, methanol; flow-rate, 60 ml/min.

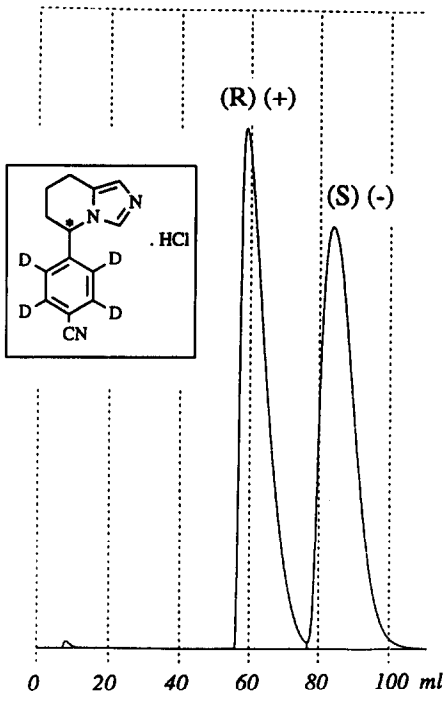


Fig. 12. Separation of the enantiomers of deuterium-labeled fadrozole (1 g) on Chiralcel OD with one recycling [79,91]. Column, 50 cm × 5 cm I.D.; mobile phase, hexane–2-propanol (9:1); flow-rate, 100 ml/min.

attractants [125] (Table 3). This effect is being increasingly documented with applications in the field of insecticides, herbicides and fungicides. Also for the separation of the enantiomers of these chiral biologically active compounds, the potential of preparative chromatography on CSPs has been recognized. The applications reported usually range between the milligram and gram scales, but larger amounts needed for field testing have already been prepared in this way, as illustrated for the resolution of up to 52 g per run of the racemic fungicide clozylacon (Fig. 13) [76,126]. In most cases, CTA-I has been used as the CSP and the recycling technique [73] has been applied in order to improve the throughput. As in the case of labelled drugs, chromatography on CSPs is the method of choice for the resolution of labelled pesticides such as the herbicide Topik (Fig. 14) [28], from which the ^{14}C-isotopic form was resolved on a 400-mg scale.

Although the development and production of chiral pesticides generally require the preparation of active material in such large amounts that a chromatographic process is not realistic, the chromatographic isolation on CSPs of the pure

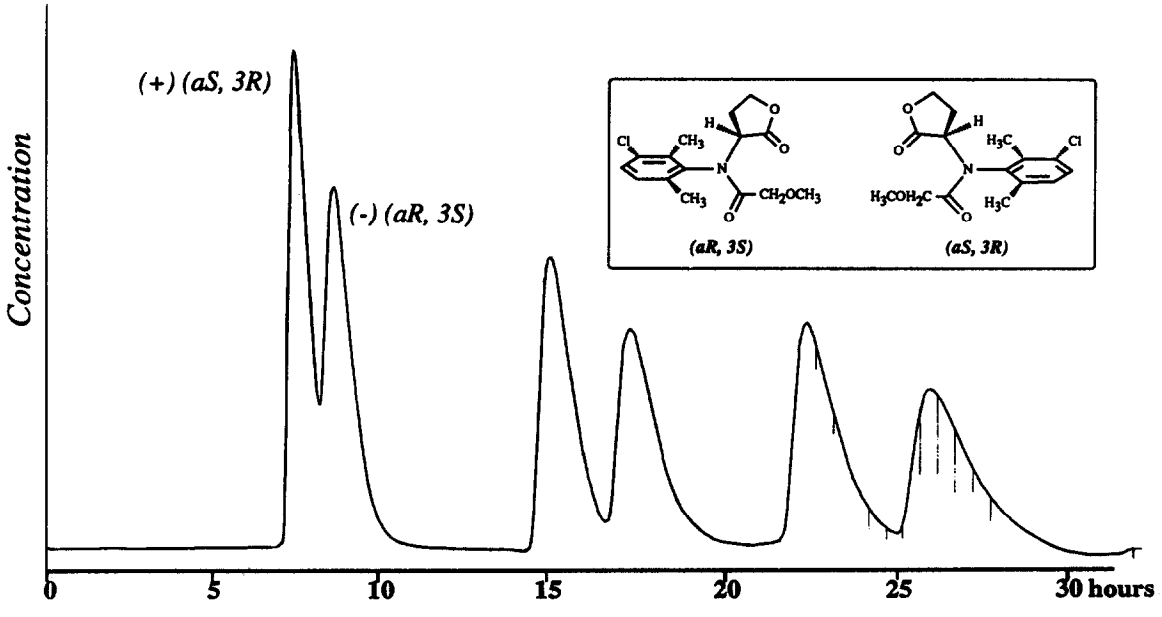
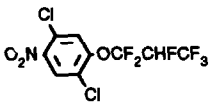
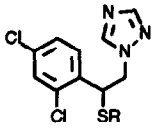
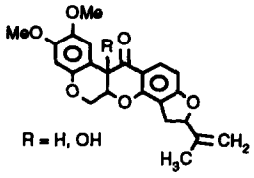
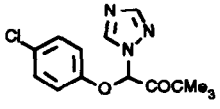
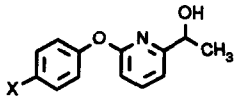
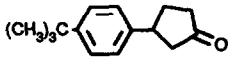
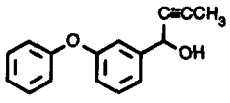
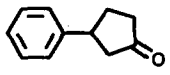
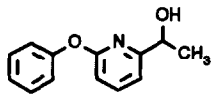
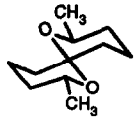
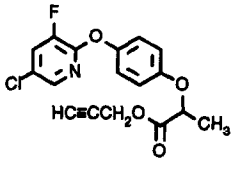
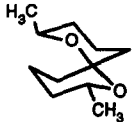
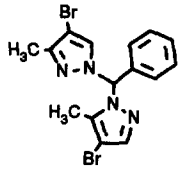
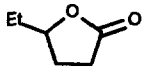
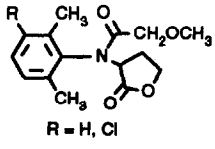


Fig. 13. Preparative separation of the enantiomers of 52 g of racemic (*aR*, 3*S*)- and (*aS*, 3*R*)-clozylacon (fungicide) on cellulose triacetate (CTA-I) with two recyclings [76,126]. Column and chromatographic conditions as in Fig. 9.

TABLE 3

PREPARATIVE RESOLUTION OF RACEMIC PESTICIDES AND PHEROMONES

	*Activity:* insecticide[a] *Sample size:* 200 mg *CSP:* TBC [127] *Column (cm):* 5 x 75		*Activity:* fungicide *Sample size:* 2 g *CSP:* CTA-I [74] *Column (cm):* 6.3 x 66
	*Activity:* insecticide *Sample size:* 5 mg *CSP:* PTrMA [128] *Column (cm):* 1 x 25		*Activity:* fungicide *Sample size:* 30 mg *CSP:* CTA-I [131] *Column (cm):* 2.5 x 30
	*Activity:* insecticide[a] *Sample size:* 10-25 mg *CSP:* CTA-I [129] *Column (cm):* 1.25 x 60		*Activity:* fungicide[a] *Sample size:* 500 mg *CSP:* MMBC [76, 132] *Column (cm):* 2.5 x 60
	*Activity:* insecticide[a] *Sample size:* 300 mg *CSP:* CTA-I [129] *Column (cm):* 5 x 60		*Activity:* fungicide[a] *Sample size:* 300 mg *CSP:* CTA-I [76, 132] *Column (cm):* 5 x 90
	*Activity:* insecticide[a] *Sample size:* 300 mg *CSP:* CTA-I [129] *Column (cm):* 5 x 60		*Activity:* pheromone *Sample size:* 140 mg *CSP:* CTA-I [133] *Column (cm):* 2.5 x 60
	*Activity:* herbicide *Sample size:* 4 g *CSP:* CTA-I [28] *Column (cm):* 5 x 60		*Activity:* pheromone *Sample size:* 80 mg *CSP:* CTA-I [133] *Column (cm):* 2.5 x 60
	*Activity:* fungicide *Sample size:* 100 mg *CSP:* CTA-I [130] *Column (cm):* 2.5 x 20		*Activity:* pheromone *Sample size:* 2.47 g *CSP:* CTA-I [134] *Column (cm):* 5 x 60
	*Activity:* fungicide *Sample size:* 1-52 g *CSP:* CTA-I [76, 126] *Column (cm):* 20 x 100		

[a] Synthesis intermediate.

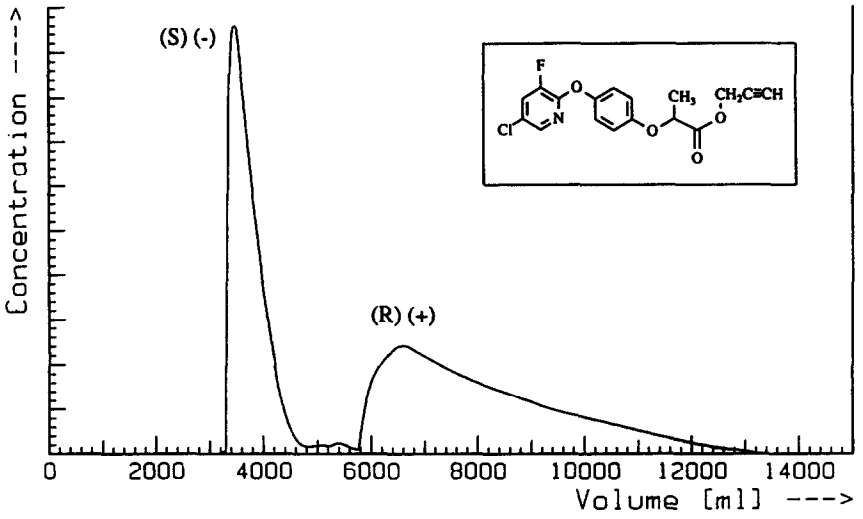


Fig. 14. Preparative separation of the enantiomers of 4 g of the racemic herbicide Topik on cellulose triacetate (CTA-I) [28]. Column and chromatographic conditions as in Fig. 10.

enantiomers in amounts adequate for performing preliminary tests can be very helpful during the first steps of the development of the compound.

6. CHIRAL SYNTHONS, CHIRAL AUXILIARIES AND CHIRAL CSP PRECURSORS

Most enantioselective syntheses start from chiral building blocks or chiral auxiliaries as a source of chiral information to be incorporated in the final product or to be transferred during the synthesis. Many chiral materials are commercially available in their optically pure form, but they are generally expensive. Chromatographic resolution on CSPs provides a useful tool for the preparation of such chiral starting materials, as illustrated with the applications summarized in Table 4. Typical examples are the chiral

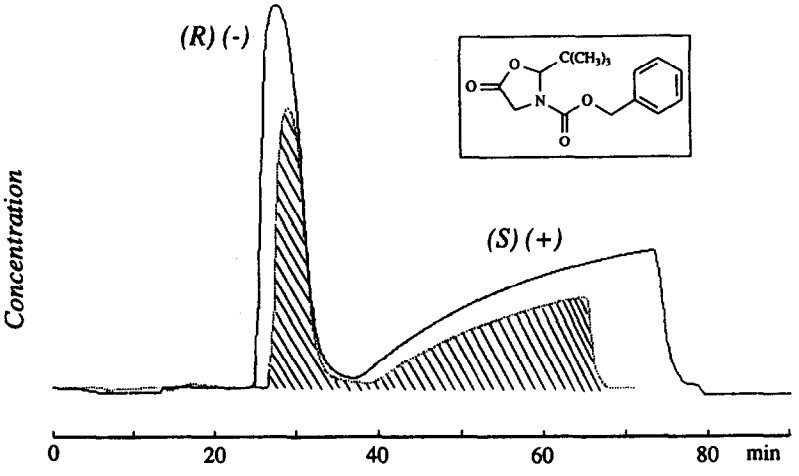
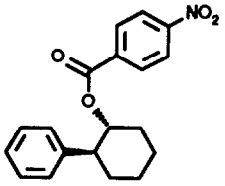
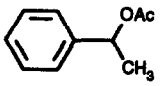
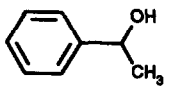
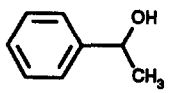
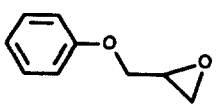
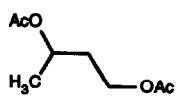
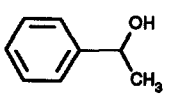
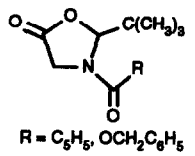
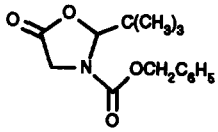
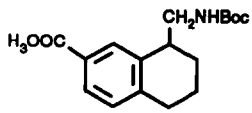
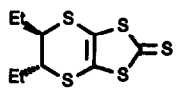
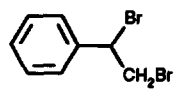
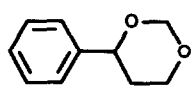
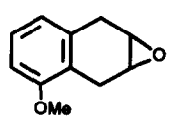


Fig. 15. Separation of the enantiomers of 3-benzyloxycarbonyl-2-*tert.*-butyloxazolidinone on Chiralcel OD [91]: influence of the sample size on the elution profile. Amount injected, 2 g (hatched chromatogram) and 3 g; column, 50 cm × 5 cm I.D.; mobile phase, hexane–ethanol (8:2); flow-rate, 50 ml/min; room temperature.

TABLE 4

PREPARATIVE SEPARATION OF THE ENANTIOMERS OF CHIRAL AUXILARIES AND CHIRAL SYNTHONS

SMBA = Simulated moving bed adsorption.

	***Sample size:*** 1.4 g ***CSP:*** CTA-I [61] ***Column (cm):*** 5 x 100
	***Sample size:*** 5-100 g ***CSP:*** CTA-I [61, 86] ***Column (cm):*** 20 x 100
	***Sample size:*** 1 g ***CSP:*** TBC [137] ***Column (cm):*** 5 x 75
	***Sample size:*** 219 mg ***CSP:*** PTrMA [29] ***Column (cm):*** 1.16 x 80.6
	***Sample size:*** 3.01 g ***CSP:*** CTA-I [61] ***Column (cm):*** 5 x 100
	***Sample size:*** 2 g ***CSP:*** TBC [137] ***Column (cm):*** 5 x 75
	***Sample size:*** SMBA ***CSP:*** Chiralcel OD [51] ***Column (cm):*** 8 (2 x 15)
	***Sample size:*** 1-5.3 g ***CSP:*** Chiraspher [135, 136] ***Column (cm):*** 10 x 40
	***Sample size:*** 0.5-3 g ***CSP:*** Chiralcel OD [91] ***Column (cm):*** 5 x 50
	***Sample size:*** 1 g ***CSP:*** PMBC [138] ***Column (cm):*** 5 x 75
	***Sample size:*** 700 mg ***CSP:*** CTA-I [28] ***Column (cm):*** 5 x 75
	***Sample size:*** 350 mg ***CSP:*** CTA-I [139] ***Column (cm):*** 4.5 x 32
	***Sample size:*** 10-150 g ***CSP:*** CTA-I [61, 76, 86] ***Column (cm):*** 20 x 100
	***Sample size:*** SMBA ***CSP:*** CTA-I [54] ***Column (cm):*** 10 (2.6 x 11)

synthons developed by Seebach *et al.* [135], which are easily separated on ChiraSpher [135,136] or on Chiralcel OD [91]. The preparative resolution of 3-benzyloxycarbonyl-2-*tert.*-butyloxazolidinone on Chiralcel OD shows an unusual band profile with increasing the sample mass (Fig. 15), reflecting that both enantiomers have different adsorption isotherms. In principle, this case represents an ideal situation for preparative application since, at least in the concen-

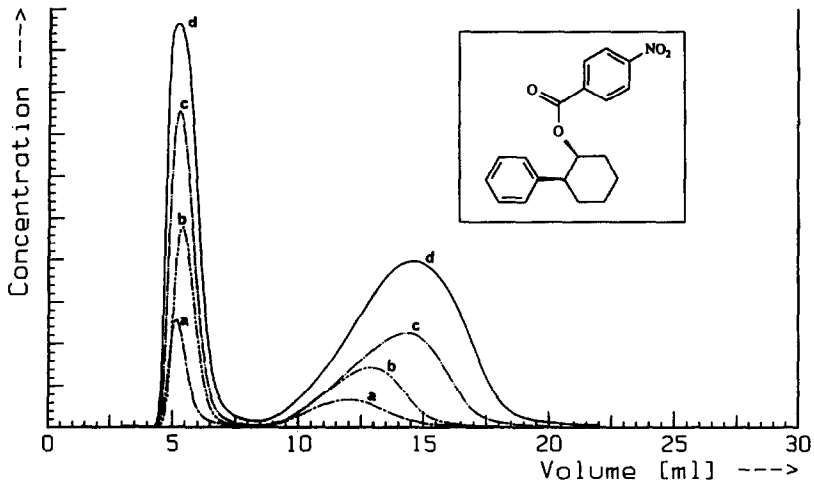


Fig. 16. Chromatographic resolution of racemic *trans*-1-phenyl-2-cyclohexanol-4-nitrobenzoate on cellulose triacetate (CTA-I) at 22°C: influence of the sample size on the elution profile [28]. HPLC column, 25 cm × 0.46 cm I.D.; mobile phase, ethanol–water (95:5); flow-rate, 0.5 ml/min; amount injected, (a) 0.6 mg in 100 ml, (b) 1.5 mg in 500 ml, (c) 3 mg in 500 ml and (d) 6.5 mg in 500 ml.

tration range investigated, there is no peak overlapping by increasing the amount of injected racemate.

The same phenomenon has been observed for the enantiomers of *trans*-1-phenyl-2-cyclohexanol-4-nitrobenzoate showing an "abnormal" elution profile at "preparative" concentrations (Fig. 16) characterized by a strong peak front and a displacement of the peak tail to higher retention volumes with increasing concentrations for the more retained enantiomer [28,61].

The examples in Table 4 demonstrate that in some instances it can be beneficial to start with an achiral material and to perform the enantiomeric separation in a later stage of the enantioselective synthesis. A unique feature of CSPs is their ability to resolve racemates lacking functional groups needed for derivatization as illustrated for phenyl-1,2-dibromoethane (Table 4) and 4-phenyl-1,3-dioxane, of which 150 g have been resolved on a CTA-I pilot column (Fig. 17) [76,86].

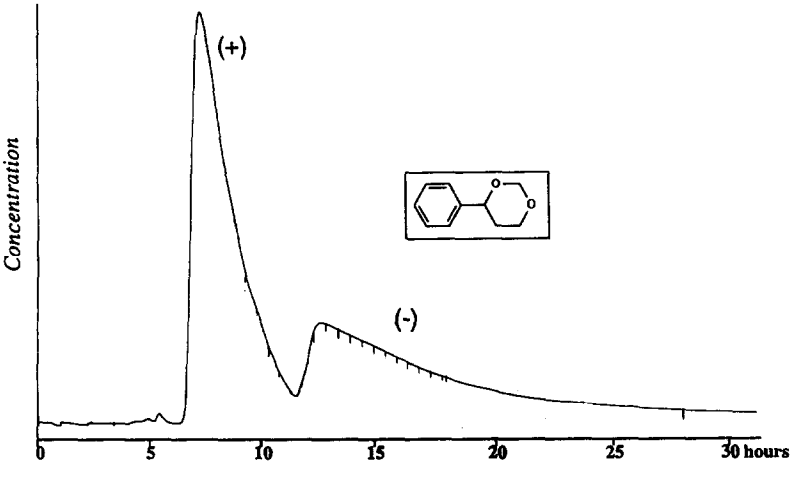
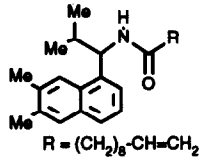
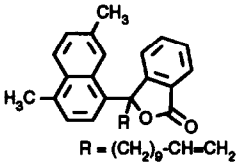
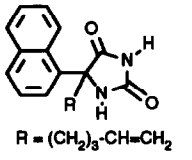
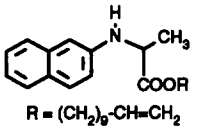
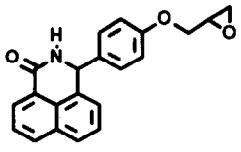
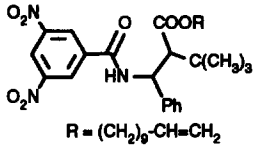


Fig. 17. Chromatographic separation of the enantiomers of 150 g of racemic 4-phenyl-1,3-dioxane on cellulose triacetate (CTA-I) [76,86]. Column and chromatographic conditions as in Fig. 9.

TABLE 5

PREPARATIVE SEPARATION OF THE ENANTIOMERS OF CSP PRECURSORS

	***Sample size:*** 3-6 g ***CSP:*** DNBPG-io [118, 140] ***Column (cm):*** 5 x 76
	***Sample size:*** 7 g ***CSP:*** DNBPG-io [118] ***Column (cm):*** 5 x 76
	***Sample size:*** 4 g ***CSP:*** DNBPG-io [118, 141] ***Column (cm):*** 5 x 76
	***Sample size:*** 1.6 g ***CSP:*** DNBPG-io [142] ***Column (cm):*** 5 x 76
	***Sample size:*** 100 g ***CSP:*** DNBPG-co [143] ***Column (cm):*** 15 x 122
	***Sample size:*** 300 mg ***CSP:*** DNB-Tyr-A [144] ***Column (cm):*** 4 x 26
	***Sample size:*** 1.92 g ***CSP:*** NAP-Al [145] ***Column (cm):*** 5 x 76

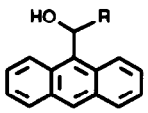
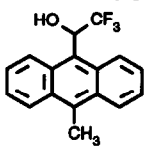
Various chiral CSP precursors, which can be considered as chiral synthons, were also resolved on a preparative scale by Pirkle and co-workers in connection with their efforts to design new CSPs and with the application of the concept of reciprocality that they developed [45,46] (Table 5). These separations clearly demonstrate the possibility to scale-up of the separations obtained on analytical Pirkle columns.

7. CHIRAL NMR SOLVATING AGENTS

Pirkle was one of the major contributors to the establishment of the chromatographic resolution of racemates on CSPs as a versatile technique, demonstrating the potential of the method with a series of new CSPs. Based on previous investigations on chiral solvating agents for NMR spectroscopy, he and his co-workers developed the concept of reciprocality and designed a series of CSPs originally based on amino acids [42,43,45,46]. One of his first preparative applications was the separation of the enantiomers of 1-(9-anthryl)-2,2,2-trifluoroethanol (TFAE), which is now generally used in NMR spectroscopy for the determination of the optical purity of optically active substances. This racemate has even become a reference compound for testing new chiral phases because it has the special property of being separated on a wide variety of CSPs. Therefore, it is not surprising that preparative separations have been performed on both Pirkle CSPs and CTA-I (Table 6). Analogues of TFAE have been preparatively resolved by Francotte *et al.* [147] on cellulose-based CSPs and the new chiral fluoroanthryl derivatives exhibit the same properties as chiral NMR solvating agents.

TABLE 6

PREPARATIVE SEPARATION OF THE ENANTIOMERS OF CHIRAL NMR SOLVATING AGENTS

Structure	Sample amount	CSP	Column dimensions [length × I.D. (cm)]	Ref.
				
R = CF_3	1–30 g	CTA-I	100 × 20	77, 86, 136
R = CF_3	1 g	DNBPG-io	76 × 5	142
R = CF_3	100 mg	DNBPG-co	50 × 35.5	146
R = CF_3	1 g	DNBLeu	76 × 5	115
R = CF_3	40 mg	DNB-Tyr-A	76 × 6	56
R = N-C_4H_9	1 g	DNBPG-io	76 × 5	142
R = CF_2CF_3	800 mg	CTA-I	100 × 5	147
R = CCl_2CF_3	3.2 g	CTA-I	100 × 5	147
R = $CF(CF_3)_2$	210 mg	MMBC	100 × 5	147
	8 g	DNBPG-io	76 × 5	142

8. CHIRAL COMPOUNDS FOR MECHANISTIC STUDIES

The retention or loss of optical purity in molecular transformations is often an important source of information on the reaction mechanism. Nevertheless, access to the optically pure enantiomers needed for such mechanistic studies is not always easy and can strongly limit the possibilities for exploiting this investigation tool. Again, the chromatographic separation of enantiomers on CSPs can constitute an easy way of

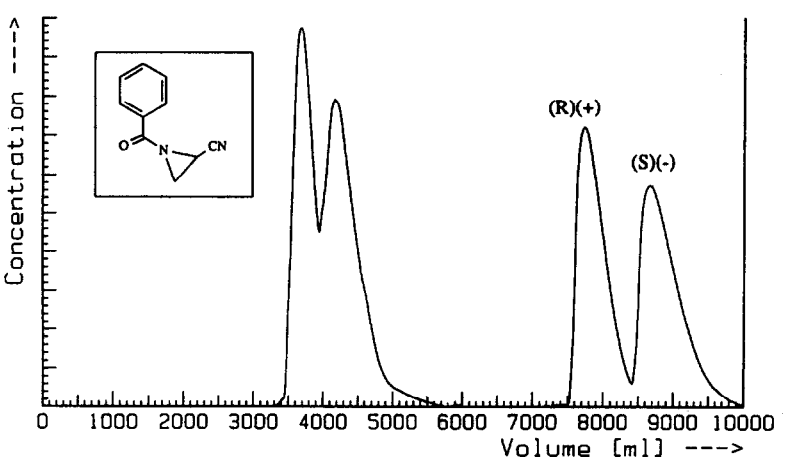
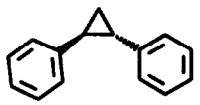
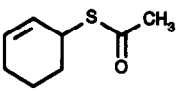
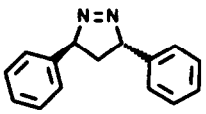
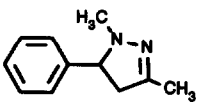
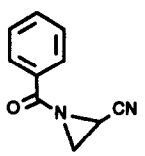


Fig. 18. Chromatographic resolution of 170 mg of racemic N-benzoylaziridine on *m*-methylbenzoylcellulose beads (MMBC) [28,150]. Column, 60 cm × 2.5 cm I.D.; mobile phase, hexane–2-propanol (8:2); flow-rate, 9 ml/min.

TABLE 7

PREPARATIVE RESOLUTION OF RACEMIC COMPOUNDS USED FOR MECHANISTIC STUDIES

	***Sample size:*** 150 mg ***CSP:*** CTA-I [148, 149] ***Column (cm):*** 2.5 x 30		***Sample size:*** 20 g ***CSP:*** CTA-I [28, 151] ***Column (cm):*** 20 x 100
	***Sample size:*** nm. ***CSP:*** CTA-I [149] ***Column (cm):*** 0.8 x 25		***Sample size:*** 300 mg ***CSP:*** CTA-I [152] ***Column (cm):*** 2.5 x 69
	***Sample size:*** 170 mg ***CSP:*** MMBC [28, 150] ***Column (cm):*** 2.5 x 60		

providing the desired optically pure intermediates. This strategy has been applied in different areas such as radical transformations [148–150] and Claisen rearrangements [151] (Table 7). The preparative resolution of 170 mg of the racemic N-benzoylaziridine derivative on *m*-methylbenzoylcellulose beads (MMBC) is shown in Fig. 18.

9. COMPOUNDS WITH CHIRAL HETEROATOMS

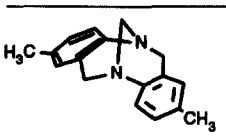
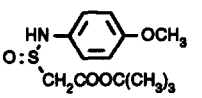
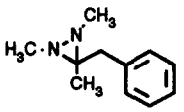
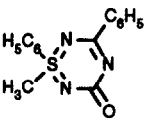
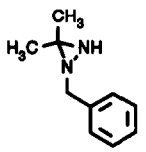
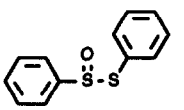
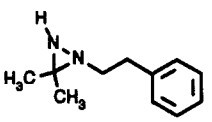
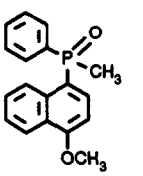
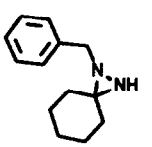
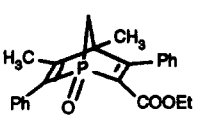
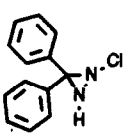
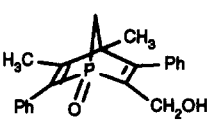
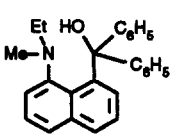
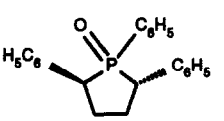
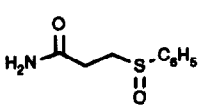
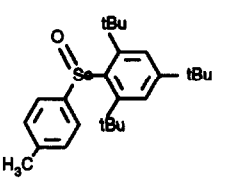
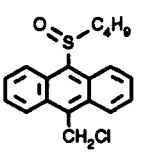
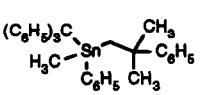
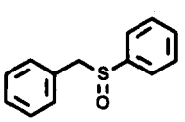
Many atoms other than carbon can be chiral. Nevertheless, it is often difficult to obtain the optically pure forms of compounds containing a chiral heteroatom by conventional approaches. Chromatography on CSPs has proved in many cases to be a powerful resolution method for such chiral substances (Table 8). For instance, Tröger's base was resolved by Prelog and Wieland [153] in 1944 into the corresponding enantiomers by chromatography on a lactose CSP. This example was the first observed case of an amine where the inversion of nitrogen is inhibited. Later, it was found that the resolution could be successfully achieved on CTA-I [7] and we reported separations of up to 65 g per run [86]. The elution profile of the enantiomers of Tröger's base (Fig. 19a) shows also the unusual form reported above for two chiral synthons (Table 4).

This behaviour has been observed by other workers [136,154] and has recently been investigated in detail by Seidel-Morgenstern and Guiochon [155], who showed that the adsorption isotherms of the two enantiomers are not similar. This effect is CSP dependent, as illustrated on Fig. 19, showing the resolution of Tröger's base under identical chromatographic conditions (column, mobile phase, flow-rate, temperature, sample size) on cellulose triacetate (CTA-I) and on dextran triacetate [28]. Moreover both polysaccharide derivatives exhibit opposite chiral recognitions.

The barrier to pyramidal inversion of the nitrogen atom is also considerably enhanced by incorporating it into a three-membered ring, but even in this case the optically active forms are stable at room temperature only when the nitrogen atom is attached to an electronegative atom such as halogen, oxygen or nitrogen. A series of compounds containing the chirality on the nitrogen atom included in a three-membered ring have been investigated, especially by Mannschreck and co-workers, who achieved the preparative resolution of the enantiomers on CTA-I [156–158].

TABLE 8

PREPARATIVE SEPARATION OF THE ENANTIOMERS OF COMPOUNDS CONTAINING CHIRAL HETEROATOMS

	***Sample size:*** 32 mg / 65 g ***CSP:*** CTA-I [86, 136] ***Column (cm):*** 20 x 100		***Sample size:*** 200 mg ***CSP:*** DNB-Tyr-A [161] ***Column (cm):*** 4 x 26
	***Sample size:*** 100 mg ***CSP:*** CTA-I [156, 157] ***Column (cm):*** 2.5 x 41		***Sample size:*** 15 mg ***CSP:*** Poly-PEA [81] ***Column (cm):*** 1 x 24
	***Sample size:*** 149 mg ***CSP:*** CTA-I [157] ***Column (cm):*** 2.5 x 41		***Sample size:*** nm. ***CSP:*** CTA-I [163] ***Column (cm):*** 1 x 25
	***Sample size:*** 100 mg ***CSP:*** CTA-I [158] ***Column (cm):*** 2.5 x 30		***Sample size:*** 500 mg / 1 g ***CSP:*** DNBPG-co [164] ***Column (cm):*** 8 x 22
	***Sample size:*** 120 mg ***CSP:*** CTA-I [158] ***Column (cm):*** 2.5 x 30		***Sample size:*** 100-150 mg ***CSP:*** DNB-Tyr-E [58, 165] ***Column (cm):*** 4 x 26
	***Sample size:*** 100 mg ***CSP:*** MMBC [28] ***Column (cm):*** 2.5 x 50		***Sample size:*** 100 mg ***CSP:*** DNB-Tyr-A [56, 58] ***Column (cm):*** 6 x 7.6
	***Sample size:*** 40 mg ***CSP:*** CTA-I [159] ***Column (cm):*** 1.2 x 50		***Sample size:*** 38 mg ***CSP:*** DNB-Tyr-E [58] ***Column (cm):*** 6 x 10
	***Sample size:*** 100 mg ***CSP:*** Chiralcel OB [160] ***Column (cm):*** 2 x50		***Sample size:*** 220 mg ***CSP:*** DNBPG-co [166] ***Column (cm):*** 1.1 x 30
	***Sample size:*** 3 g ***CSP:*** DNBPG-io [142] ***Column (cm):*** 5 x 76		***Sample size:*** 100 mg ***CSP:*** CTA-I [167] ***Column (cm):*** 2.8 x 66
	***Sample size:*** 100 mg ***CSP:*** DACH-DNB [162] ***Column (cm):*** 5 x 76		

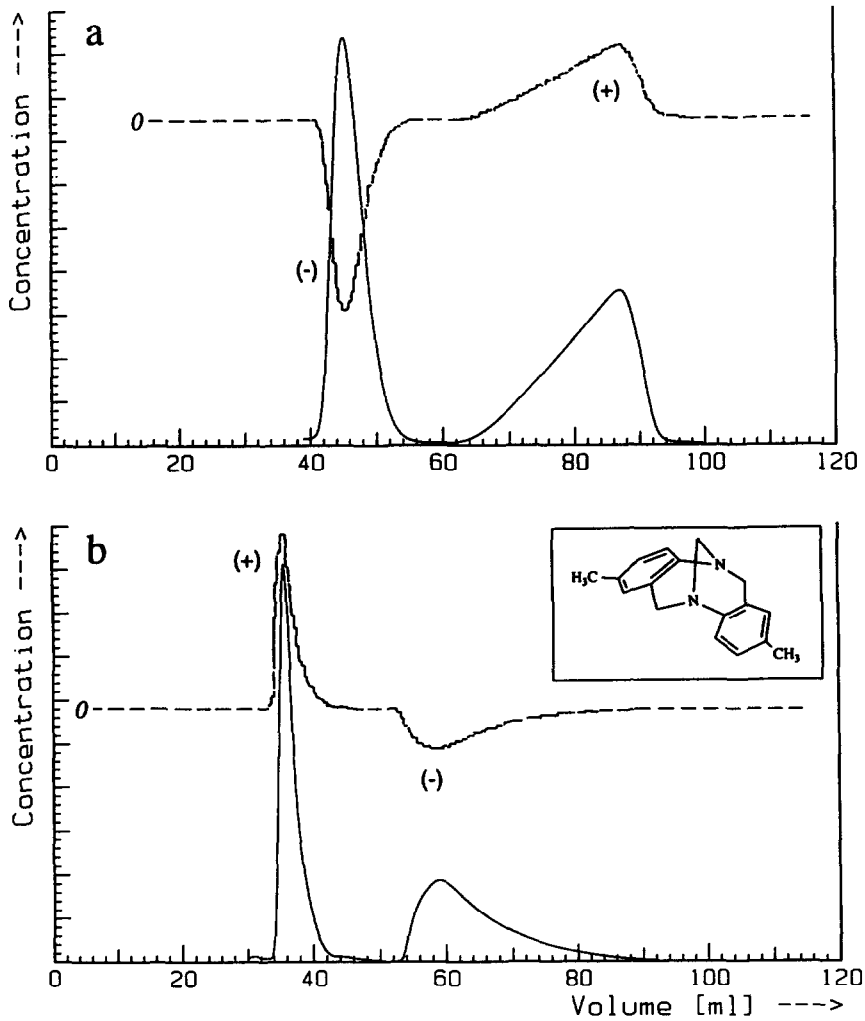


Fig. 19. Chromatographic resolution of Tröger's base on (a) cellulose triacetate and (b) dextran triacetate [28]. The dashed traces are the optical rotation at 365 nm. Column, 30 cm × 1.25 cm I.D.; mobile phase, ethanol–water (95:5); flow-rate, 0.5 ml/min; temperature, 22°C; sample size, 3.4 mg in 0.5 ml.

Enantiomers of various chiral sulphur (sulphoxide and sulphimine), chiral phosphorus and even chiral selenium and chiral tin compounds have also been chromatographically separated in a simple way and up to the gram scale on CSPs (Table 8). Chiral sulphur and phosphorus compounds include also several drugs such as ifosfamide (Table 2). These examples illustrate very well the broad applicability of the technique, demonstrating that the chirality of very different types of elements can be recognized. This tool is especially useful for chiral compounds not easily resolvable by other methods.

10. ENANTIOMERS OF ALLENES, CUMULENES AND SPIRO DERIVATIVES

Many molecules are chiral even in the absence of asymmetric atoms. Allenes, cumulenes and spiranes (Fig. 20) being to a class of structures that can be chiral because they possess a binary C_2 symmetry. Such chirality can also be recog-

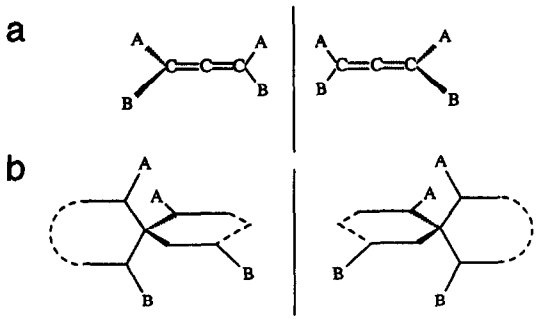


Fig. 20. Structures with axial chirality: (a) allene; (b) spiro.

nized by a CSP and some applications have been reported (Table 9).

All resolutions were performed on CTA-I or PTrMA as a CSP, showing that the structure of both polymeric materials is particularly adapted to recognizing axial chirality.

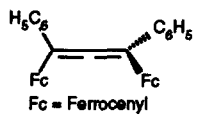
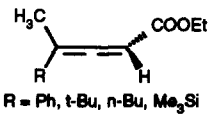
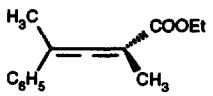
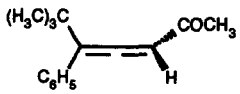
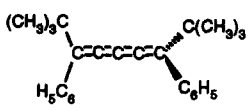
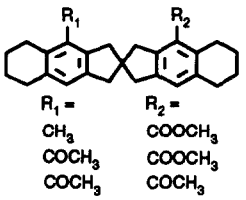
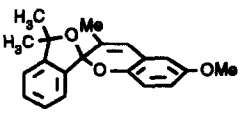
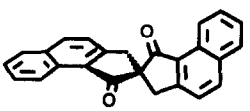
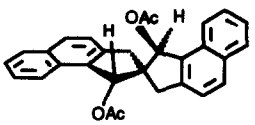
11. CHIRAL COMPOUNDS WITH RESTRICTED ROTATION

Molecules containing adjacent π-systems which cannot adopt a coplanar conformation because of rotational restrictions due to sterical hindrance can exist in two mirror forms depending on the substitution on the π-system. This is the case for some dienes or olefins (Fig. 21a) (Table 10), for some non-planar amides, thioamides and enamides (Fig. 21b) (Table 10) and for the biphenyl or binaphthyl type of compounds (Fig. 21c) (Table 11). Especially for molecules lacking functional groups such as *cis,trans*-1,3-cyclooctadiene or diisopropyl-2-dimethylstyrene (Table 10), chromatography on CSPs have proved to be a unique way to access the enantiomers. Also for non-planar amides and thioamides, which cannot be resolved via dia-

TABLE 9

PREPARATIVE RESOLUTION OF RACEMIC ALLENES, CUMULENES AND SPIRO COMPOUNDS

nm. = Not mentioned.

 Fc = Ferrocenyl	***Sample size:*** nm. ***CSP:*** CTA-I [168] ***Column (cm):*** 2.5 x 30
 R = Ph, t-Bu, n-Bu, Me_3Si	***Sample size:*** nm. ***CSP:*** CTA-I [65] ***Column (cm):*** 2.5 x 16
	***Sample size:*** nm. ***CSP:*** CTA-I [65] ***Column (cm):*** 2.5 x 16
	***Sample size:*** nm. ***CSP:*** CTA-I [65] ***Column (cm):*** 2.5 x 16
	***Sample size:*** 150 mg ***CSP:*** CTA-I [169] ***Column (cm):*** 2 x 100
 R_1 = CH_3, $COCH_3$, $COCH_3$; R_2 = $COOCH_3$, $COOCH_3$, $COCH_3$	***Sample size:*** 70 mg / 3 g ***CSP:*** CTA-I [73, 74] ***Column (cm):*** 6.3 x 66
	***Sample size:*** nm. ***CSP:*** CTA-I [170] ***Column (cm):*** nm.
	***Sample size:*** nm. ***CSP:*** PTrMA [171] ***Column (cm):*** nm.
	***Sample size:*** nm. ***CSP:*** PTrMA [171] ***Column (cm):*** nm.

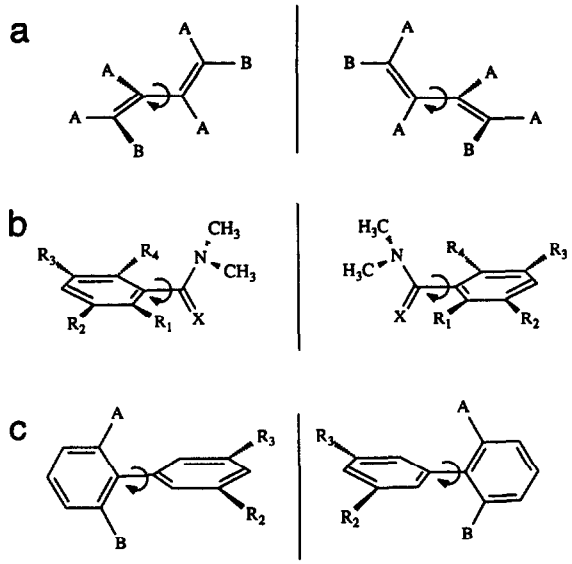


Fig. 21. Molecules with restricted rotation (indicated with an arrow): (a) butadiene; (b) aryl(thio)amides; (c) biphenyl.

stereoisomers, this is the only method for preparing the enantiomers. This class of molecule has been particularly investigated by Mannschreck and co-workers [175–177], who isolated the enantiomers by preparative resolution on CTA-I and investigated the barrier to rotation (Table 10).

Biphenyl derivatives (Fig. 21c) which are disubstituted in the 2-position are non-planar as a consequence of the steric interaction between the substituents. When both *ortho* substituents are different, the molecule possesses C_2 symmetry and is chiral. Nevertheless, to observe the two optical antipodes (atrope isomers), it is necessary for the barrier to rotation of the biphenyl bond to be large enough (higher than 80–90 kJ mol^{-1}). The energy barrier to rotation depends on the nature of the substituent. The typical hindered rotation of the biphenyl system is also found in related structures such as

TABLE 10

PREPARATIVE RESOLUTION OF RACEMIC COMPOUNDS WITH RESTRICTED ROTATION

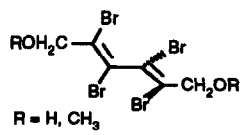
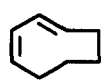
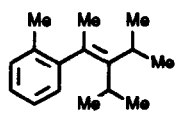
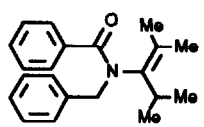
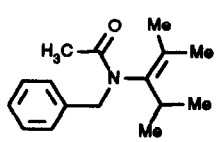
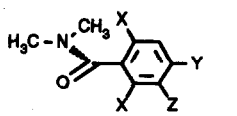
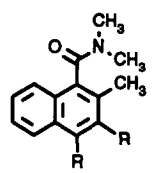
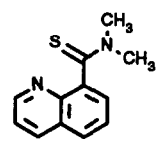
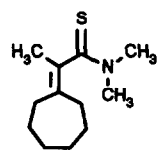
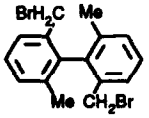
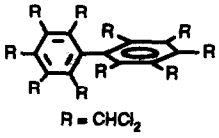
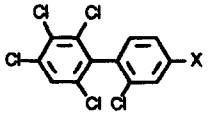
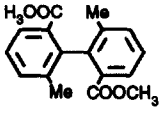
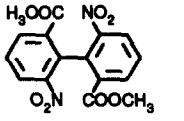
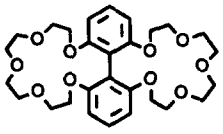
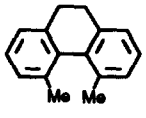
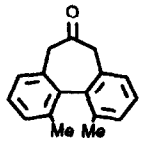
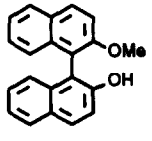
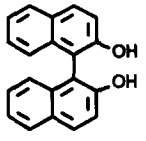
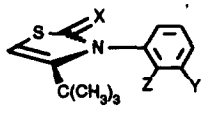
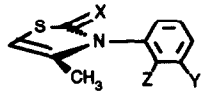
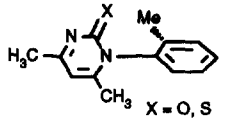
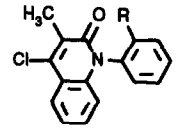
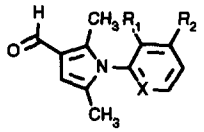
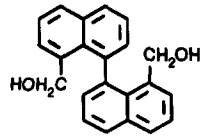
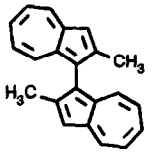
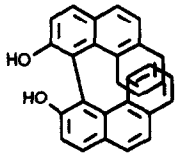
 R = H, CH_3	***Sample size:*** 100-300 mg ***CSP:*** CTA-I [172] ***Column (cm):*** 2.5 x 30
	***Sample size:*** 30 mg ***CSP:*** CTA-I [173] ***Column (cm):*** 2.5 x 30
	***Sample size:*** 10 mg ***CSP:*** CTA-I [174] ***Column (cm):*** 2.5 x 30
	***Sample size:*** 200 mg ***CSP:*** CTA-I [175] ***Column (cm):*** 2.5 x 30
	***Sample size:*** 500 mg ***CSP:*** CTA-I [175] ***Column (cm):*** 2.5 x 30
 X = CH_3, Br, I, OCH_3 Y = H, CH_3, Br, I Z = CH_3, NH_2, NO_2, Cl, I	***Sample size:*** 30-40 g ***CSP:*** CTA-I [176] ***Column (cm):*** 2.5 x 30
	***Sample size:*** 30-40 mg ***CSP:*** CTA-I [176] ***Column (cm):*** 2.5 x 30
	***Sample size:*** nm. ***CSP:*** CTA-I [177] ***Column (cm):*** 0.8 x 25
	***Sample size:*** 30 mg ***CSP:*** CTA-I [131] ***Column (cm):*** 2.5 x 30

TABLE 11

PREPARATIVE RESOLUTION OF BIPHENYL-TYPE RACEMATES

nm. = Not mentioned.

	***Sample size:*** 5-50 mg ***CSP:*** CTA-I [168] ***Column (cm):*** 2.5 x 30	
	***Sample size:*** nm. ***CSP:*** Chiralcel OD [178] ***Column (cm):*** 2.5 x 30	
	***Sample size:*** 80 mg ***CSP:*** CTA-I [179, 180] ***Column (cm):*** 2.5 x 30	
	***Sample size:*** 5-50 mg ***CSP:*** CTA-I [168] ***Column (cm):*** 2.5 x 30	
	***Sample size:*** 1 g ***CSP:*** CTA-I [74] ***Column (cm):*** 6.3 x 66	
	***Sample size:*** 53 mg ***CSP:*** CTA-I [181] ***Column (cm):*** 2 (1 x 60)	
	***Sample size:*** nm. ***CSP:*** CTA-I [182] ***Column (cm):*** 2.5 x 30	
	***Sample size:*** 50 mg / 1 g ***CSP:*** CTA-I [74, 168, 182] ***Column (cm):*** 6.3 x 66	
	***Sample size:*** 1 g ***CSP:*** BNBPG-io [142] ***Column (cm):*** 5 x 76	
	***Sample size:*** 40 mg ***CSP:*** PTrMA [188] ***Column (cm):*** 2.2 x 30	
	***Sample size:*** 100 mg ***CSP:*** CTA-I [183, 184] ***Column (cm):*** 2.5 x 20	
	***Sample size:*** 80-100 mg ***CSP:*** CTA-I [184, 185] ***Column (cm):*** 2.5 x 20	
	***Sample size:*** 80-100 mg ***CSP:*** CTA-I [185] ***Column (cm):*** 2.5 x 20	
	***Sample size:*** 1-20 mg ***CSP:*** CTA-I [186] ***Column (cm):*** 2.5 x 30	
	***Sample size:*** 1.3-5 mg ***CSP:*** CTA-I [187] ***Column (cm):*** 2.5 x 30	
	***Sample size:*** 1.6 g ***CSP:*** CTA-I [74] ***Column (cm):*** 6.3 x 66	
	***Sample size:*** nm. ***CSP:*** PTrMA [189] ***Column (cm):*** 0.46 x 25	
	***Sample size:*** nm. ***CSP:*** PTrMA [190] ***Column (cm):*** 0.46 x 25	

binaphthyl derivatives and heterocyclic analogues, which have been resolved in their corresponding enantiomers by preparative chromatography on diverse CSPs, mainly CTA-I (Table 11). Applications where a nitrogen–carbon bond is involved also include biologically active compounds such as the hypnotic drug methaqualone [77,78] (Table 2) and the fungicide clozylacon [76,126] (Table 3). Some chiral polychlorinated biphenyl derivatives have also been found to exhibit interesting biological activities [179,180] and the enantiomers were obtained by chromatographic resolution on CTA-I.

12. ENANTIOMERS OF PLANAR CHIRAL COMPOUNDS

In addition to the different classes of compounds discussed above and possessing a centre or an axis of chirality, there are numerous molecules which have a plane of chirality as a stereogenic unit. This kind of chiral structure, including ansa compounds, cyclophane derivatives (Fig. 22a), some metallocenes and bridged annulenes, have been exhaustively reviewed by Schlögl and methods for preparing the optically active forms were discussed [191].

As that time, about 10 years ago, the potential of chromatography on CSPs to resolve this type of racemate was already recognized, but with the development of the technique, numerous new applications have since appeared (Table 12). In many instances, the optical antipodes could be isolated for the first time using the chromatographic method.

13. COMPOUNDS WITH HELICAL OR PROPELLER CHIRALITY

They are other types of molecules in which steric strain prohibits a planar conformation, leading to the existence of non-superimposable mirror images. Typical examples are polyaromatic compounds building a helical structure and compounds having a propeller-type arrangement (Fig. 22b and c). Table 13 gives a representative list of the preparatively resolved racemates exhibiting helical chirality, including the shortest members of this class, namely phenanthrene derivatives. Numerous structures of this type were investigated by Mannschreck and coworkers, who showed that CTA-I is particularly appropriate for recognizing this helical chirality [131,199,200].

Molecules with propeller chirality are usually difficult to resolve into the corresponding enantiomers. Chromatographic separation on CSPs provides an elegant method to fulfil this task. Reported applications are given in Table 13 and were performed on CTA-I and PTrMA. Both

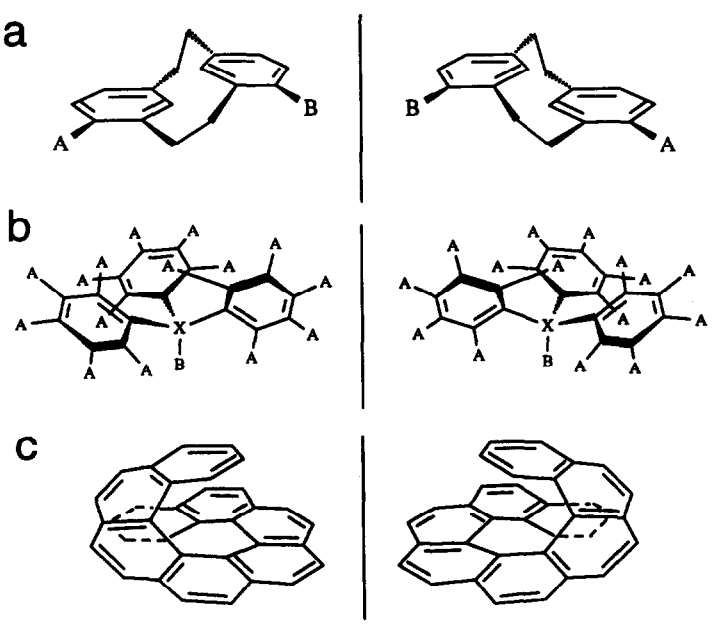
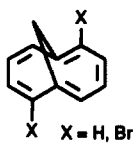
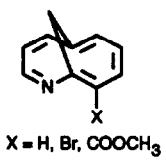
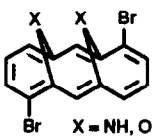
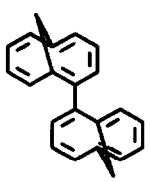
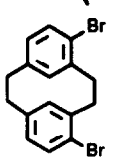
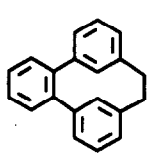
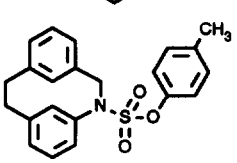
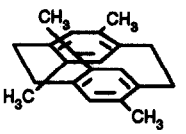
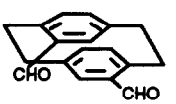
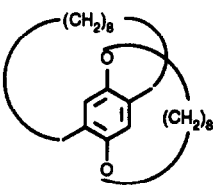
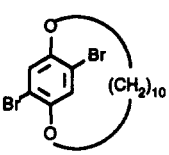
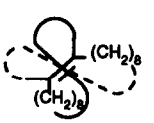


Fig. 22. (a) *m*-Cyclophane; (b) propeller; (c) helicene.

TABLE 12

PREPARATIVE SEPARATION OF THE ENANTIOMERS OF PLANAR CHIRAL COMPOUNDS

nm. = Not mentioned.

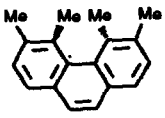
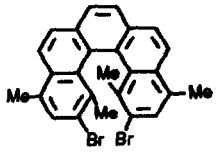
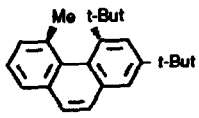
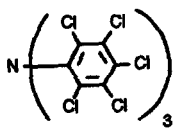
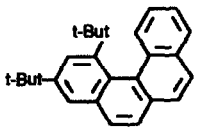
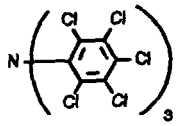
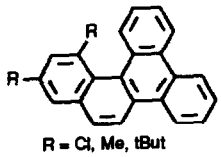
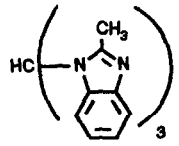
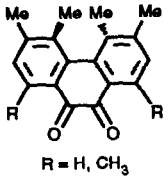
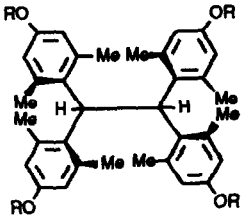
 X = H, Br	***Sample size:*** 10-450 mg ***CSP:*** CTA-I [192, 193] ***Column (cm):*** 6.3 x 66
 X = H, Br, $COOCH_3$	***Sample size:*** 10-20 mg ***CSP:*** CTA-I [168, 194] ***Column (cm):*** 2.5 x 30
 X = NH, O	***Sample size:*** 20 mg ***CSP:*** CTA-I [193] ***Column (cm):*** 2.5 x 30
	***Sample size:*** nm. ***CSP:*** CTA-I [192] ***Column (cm):*** 6.3 x 66
	***Sample size:*** 5-500 mg ***CSP:*** CTA-I [74, 168] ***Column (cm):*** 6.3 x 66
	***Sample size:*** 50 mg ***CSP:*** CTA-I [195] ***Column (cm):*** 2.5 x 30
	***Sample size:*** 10 mg ***CSP:*** CTA-I [196] ***Column (cm):*** nm.
	***Sample size:*** 70 mg ***CSP:*** PTrMA [29] ***Column (cm):*** 0.95 x 37.5
	***Sample size:*** 5 mg ***CSP:*** Chiralcel OD [198] ***Column (cm):*** 0.46 x 25
	***Sample size:*** 70 mg ***CSP:*** PTrMA [29] ***Column (cm):*** 0.95 x 37.5
	***Sample size:*** 250 mg ***CSP:*** CTA-I [197] ***Column (cm):*** 3.1 x 40
	***Sample size:*** 200 mg ***CSP:*** PTrMA [29] ***Column (cm):*** 1.16 x 80.6

polymeric stationary phases seems to be particularly adapted for recognizing helical- and propeller-type chirality, maybe because these polymers are themselves known to adopt a helical conformation. This observation points out a similarity concept, in contrast to the reciprocality concept developed by Pirkle, illustrating that a certain "type of chirality" is better recognized by an environment having a chirality based on the same type of stereogenic unit.

TABLE 13

PREPARATIVE RESOLUTION OF RACEMATES WITH HELICAL OR PROPELLER CHIRALITY

nm. = Not mentioned.

	*Type of chirality:* helical *Sample size:* 5 mg *CSP:* CTA-I [131, 199] *Column (cm):* 2.5 x 30		*Type of chirality:* helical *Sample size:* nm. *CSP:* PTrMA [201] *Column (cm):* nm.
	*Type of chirality:* helical *Sample size:* 10 mg *CSP:* CTA-I [199] *Column (cm):* 2.5 x 30		*Type of chirality:* propeller *Sample size:* 50 mg *CSP:* CTA-I [202] *Column (cm):* 2 x 38
	*Type of chirality:* helical *Sample size:* 10 mg *CSP:* CTA-I [199] *Column (cm):* 2.5 x 30		*Type of chirality:* propeller *Sample size:* 6 mg *CSP:* PTrMA [203] *Column (cm):* 2.2 x 30
	*Type of chirality:* helical *Sample size:* 10 mg *CSP:* CTA-I [199] *Column (cm):* 2.5 x 30		*Type of chirality:* propeller *Sample size:* 15 mg *CSP:* CTA-I [204] *Column (cm):* 2.5 x 20
	*Type of chirality:* helical *Sample size:* nm. *CSP:* CTA-I [200] *Column (cm):* 2.5 x 30		*Type of chirality:* propeller *Sample size:* nm. *CSP:* CTA-I [205] *Column (cm):* 2.5 x 30

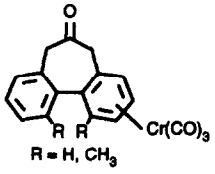
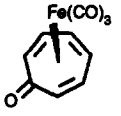
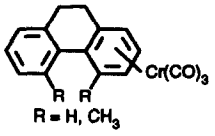
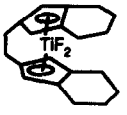
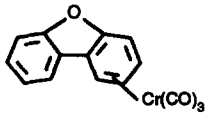
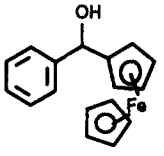
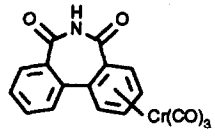
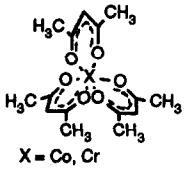
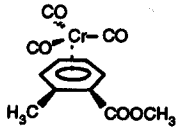
14. CHIRAL METALLOCENES

An interesting application of preparative resolution on CSPs is the separation of the enantiomers of organometallic compounds. Chiral metallocenes are generally difficult to obtain in their optically pure forms. Owing to the very mild and neutral experimental conditions usually applied in chromatography on CSPs, the method is particularly appropriate for resolving racemic metallocenes. In addition to cellulose triacetate (CTA-I), the 3,5-dimethylphenyl carbamate derivative of cellulose (Chiralcel OD) seems to be particularly appropriate for the resolution of racemic metallocenes, as demonstrated in various recent analytical applications [206–208]. Most of the preparative applications that have been reported are summarized in Table 14, which contains examples referring to the different kinds of chirality discussed in this review, namely centro-, axial- or planar-chiral and propeller. Fig. 23 shows the chromatographic separation of the enantiomers of a racemic titanocene derivative [211].

TABLE 14

PREPARATIVE RESOLUTION OF RACEMIC METALLOCENES

nm. = Not mentioned.

	***Sample size:*** 2-5 mg ***CSP:*** CTA-I [168, 182] ***Column (cm):*** 2.5 x 30		***Sample size:*** nm. ***CSP:*** PTrMA [210] ***Column (cm):*** 1.67 x 25
	***Sample size:*** 2-5 mg ***CSP:*** CTA-I [182] ***Column (cm):*** 2.5 x 30		***Sample size:*** 300 mg ***CSP:*** CTA-I [211] ***Column (cm):*** 5 x 60
	***Sample size:*** 20 mg ***CSP:*** CTA-I [209] ***Column (cm):*** 2.5 x 30		***Sample size:*** 40 mg ***CSP:*** CTA-I [73] ***Column (cm):*** 2.5 x 30
	***Sample size:*** 20 mg ***CSP:*** CTA-I [209] ***Column (cm):*** 2.5 x 30		***Sample size:*** nm. ***CSP:*** PTrMA [212] ***Column (cm):*** 0.46 x 75
	***Sample size:*** 500 mg ***CSP:*** CTA-I [74] ***Column (cm):*** 6.3 x 66		

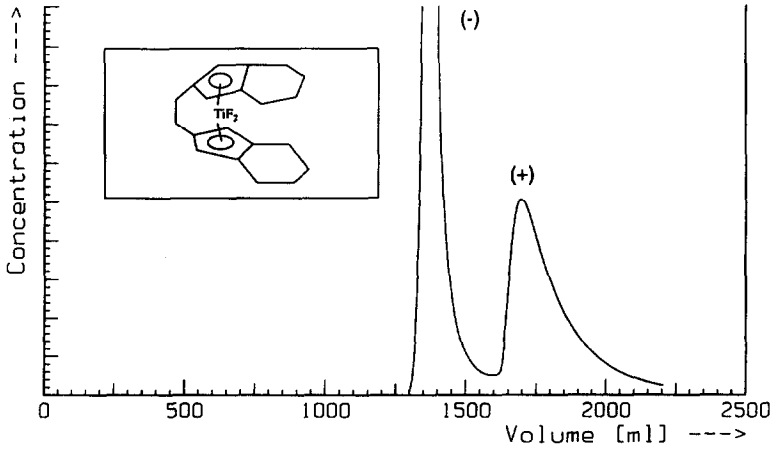


Fig. 23. Preparative chromatographic resolution of 300 mg of the racemic difluorotitanocene derivative (Table 14) on cellulose triacetate (CTA-I) [211]. Column and chromatographic conditions as in Fig. 10.

15. CONCLUSIONS

A variety of CSPs are now available for the preparative resolution of racemic compounds and the technique has been used to separate the enantiomers of molecules possessing different types of chirality (central, axial, planar, helical). Interest in the method has been especially recognized in the pharmaceutical field because it constitutes a powerful and widely applicable tool for preparing the enantiomers of potential new drugs. Moreover, numerous racemic molecules that could not be resolved by conventional methods, especially those lacking functional groups, were separated for the first time into their antipodes by chromatography on CSPs. For large-scale separations of up to kilograms of racemate, CTA-I has been preferred, probably because of the relatively low cost of this CSP. Further, CTA-I exhibits good chiral recognition not only for centro-chiral compounds but also for molecules with axial, planar or helical chirality. Preparative applications on other cellulose derivatives have been reported, but the feasibility of large-scale separations has still to be demonstrated. Some drugs were particularly well resolved on poly(meth)acrylamide CSP types. Chiral sulphur and phosphorus compounds were mainly resolved on π-acid CSPs. All these examples show that, even if new developments are to be expected in this field, the currently available CSPs already allow the optically pure enantiomers of many racemates to be isolated and to contribute actively to the investigation of the fascinating area of chirality.

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