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Review

Preparative chromatographic separation of enantiomers

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ABSTRACT

The potential of the chromatographic separation of enantiomers on a preparative scale as a tool for the isolation of optically pure compounds is gaining increasing recognition. This review surveys the different chiral stationary phases (CSPs) used for preparative chromatography, emphasizing the advantages and drawbacks of each. The strategy to be followed for preparative separations is discussed and tables summarizing separations reported in the literature give an overview of practical applications. Cellulose triacetate has been used most frequently, probably because of its broad application range and its low production costs in comparison with more recently introduced CSPs. Nevertheless, the high efficiency of some of the novel CSPs is likely to contribute to the further development and expansion of the method.

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1. INTRODUCTION

Owing to the increasing demand for optically active compounds, the development of methods for obtaining optically pure isomers is being intensively pursued. The preparation of optically active compounds has become very important for the development of new biologically active substances containing one or several chiral centres, because it is now apparent that many chiral drugs and agrochemicals (herbicides, fungicides, insecticides, etc.) display different activity and toxicity profiles with respect to their absolute configuration.

For a long time the major source of optically active compounds was nature (chiral pool). In the last few decades, however, numerous synthetic strategies and methods (including enzymatic reactions) have been developed to prepare an increasing number of optically active building blocks or chiral auxiliary reagents. Many approaches have been established on the basis of optically active catalysts which transfer their chiral information during the creation step of new centres of chirality. Among the different methods, chromatographic resolution on chiral stationary phases can be regarded as a useful alternative for the preparation of optically pure compounds and belongs to the methods by which the material carrying the chiral information is not consumed but can be used repetitively (like a catalyst).

The direct separation of enantiomers by chromatography is now widely used and a large number of chiral columns are commercially available, allowing many analytical problems to be solved (optical purity of compounds resulting from enantioselective synthesis, pharmacological studies, etc.). However, only a limited number of preparative applications have been reported. Nevertheless, chiral stationary phases (CSPs) can be used not only as an analytical tool, but also for the production of optically active materials. The method is especially attractive for industry because it allows the rapid and easy supply of amounts of materials suitable for biological testing, toxicological studies and even, in a later stage, clinical or field testing. Also, data concerning the activity and toxicity profiles of the individual pure enantiomers are now systematically required by the authorities for all new chiral drugs submitted for registration. At least during the preliminary test phase of a chiral biologically active substance, chromatography can replace the often lengthy elaboration of an enantioselective synthesis (Fig. 1). Taking in account that only a limited number of drugs proceed to more intensive investigations, a systematic synthesis would necessitate much time and manpower before starting the preliminary tests needed to decide about the further development of either a pure enantiomer of the racemate. Additionally, if the drug has been selected for further study,

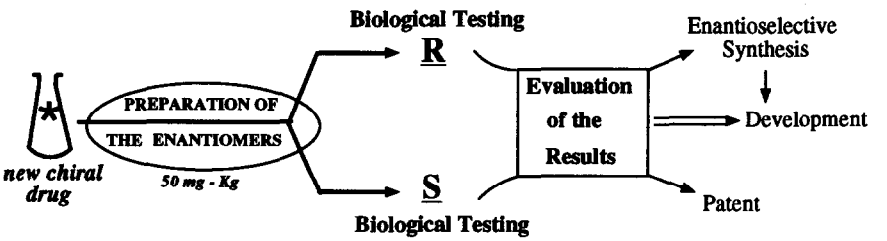


Fig. 1. Pathway for the development of a new chiral drug.

chromatography can continue to supply more enantiomerically pure substance for the biological investigations while an enantioselective synthesis is being developed. Moreover, the chromatographic method offers the advantage of furnishing both enantiomers obviously required for comparative biological testing. For preliminary tests, amounts ranging between 100 and 200 mg are generally sufficient and therefore we have chosen arbitrarily 150 mg as the lowest amount of separated racemate to be reported in this review. A non-negligible number of separations (about 160) have been performed for amounts ranging between 5 and 150 mg, but the limitation to 150 mg will not modify the conclusions of this review regarding the usefulness of the different chiral stationary phases used for preparative applications.

The chromatographic separation of enantiomers does not differ basically from the classical methods of chromatography, taking advantage of the difference of the interaction energies between two different solutes and a stationary phase. In a chiral environment two enantiomers can be considered as physically distinct compounds. Owing to the opposite configurational arrangement of their atoms in the space, enantiomers may interact differently with a chiral surrounding. The chiral differentiation can be exerted either by a chiral mobile phase or by a chiral stationary phase. Thus the difference from "normal chromatography" resides only in the type of mobile or stationary phase to be used. For this reason, this review will not cover the "indirect separation" of enantiomers on an achiral stationary phase after derivatization to diastereoisomers with an optically active reagent. This last method is only a special application of classical chromatography and has recently been reviewed [1,2]. Emphasis will be placed on the advantages and problems specific to the direct separation of enantiomers using chiral phases.

This paper is focused on preparative separations mostly performed under medium-pressure conditions, which are technically the most appropriate on a large scale. As "preparative" we shall consider any separation involving the isolation of each enantiomer in the limits of the amounts defined above for further use, *e.g.*, for the determination of the chiroptical properties, for biological tests or as chiral intermediates in synthesis.

The potential of the chromatographic method for the separation of enantiomers on a preparative scale using chiral stationary phases was recognized during the first attempts on lactose by Henderson and Rule in 1939 [3] and later by Lecoq [4] and Prelog and Wieland [5]. Since these pioneering studies, the method has undergone a spectacular development owing to the concomitant evolution of the chromatographic techniques and the design of numerous new chiral phases. Different reasons can be given for this strong development: (1) the importance of the relationship between the stereochemistry of a chiral compound and its biological activity has generally been recognized; (2) in parallel, the enantioselective synthesis has been considered as a new, promising challenge in organic chemistry and needed new, reliable analytical support; (3) in many instances it has been found that chromatography could be the most efficient and rapid method for the preparation of enantiomers and in some instances it is even the only alternative (atrope isomers, no possibility of derivatization,

etc.). The large number of chiral high-performance liquid chromatographic (HPLC) and gas chromatographic (GC) columns which have been introduced [6,7] in recent years clearly accounts for this development. This analytical tool has been welcome for solving the increasing number of problems related to stereochemistry in biological tests and pharmacokinetic studies required for the development of new chiral agrochemicals or drugs. Nevertheless, the preparative potential of the method has been somewhat neglected until now. This might change rapidly because of the growing importance of isolating the optically pure enantiomeric forms of new chiral biologically active compounds as discussed above.

As mentioned above, chromatographic resolutions can be achieved by using an achiral stationary phase and a chiral solvent or chiral additive in the mobile phase. This last technique has been applied successfully on an analytical scale and has recently been reviewed [8], but owing to the difficulties in recovering the separated enantiomers (contaminated with the additive), no preparative applications have been reported. Therefore, this review will be focused exclusively on the use of chiral stationary phases. Obviously, chiral stationary phases can also be used for the separation of diastereoisomeric mixtures or even achiral compounds, as exemplified by the numerous applications on the commercially available cellulose-based ion exchangers or dextran (Sephadex), but these utilizations are outside the scope of this review. Also, as we defined above, we limited to 150 mg the minimum amount of separated racemate to be reported in this work and we did not consider the separations if the injected amount is not specified, except if the dimensions of the column suggested that large amounts had been chromatographed.

It must be emphasized that this review should help above all those workers who want to use the chromatographic enantiomer separation method on a preparative scale. It is intended to give a general outline of the different applications reported in the literature, showing the advantages and disadvantages of the various chiral stationary phases developed up to now for this purpose.

2. PROPERTIES OF CHIRAL PHASES

2.1. Requirements for preparative separations

2.1.1. General properties

Most problems related to preparative chromatography on chiral stationary phases are not specific to chirality and can be treated similarly to those encountered in any preparative chromatographic separation. The general scale-up problems in chromatography have been discussed in different reviews from theoretical and practical points of view [9–16]. Nevertheless, in chiral separations, some properties can be determining for the choice of the chiral phase and a general strategy to be followed for performing preparative separations is presented in the next section. Depending on the analytical or preparative purpose, the properties required for chromatographic materials used as stationary phases are different. For instance, whereas the loading capacity of the stationary phase is not determining for analytical separations, the efficiency (resolution) is a very important factor. For preparative purposes, however, the loading capacity of the sorbent is very important. General features have to be considered in developing or using stationary phases for preparative separations:

wide availability;
easy preparation of the phase;
relatively low preparation costs;
durability (chemical and mechanical stability);
high loading capacity of the phase; and
broad range of applicability.

The development of a chiral stationary phase which would simultaneously fulfil all these requirements is probably impossible. Still, a number of phases have already been used successfully for resolutions on a preparative scale.

2.1.2. Resolution

The factors affecting the resolving power are, of course, the same as for more conventional chromatographic supports, *i.e.*, particle size, porosity, surface area, elution rate, column dimensions, etc. [17]. Owing to the utilization (in general) of materials with a larger particle size, the

chromatographic performance of preparative separations is lower than that of the corresponding analytical separations. Therefore, a separation factor of at least 1.2–1.3 is generally a prerequisite for attempting a preparative separation. This value is a lower limit if the resolution factor is small; it can be less, however, when for example recycling techniques are used [18].

2.1.3. Isolation

An important point in preparative applications is that the resolved compounds should be easily isolated. This problem has to be considered particularly in cases where additives are used in the mobile phase which have to be removed from the desired solute after the separation.

2.1.4. Derivatization

If the racemate has to be derivatized to improve the separability, special attention has to be paid to the choice of the derivatizing group to make sure that no racemization occurs during the step involving the cleavage of this group.

2.1.5. Mobile phases

The choice of the mobile phase can also be a limiting factor because of the physico-chemical properties of the stationary phase (dissolution or swelling). In addition, when flammable solvents are used on a large scale, special safety requirements are imposed. The price of the mobile phase becomes relevant in preparative applications.

2.2. Chemical and physical properties of chiral stationary phases

When using chiral stationary phases, one must pay attention to some chromatographic properties that are usually neglected. An appreciable number of chiral phases used for chromatographic resolutions are based on organic polymeric materials which can exhibit some restrictions regarding their chemical, physical or mechanical properties. On the other hand, numerous chiral phases have been developed by fixing or coating chiral low-molecular-weight compounds or macromolecules on silica gel used as a mechanical support. In these instances only a part of the chromatographic material is capable of chiral discrimination. This can reduce appreciably the total loading capacity of the stationary phase.

2.2.1. Chemical and physical properties

There are fundamentally two major classes of chiral stationary phases: (1) polymeric materials in the pure form or as a coating on a macroporous mechanically stable support; and (2) materials obtained by chiral chemical modifications of the surface of a stable supporting phase (mostly silica gel).

If they are not cross-linked, most polymeric materials are more or less soluble in numerous solvents. For example, cellulose triacetate (CTA), which has proved to a very useful chiral stationary phase for preparative purposes [19–22], cannot be used with chlorinated alkanes as mobile phases because they dissolve the polymer. Other solvents in which cellulose triacetate is strongly swelling are also excluded. This dependence is listed in Table 1 for various solvents, ethanol–water (95:5) being the usual mobile phase composition used with CTA I and causing a swelling of *ca.* 40%. In solvents such as toluene–dioxane (1:1) or nitrobenzene, the swelling becomes too great and in tetrahydrofuran cellulose triacetate forms a gel. Further cellulose derivatives have been introduced for the chromatographic resolution of racemates [23] and generally show the same limitations regarding the mobile phases. Furthermore, this effect can depend on the molecular weight or on the crystallinity of the polymer used. This information is rarely specified for commercial materials. Despite the above limitations, cellulose derivatives are probably the most often used chiral phases for preparative separations because of their broad applicability and/or their high loading capacity. Attempts have been made to improve the mechanical stability of the cellulosic derivatives by cross-linking [24], but they were not very successful, presumably owing to the alteration of the crystal structure by the introduction of covalent bonds between the polymeric chains. The importance of the crystal struc-

TABLE I

SWELLING OF 0.9 ml (0.5 g) OF CELLULOSE TRIACETATE (CTA I) IN DIFFERENT SOLVENTS

Solvent	Volume after swelling (ml)	Swelling (ml/ml CTA)
Diisopropyl ether	0.90	1.00
Hexane	1.00	1.11
Cyclohexane	1.05	1.16
Toluene–cyclohexane (1:1)	1.05	1.16
Diethyl ether	1.05	1.16
Toluene	1.10	1.22
Cyclohexene	1.10	1.22
Ethanol	1.20	1.33
Ethanol–water (95:5)	1.25	1.39
Toluene–dioxane (1:1)	1.80	2.00
Nitrobenzene	3.00	3.33
Dimethoxyethane	3.50	3.90
Dioxane	4.30	4.77
Tetrahydrofuran	Gel	–

ture (order) for the resolving power in these types of chiral sorbents has been investigated by Francotte *et al.* [25].

Strong swelling has also been reported for the polyacrylamide derivatives developed in the pure polymeric form some years ago by Blaschke and Donow [26]. Although these materials had been cross-linked, they could only be used under very-low-pressure conditions owing to their gel structure in most solvents. In order to improve the mechanical properties, these materials were later polymerized (graft polymerization) on the surface of silica gel, but consequently the loading capacity was reduced owing to the smaller amount of chiral material per unit mass.

The chemical stability of the stationary phase can also be a limiting factor. Indeed, phases bearing reactive chemical functions can be altered by injection of certain solutes. For example, nucleophilic amines exhibit very strong tailing or are no longer eluted from cellulose triacetate or some polyacrylamides, suggesting that they react with stationary phases bearing labile ester functions.

2.2.2. Loading capacity

Numerous chiral sorbents introduced for the chromatographic resolution of racemates have been obtained by fixing chiral compounds covalently or ionically on the modified surface of silica gel. These compounds are small molecules such as amino acid derivatives in the case of the Pirkle's phases, for instance [27], or high-molecular-weight compounds such as cyclodextrins [28], polypeptides [29] or polymethacrylamides [30]. Macroporous silica gel has also been used as an inert support for coating with polymeric materials [23]. Although the mechanical properties of these materials are generally satisfactory, they have the disadvantage that only a part of the sorbent contains the chiral information capable of differentiation between the enantiomers. Obviously, this feature will be a determining factor for the loading capacity of the material. A stationary phase mostly composed of silica gel with only a few chiral elements will be rapidly overloaded. In this event, even if the phase exhibits useful properties for analytical purposes, it will not be appropriate for preparative applications. This restriction has also to be considered when only a small part of the chiral material seems to be involved in the chiral recognition process. This is the case for the protein-based phases, which generally show a very low capacity. Presumably only a limited number of "active sites" of the macromolecule are involved in the interaction.

Further, not only the number but also the availability of the potential interaction sites is important to obtain a useful preparative sorbent. In fact, this last factor is related to the physical properties of the material such as surface area or porosity, which are general features to be considered about sorbents for chromatography. These properties are not necessarily conserved on modifying the surface of silica gel with chiral materials but they are rarely discussed in the literature.

2.3. Strategy for preparative separations

The general strategy to be followed for performing a preparative chiral separation is summarized schematically in Fig. 2.

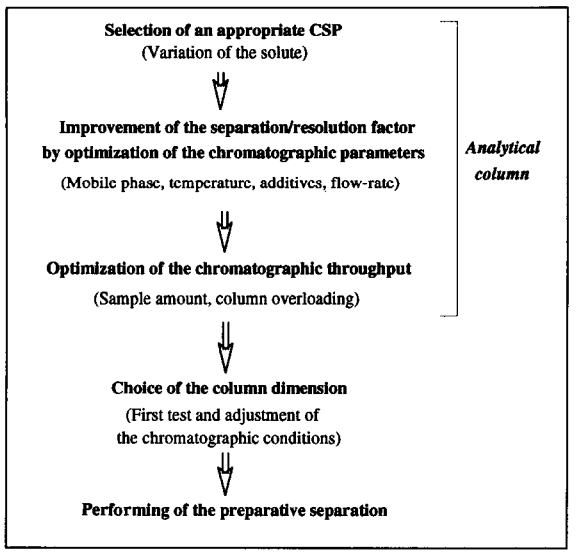


Fig. 2. General strategy for a preparative chiral separation.

2.3.1. Selection of the CSP

Before starting a preparative chiral separation, it is obvious to identify a chiral stationary phase exhibiting a good chiral recognition ability. This is usually done with an analytical column because it is less substance and time consuming. Predictions based on structural requirements or empirical rules [6,31] can help in selecting the appropriate CSP, but as the number of CSPs available (see Table 2) for separations of amounts larger than 100 mg is limited, the selection can be carried out by screening the different columns. The electronical chiral database "Chirbase", recently introduced by Roussel *et al.* [32], can also be a useful tool for selecting the CSP because it allows a search for separations of racemates reported in the literature on the basis of structural fragments. However, many examples have demonstrated that small alterations of the structure of the solute can have unpredictable and determining influences on the separation. Therefore, it is still always preferable to rely on screening. The required amount of separated enantiomers can also impose a restriction on the choice of the CSP. If large amounts are desired, larger columns have to be used and these columns can be very expensive.

The possibility of substantially improving the separation by achiral derivatization of the solute should also not be neglected. It has been shown that this strategy can lead to successful resolutions of racemates (Fig. 3) which otherwise could not be separated on a defined CSP [20,33,34]. Finally, if the desired compound from a reaction sequence cannot be separated, it is often possible to achieve resolution on a precursor.

2.3.2. Improvement of the separation/resolution factor

The most important point to ensure success in resolving a racemate is to work under high selectivity conditions, *i.e.*, under conditions which give the highest separation factor. The selectivity is first determined by the recognition ability of the CSP, but it can be also influenced by the mobile phase composition, the presence of additives, pH, temperature, etc. [35–43]. These different parameters can be optimized on an analytical column, while good solubility of the solute and rea-

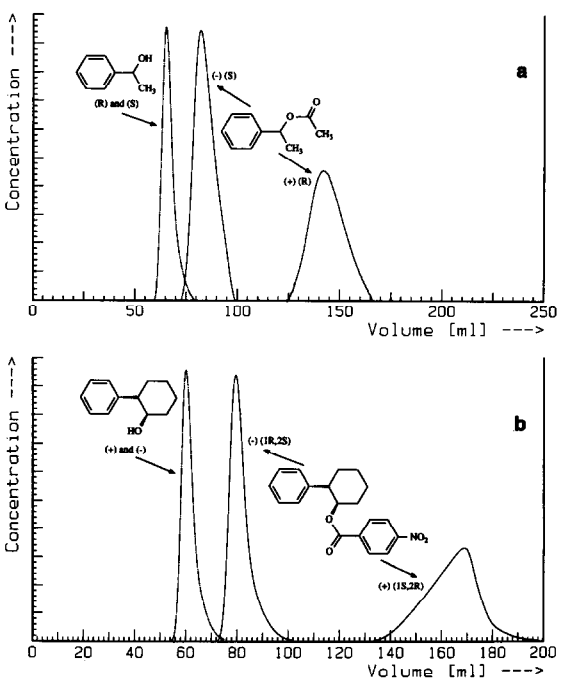


Fig. 3. Chromatograms of (a) racemic 1-phenylethanol and its acetate derivative and (b) 2-phenyl-*trans*-cyclohexanol and its *p*-nitrobenzoate derivative on cellulose triacetate (CTA I). Column, 60 × 1.25 cm I.D.; mobile phase, ethanol–water (95:5); flow-rate, 0.5 ml/min.

TABLE 2

COMMERCIALLY AVAILABLE CHIRAL STATIONARY PHASES FOR PREPARATIVE SEPARATIONS (100 mg AND MORE PER RUN)

Type of CSP	Name	Chiral unit
Cyclodextrin phases	Cyclobond I	β-Cyclodextrin
	Cyclobond I acetylated	β-Cyclodextrin, acetylated
	Cyclobond I DMP	β-Cyclodextrin 3,5-dimethylphenylcarbamate
	Cyclobond I PT	β-Cyclodextrin *p*-methylbenzoate
	Cyclobond I RN	β-Cyclodextrin (*R*)-naphthylethylcarbamate
	Cyclobond I SN	β-Cyclodextrin (*S*)-naphthylethylcarbamate
	Cyclobond I SP	β-Cyclodextrin, (*S*)-hydroxypropyl
	Cyclobond I RSP	β-Cyclodextrin, (*R,S*)-hydroxypropyl
	Cyclobond II	γ-Cyclodextrin
	Cyclobond II acetylated	γ-Cyclodextrin, acetylated
	Cyclobond III	α-Cyclodextrin
	Cyclobond III acetylated	α-Cyclodextrin, acetylated
	Si100 α-cyclodextrin	α-Cyclodextrin
	Si100 β-cyclodextrin	β-Cyclodextrin
	Si100 γ-cyclodextrin	γ-Cyclodextrin
Cellulose-based phases	Chiralcel OA	Cellulose acetate (CTA II)
	Chiralcel OB	Cellulose benzoate
	Chiralcel OC	Cellulose phenylcarbamate
	Chiralcel OD	Cellulose 3,5-dimethylphenylcarbamate
	Chiralcel OF	Cellulose 4-chorophenylcarbamate
	Chiralcel OG	Cellulose 4-methylphenylcarbamate
	Chiralcel OJ	Cellulose 4-methylbenzoate
	Chiralcel OK	Cellulose cinnamate
	Chiralcel CA-1	Cellulose triacetate (CTA I)
	Cellulose triacetate	Cellulose triacetate (CTA I)
	CONBRIO-TAC	Cellulose triacetate (CTA I)
Polyacrylamide phase	ChiraSpher®	Poly-[(*S*)-N-acryloylphenylalanine ethyl ester]
π-Acid and π-base phases	Bakerbond DNBPG (covalent)	Dinitrobenzoylphenylglycine
	Bakerbond DNBLeu (covalent)	Dinitrobenzoylleucine
	Pirkle Prep-DPG	Dinitrobenzoylphenylglycine
	Pirkle Prep-LPG	Dinitrobenzoylleucine
	Si100-DNB-Leu (L- or D-)	Dinitrobenzoylleucine (L- or D-)
	Si100-DNB-Phgly (L- or D-)	Dinitrobenzoylphenylglycine (L- or D-)
	SUMICHIRAL OA-1000	(*S*)-Naphthylethylamine
	SUMICHIRAL OA-2000	(*R*)-Phenylglycine dinitrobenzoylamide
	SUMICHIRAL OA-2100	(*R*)-Phenylglycine, (*S*)-chlorophenylisovaleric acid
	SUMICHIRAL OA-2200	(*R*)-Phenylglycine, (1*R*,3*R*)-chrysanthemic acid
	SUMICHIRAL OA-2500	(*R* or *S*)-Naphthylglycine dinitrobenzoylamide
	SUMICHIRAL OA-3000	(*S*)-Valine *tert.*-butylurea
	SUMICHIRAL OA-3100	(*S*)-Valine dinitrophenylurea
	SUMICHIRAL OA-3200	(*S*)-*tert.*-Leucine dinitrophenylurea
	SUMICHIRAL OA-3300	(*R*)-Phenylglycine dinitrophenylurea
	SUMICHIRAL OA-4000/4100	(*S*)-Valine, (*S* or *R*)-Naphthylethylamine
	SUMICHIRAL OA-4400/4500	(*S*)-Proline, (*S* or *R*)-naphthylethylamine
	SUMICHIRAL OA-4600/4700	(*S*)-*tert.*-Leucine, (S *or R*)-naphthylethylamine
	SUMICHIRAL OA-4800/4900	(*S*)-Indoline-2-carboxylic acid, (*S or R*)-naphthylethylamine
	Spherisorb Chiral-1	(*R*)-Phenylethylamine urea

Carrier	Particle size (μm)	Supplier[a]
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	20 or 40	Astec
Bonding on silica gel	30	Serva
Bonding on silica gel	30	Serva
Bonding on silica gel	30	Serva
Polymer coating on silica gel	20	Diacel
Polymer coating on silica gel	20	Daicel
Polymer coating on silica gel	20	Daicel
Polymer coating on silica gel	20	Daicel
Polymer coating on silica gel	20	Daicel
Polymer coating on silica gel	20	Daicel
Polymer coating on silica gel	20	Daicel
Polymer coating on silica gel	20	Daicel
Pure polymer	20	Daicel
Pure polymer	15–25 or 25–40	Merck
Pure polymer	15–25	Perstorp Biolytica
Polymer grafting on silica gel	25	Merck
Bonding on silica gel	40	Baker
Bonding on silica gel	40	Baker
Bonding on silica gel	9,17 or 35	Regis
Bonding on silica gel	9,17 or 35	Regis
Bonding on silica gel	30	Serva
Bonding on silica gel	30	Serva
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	15 or 30	SCAS
Bonding on silica gel	?	Phase Sep

(Continued on p. 10)

TABLE 2 (*continued*)

Type of CSP	Name	Chiral unit
Phases for ligand-exchange chromatography	Chirosolve L- or D-pip	Pipecolic acid
	Chirosolve L-or D-phe	Phenylalanine
	Chirosolve L- or D-val	Valine
	Chirosolve L- or D-hypro	Hydroxyproline
	Chirosolve L- or D-pro	Proline
	Chirosolve L- or D-porretine	Porretine
	Si100 Hypro-Cu	Hydroxyproline
	Si100 Pro-Cu	Proline
	Si100 Val-Cu	Valine

[a] Astec: Advanced Separation Technologies (Whippany, NJ, USA). Baker: J. T. Baker (Phillipsburg, NJ, USA). Daicel: Daicel Chemical Industries (Tokyo, Japan). JPS Chimie (Bevaix, Switzerland). Merck: E. Merck (Darmstadt, Germany). Perstorp Biolytica: Perstorp Biolytica (Lund, Sweden). Phase Sep: Phase Separations (Clwyd, UK). Regis: Regis Chemical (Morton Grove, IL, USA). Serva: Serva Feinbiochemica (Heidelberg, Germany).

sonable retention times should be maintained.

The resolution factor is also determining because a good resolution means narrow peaks and consequently allows the sample throughput to be enhanced, improving the yield. It is well known that the particle size and the column packing have a strong influence on the column efficiency [17], but as the available preparative columns are usually prepacked, these parameters cannot be modified by the user. For CSPs which are available in bulk, material with larger particle sizes are usually proposed for preparative separations.

There are different types of columns and the slurry technique is usually the most convenient for packing. For CSPs used under low-pressure conditions (*e.g.*, cellulose triacetate) it is recommended to use glass columns, which allows any irregularities in the packing to be seen. For chiral packing materials with good mechanical stability, it is advantageous to use stainless-steel columns packed at high pressure. Different column hardware is available and has recently been reviewed [15,44].

The mobile phase composition can also influence the resolution factor [39,40] but this effect is unpredictable. Moreover, the presence of additives can considerably enhance the resolution. For example, the addition of 0.1% of diethylamine to the mobile phase when using the cellulose carbamate CSPs Chiralcel OC and OD generally reduces the tailing, thus improving the resolution [39]. Finally, the flow-rate can be varied to optimize the resolution factor [17], provided that the throughput is acceptable.

2.3.3. Optimization of the chromatographic yield

When the chromatography has to be repeated many times to obtain the desired amount of pure enantiomers, it is recommended to optimize the sample throughput by determining the maximum overloading conditions for a defined racemate, using an analytical column. The time factor and the mobile phase volume are more critical for preparative than for analytical separations. Guidelines for optimizing the chromatographic yield have been discussed by Tambuté *et al.* [43], Kinkel *et al.* [45] and Miller and Bush [46].

2.3.4. Column dimensions

The choice of the column dimensions is usually determined by the amount of racemate which has to be resolved. When the column and/or the packing material are very expensive, it is sometimes preferable to use a smaller column and to repeat the chromatography several times. This choice is strongly dependent on the available financial means.

Carrier	Particle size (μm)	Supplier[a]
Copolymer (polyacrylamide)	40–80 or < 40	JPS Chimie
Copolymer (polyacrylamide)	40–80 or < 40	JPS Chimie
Copolymer (polyacrylamide)	40–80 or < 40	JPS Chimie
Copolymer (polyacrylamide)	40–80 or < 40	JPS Chimie
Copolymer (polyacrylamide)	40–80 or < 40	JPS Chimie
Copolymer (polyacrylamide)	40–80 or < 40	JPS Chimie
Bonding on silica gel	30	Serva
Bonding on silica gel	30	Serva
Bonding on silica gel	30	Serva

2.3.5. Performing preparative separations

If the chromatography has to be carried out many times, it is advantageous to automate the process. In this instance it is necessary to perform a test to determine exactly the retention times, the amount of mobile phase needed per run, the fractionation and, if necessary, to readjust the amount of sample injected. The recycling technique [18], consisting in passing the sample a second or more times through the column, can be very useful (Fig. 4), especially when the separation factor is relatively low [47–49]. The entire sample can be recycled but generally it is preferable to isolate the pure fractions and to recycle only the mixed fractions. In this instance a correct fractionation is critical. This can be checked by injecting selected fractions on an analytical column. The recycling technique also has the advantage of saving considerable amounts of mobile phase.

Monitoring of liquid chromatography is usually performed by UV detection. Polarimetric detection can be coupled with UV detection and is particularly useful for compounds that are not UV absorbing. Moreover, the simultaneous online recording of the concentration (UV) and optical rotation (polarimetry) allows (with appropriate software) the continuous measurement of the specific rotation of the eluate, allowing it to be determined at which point the eluate contains a mixture of the enantiomers.

Finally, it is important to check the optical purity of the isolated fractions, and this is most appropriately done by using an analytical column exhibiting good selectivity (not necessarily the same chiral phase as used for the preparative separation).

In the next section the different chiral phases used for preparative purposes under chromatographic conditions will be reviewed, with emphasis on their respective chromatographic performance and/or limitations. Our objective is not to compile a comprehensive review, but merely to demonstrate the usefulness of the LC method for separating enantiomers on a preparative scale and to point out which kind of materials have been used for this purpose up to now. Indeed, a number of analytical and/or HPLC applications reported in different publications could probably be transposed to the preparative scale, but they

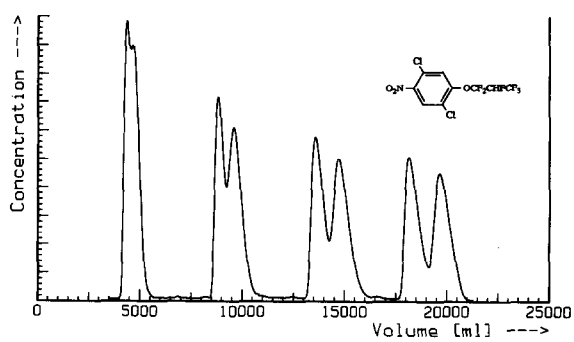


Fig. 4. Chromatographic resolution of 500 mg of racemic 3-(2,5-dichloro-4-nitrophenoxy)-1,1,1,2,3,3-hexafluoropropane (insecticide intermediate) on benzoylcellulose beads with three recyclings. Column, 75 × 5 cm I.D.; mobile phase, hexane–2-propanol (95:5); flow-rate, 12 ml/min.

will not be included here. For details of the interaction mechanism, we refer to the original literature in most instances.

3. CHIRAL STATIONARY PHASES FROM NATURALLY OCCURRING POLYMERS AND THEIR DERIVATIVES

3.1. Cellulose, dextran and starch

In addition to the disaccharide lactose [3–5], the polysaccharides cellulose, starch and dextran (Fig. 5) were among the first chiral materials to be used as stationary phases for the resolution of racemic compounds. All these naturally occurring polysaccharides have the same basic chiral unit, namely a D-glucopyranose ring, but they differ in the mode of binding of the glucose moieties. In cellulose the glucose rings are linked in the β-1,4-position instead of α-1,4- as in amylose (the main component of starch). In dextran, the bond is mainly found in the α-1,6-position and with lower frequency in the α-1,3- and α-1,4-positions.

In the 1950s, Krebs and co-workers [50–52] reported the preparative separation of various racemic compounds on starch and cellulose, but in most instances only partial resolutions could be achieved. Nevertheless, they showed the potential of these sorbents for preparative purposes. Later, further attempts with these underivatized polysaccharides as CSPs were published (Tables 3 and 4), but remained very limited because in most instances again only partial resolutions could be achieved. Moreover, only very polar and hydrophilic compounds could be chirally discriminated. This has been successfully demonstrated by Hess *et al.* [57], who separated some atrope isomers on starch. The high polarity and/or hydrophilicity of these materials seem to lead only to non-chiral discriminating interactions. Nevertheless, Sephadex® ion exchangers were found to be appropriate for the chromatographic resolution of various metal complexes (cobalt, nickel, rhodium, etc.) and have recently been used [58,62]. These sorbents are derived from dextran, cross-linked with epichlorohydrin and then modified by introducing ion-exchanging groups (cations or anions) by reaction on some hydroxyl groups. This application has been reviewed [63].

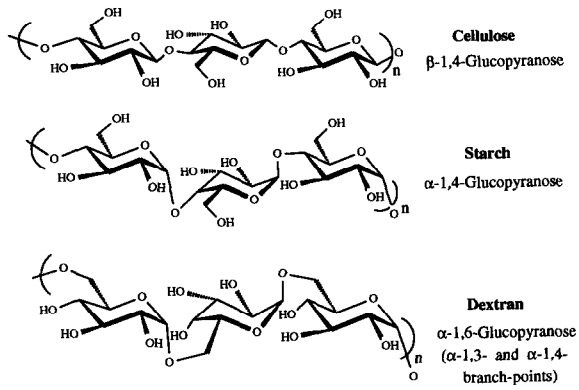


Fig. 5. Structures of cellulose, starch and dextran.

In conclusion, although there are no restrictions regarding availability, chemical stability or price limit on the use of unmodified polysaccharides as stationary phases, their poor selectivities seem to be the major reason for their relatively limited utilization.

3.2. Cellulose triacetate

In contrast to unmodified cellulose, numerous cellulose derivatives exhibit excellent chromatographic properties as chiral stationary phases. The most often used derivative is cellulose triacetate (Fig. 6), a very versatile and very efficient sorbent for the chromatographic resolution of racemates.

3.2.1. Cellulose triacetate I

As early as 1966 [64–66], it was found that partially acetylated cellulose could be used to achieve the chromatographic resolution of racemic compounds, but the potential of cellulose acetate for this purpose was definitively established by Hesse and Hagel [67], who introduced the completely acetylated material which turned out to be much more efficient.

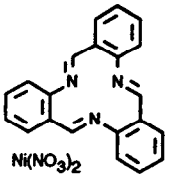
It must be emphasized that the crystal structure of the polymeric material has a determining influence on the chromatographic properties and the chiral recognition ability [25]. Indeed, cellulose triacetate exists in at least two different crys-

tal forms and only the so-called CTA I structure (cellulose triacetate, crystal form I) shows a large range of applications. This material is prepared under heterogeneous acetylation conditions which presumably preserve the original supramolecular structure of the starting cellulose [68]. Under these conditions, there is no dissolution of either the cellulose or the cellulose triacetate produced. If the cellulose ester is dissolved (during or after the acetylation) an amorphous or another crystal form (called CTA II, thermodynamically more stable than CTA I) is obtained, depending on the isolation conditions. CTA II shows completely different properties and generally has a much poorer resolving power [25].

A wide range of structurally different racemates have been resolved on CTA I and this sorbent is so far the most widely used for separations on a preparative scale (Table 5). Most of the reported chromatographic resolutions have been performed under medium-pressure conditions (Figs. 7 and 8). There are different reasons for the popularity of this sorbent, but the easy and cheap preparation, its high versatility and its high loading capacity are certainly the main advantages. These advantages largely compensate for the limitation imposed by the dissolution or strong swelling of the cellulose triacetate CSP in numerous solvents such as chlorinated alkanes, tetrahydrofuran (THF), dioxane, acetone and dimethylformamide (DMF). Usually a mixture of an alcohol (preferentially ethanol or methanol) and water is used and gives very good results. In some instances it has been shown that mixtures of alkanes and alcohols may yield a better selectivity [19,69]. One drawback of CTA I is the relatively slow kinetics of the adsorption–desorption process, resulting in peak broadening and thereby a decrease in resolution (efficiency). Data concerning the influence of the flow-rate, temperature, eluent composition, pressure and particle size on the enantioselectivity, efficiency and retention of optical isomers and also the determination of the loading capacity of CTA I have been reported by Rimböck *et al.* [70] and recently by Rizzi [38] and by Isaksson *et al.* [41].

TABLE 3

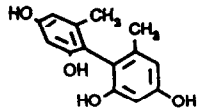
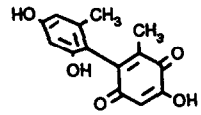
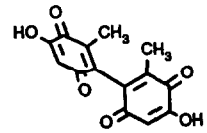
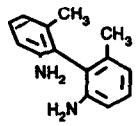
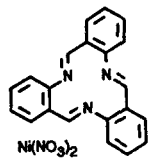
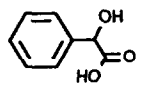
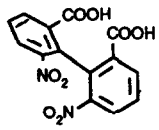
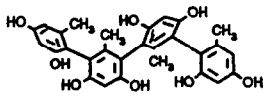
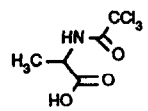
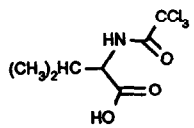
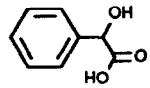
CHIRAL SPEAPARATIONS ON CELLULOSE AND CARBOXYMETHYLCELLULOSE

Structure	Sample mass	Column length × I.D. (cm)	Flow-rate (ml/h)	Purpose	Ref.
Various alkylenediamine Co(III) complexes	1.5 g	30 × 7.5	–[a]	Chiroptical properties	60
Catechin	800 mg	130 × 5	70	Chiroptical properties	59
Epicathecin	450 mg	130 × 5	70	Chiroptical properties	59
	200 mg	60 × 2.2	30	Chiroptical properties	53
Hexakis(2-aminoethanethiolo)tricobalt(III) cation	500 mg	38 × 2.2	210	Chiroptical properties	54
[Co(N,N′-dimethylethylenediamine-N,N′-diacetate)]$^+$	300 mg	70 × 2.5	120	Chiroptical properties	61

[a] Not given.

TABLE 4

CHIRAL SEPARATIONS ON STARCH

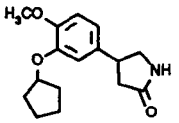
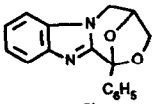
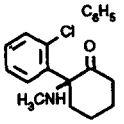
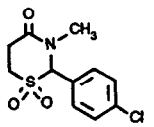
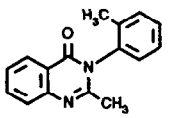
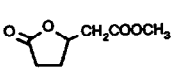
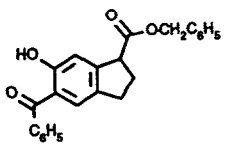
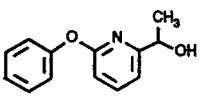
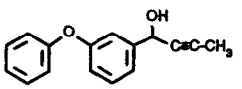
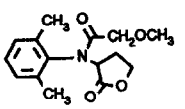
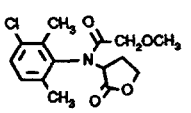
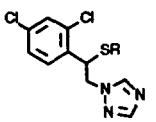
Structure	Sample mass	Column length × I.D. (cm)	Retention parameters (ml)		α^a	Flow-rate (ml/h)	Purpose	Ref.
	600 mg	116 × 4.4	135	630	–[b]	–	Chiroptical properties	56
	364 mg	80 × 3.9	–	–	–	–	Chiroptical properties	56
	402 mg	114 × 3.9	400	500	–	–	Chiroptical properties	56
	410 mg	100 × 5	4070	5820	–	–	Chiroptical properties	56
	160 mg	60 × 2.2	–	–	–	12	Chiroptical properties	53
	0.25–2 g	149 × 1.6	–	–	–	–	Chiroptical properties	58
	500 mg	136 × 5.2	6100	7300	–	70	Chiroptical properties	57
	200 mg	34 × 5.5	850	1950	–	13	Chiroptical properties	57
	480 mg	110 × 2.2	160	170	–	–	Chiroptical properties	55
	601 mg	114 × 2.2	125	140	–	–	Chiroptical properties	55
	618 mg	110 × 2.2	280	280	–	–	Chiroptical properties	55

[a] Separation factor.

[b] Dashes: not given.

TABLE 5

CHROMATOGRAPHIC SEPARATIONS ON CTA I

Structure	Sample mass	Column length × I.D. (cm)	Retention parameters (k'_1/k'_2 or ml)		α[a]	Flow-rate (ml/h)	Purpose	Ref.
	205 mg	85 × 2.4	510	860	–[b]	50	Drug	79
	2.1 g	70 × 3.8	1100	2500	–	90	Drug	79
	450 mg	70 × 3.8	1150	1800	–	90	Drug	79
	250 mg	36 × 2.5	2.31/3.12		1.35	60	Drug	80
	700 mg	70 × 3.8	2800	3300	1.28	92	Drug	82
	300 mg	30 × 2.5	–	–	–	420	Drug	81
	4.2 g	100 × 5	2900	4000	–	300	Drug	83
	200 mg	60 × 5	2200	3700	1.91[c]	300	Drug	84
	20 g	100 × 20	60 000	97 000	1.94	5000	Drug	78
	300 mg	60 × 5	0.50[c]/0.74[c]		1.48[c]	30	Biocide	85
	300 mg	60 × 5	1.08[c]/1.49[c]		1.38[c]	30	Biocide	85
	2 g	100 × 5	2020	2577	1.90	300	Fungicide	86
	1.5 g	100 × 5	2572	3197	1.53	300	Fungicide	86
	0.87 g	100 × 5	2315	2660	1.38	300	Fungicide	86
	2 g	66 × 6.3	0.45/0.48		1.04[d]	360	Fungicide	49

(Continued on p. 16)

TABLE 5 *(continued)*

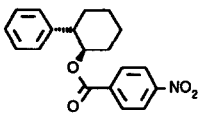
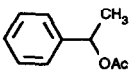
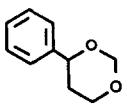
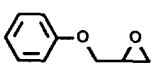
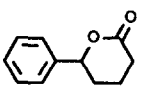
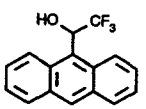
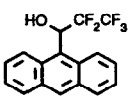
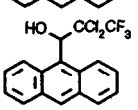
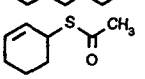
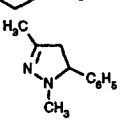
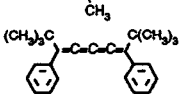
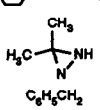
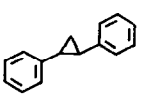
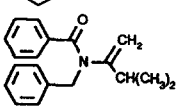
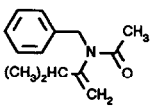
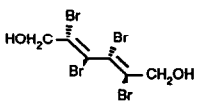
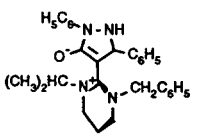
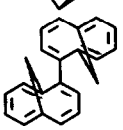
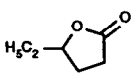
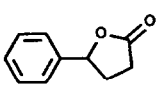
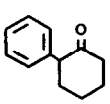
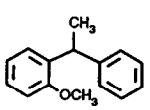
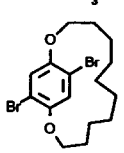
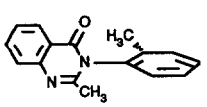
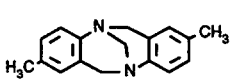
Structure	Sample mass	Column length × I.D. (cm)	Retention parameters (k'_1/k'_2 or ml)	α[a]	Flow-rate (ml/h)	Purpose	Ref.
	1.4 g	100 × 5	1.31[c]/4.10[c]	3.12[c]	320	Chiral auxiliary	20
	5 g	100 × 5	1.11[c]/2.82[c]	2.54[c]	320	Chiral auxiliary	20
	100 g	100 × 20	31 000 47 000	2.22	5000	Chiral auxiliary	78
	10 g	60 × 5	3.82[c]/15.36[c]	4.02[c]	320	Chiral auxiliary	20
	150 g	100 × 20	33 000 60 000	3.93	5000	Chiral auxiliary	78
	3.01 g	100 × 5	3.08[c]/5.53[c]	1.80[c]	320	Chiral auxiliary	20
	3 g	50 × 2.5	1.27[c]/29.7[c]	23.32[c]	320	Chiral auxiliary	20
	2.5 g	40 × 10	1.21[c]/2.78[c]	2.30	720	NMR shift reagent	45
	1 g	24 × 4	– –	–	–	NMR shift reagent	70
	45 g	100 × 20	33 600 47 700	2.47	5000	NMR shift reagent	78
	800 mg	100 × 5	1750 2250	1.99	320	NMR shift reagent	87
	3.2 g	100 × 5	1900 3200	3.26	320	NMR shift reagent	87
	20 g	100 × 20	31 250 37 500	–[d]	5000	Mechanistic study	88
	300 mg	69 × 2.5	– –	1.32	580	Mechanistic study	89
	150 mg	100 × 2	– –	–	–	Thermal racemization	90
	149 mg	41 × 2.5	450 490	–	139	Thermal racemization	91
	150 mg	30 × 2.5	200 300	–	140	Thermal racemization	92
	200 mg	30 × 2.5	– –	–	–	Thermal racemization	93

TABLE 5 (*continued*)

Structure	Sample mass	Column length × I.D. (cm)	Retention parameters (k'_1/k'_2 or ml)		α^a	Flow-rate (ml/h)	Purpose	Ref.
	500 mg	30 × 2.5	–	–	–	–	Thermal racemization	93
	300 mg	30 × 2.5	–	–	–	120	Thermal racemization	94
	200 mg	41 × 2.5	–	–	–	139	Thermal racemization	95
	450 mg	66 × 6.3	–	–	–[d]	–	Chiroptical properties	96
	2.47 g	60 × 5	2100	2400	1.41[c]	300	Chiroptical properties	97
	5 g	60 × 5	600	1700	6.03[c]	300	Chiroptical properties	97
	250 mg	40 × 3.1	450	700	–	300	Application	68
	250 mg	40 × 3.1	–	–	–	300	Application	68
	250 mg	16 × 2	–	–	–	300	Application	68
	250 mg	24 × 4	1.12[c]/2.88[c]		2.57[c]	–	Application	70
	65 g	100 × 20	34 000	65 000	2.08	5000	Application	78

(Continued on p. 18)

TABLE 5 (*continued*)

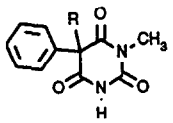
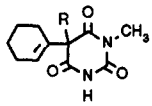
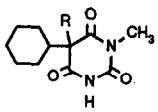
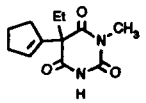
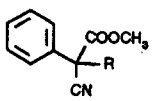
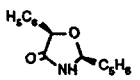
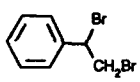
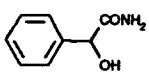
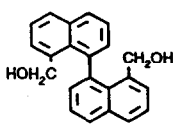
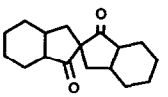
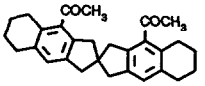
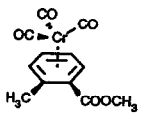
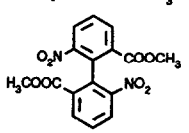
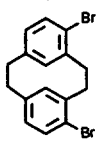
Structure	Sample mass	Column length × I.D. (cm)	Retention parameters (k'_1/k'_2 or ml)		α^a	Flow-rate (ml/h)	Purpose	Ref.
								
$R=CH_3$	160 mg	85 × 2.5	1620	2500	1.40	50	Application	98
$R=C_2H_5$	201 mg	85 × 2.5	1200	2400	2.30	50	Application	98
$R=n\text{-}C_3H_7$	140 mg	85 × 2.5	520	–	1.20	50	Application	98
								
$R=CH_3$	161 mg	85 × 2.5	900	1250	1.70	50	Application	98
$R=C_2H_5$	209 mg	85 × 2.5	600	980	2.00	50	Application	98
								
$R=CH_3$	200 mg	85 × 2.5	600	880	1.90	50	Application	98
$R=C_2H_5$	205 mg	85 × 2.5	510	850	2.40	50	Application	98
	257 mg	85 × 2.5	510	–	1.19	50	Application	98
								
$R=CH_3$	220 mg	85 × 2.5	700	–	1.80	50	Application	98
$R=C_2H_5$	230 mg	85 × 2.5	820	–	1.50	50	Application	98
$R=iso\text{-}C_3H_7$	191 mg	85 × 2.5	400	–	1.04	50	Application	98
	250 mg	32 × 4.5	90	147	1.77[c]	100	Application	99
	350 mg	32 × 4.5	160	200	1.50[c]	100	Application	99
	1.87 g	40 × 10	0.65[c]/0.97[c]		1.49[c]	5400	Application	45
	1.6 g	66 × 6.3	0.40/0.56		1.4[d]	360	Application	49

TABLE 5 (*continued*)

Structure	Sample mass	Column length × I.D. (cm)	Retention parameters (k'_1/k'_2 or ml)	α[a]	Flow-rate (ml/h)	Purpose	Ref.
	1 g	66 × 6.3	1.44/2.62	1.82	360	Application	49
	2 g	66 × 6.3	0.42/0.71	1.68[d]	360	Application	49
	500 mg	66 × 6.3	1.49/1.74	1.17[d]	360	Application	49
	1 g	66 × 6.3	2.80/3.36	1.20[d]	360	Application	49
	500 mg	66 × 6.3	0.70/0.94	1.34[d]	360	Application	49

[a] Separation factor.
[b] Dashes: not given.
[c] From analytical data.
[d] With recycling.

Although a number of theoretical investigations have been carried out on the interaction mechanism of CTA I with chiral molecules [71–73], the real mode of complexation has still not been elucidated because of the complex structure of the polymer, which seems to be characterized by the presence of multiple "interaction sites" [20,74]. Nevertheless, the determining influence of the size of structures to be resolved [20,75] and the possibility of separating enantiomers of totally apolar molecules [76,77] point to the large contribution of a mechanism involving an intercalation of the solutes between the polymeric chains (shape-selective adsorption or inclusion chromatography). This explanation does not exclude that for very large molecules, which cannot penetrate the polymer matrix or which are very polar, a simple adsorptive interaction on the surface occurs.

The multiple interaction possibilities render a prediction of separation very difficult, but on the other hand this explains the broad applicability which, combined with the high loading capacity, have certainly largely contributed to the extensive used of CTA I for preparative purposes. Its usefulness is demonstrated in Table 5 by the number of applications. The amounts generally injected range between 10 mg and 10 g, but separations of up to 200 g of racemate in one run have also been reported on a pilot scale [78]. Some aspects of preparative separations on CTA I have been already discussed earlier [45,49].

3.2.2. Cellulose triacetate II

As already mentioned before, cellulose triacetate can exist in different polymorphic forms which are readily distinguished by X-ray diffraction [25,100]. Once cellulose triacetate has been

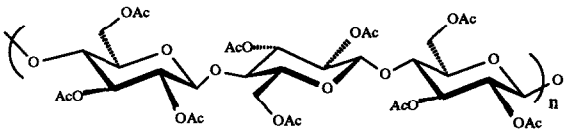


Fig. 6. Structure of cellulose triacetate. Ac = Acetyl.

dissolved (during or after the preparation), it has (under certain conditions such as slow precipitation, annealing and coating) a strong tendency to recrystallize, yielding the thermodynamically more stable crystalline form II. In the pure polymeric form this material exhibits only a poor resolving power and in some instances shows a completely different chiral selectivity to CTA I [25]. However, as a coating on an inorganic support (silica gel), it was found that the chromatographic performance could be enhanced (controlled particle size, more exchange surface, etc.) [101]. Although the chromatographic properties (efficiency and mechanical stability) of this coated form of CTA marketed by Daicel under the trade name Chiralcel OA are better that those of CTA I, the chiral recognition ability is much inferior to that of CTA I. This difference is directly related to the interaction mechanisms, which are fundamentally different for the two types of cellulose triacetate. CTA I mostly operates by a generally strongly selective inclusion mechanism, characterized by slow sorption kinetics, and CTA II probably by a more rapid but less selective adsorption–desorption process. Only one preparative separation on coated CTA II has so far been reported [102].

3.3. Benzoylcellulose derivatives

Further cellulose ester derivatives have been prepared using the same coating procedure on silica gel as discussed above for cellulose triacetate II [23]. A variety of benzoyl derivatives have been reported and they show very different selectivities, depending on the substituent on the aromatic moiety. Benzoylcellulose (Fig. 9) and *p*-methylbenzoylcellulose coated on silica gel are commercially available (Chiralcel OB and Chiralcel OJ; Daicel) either as a filled HPLC column

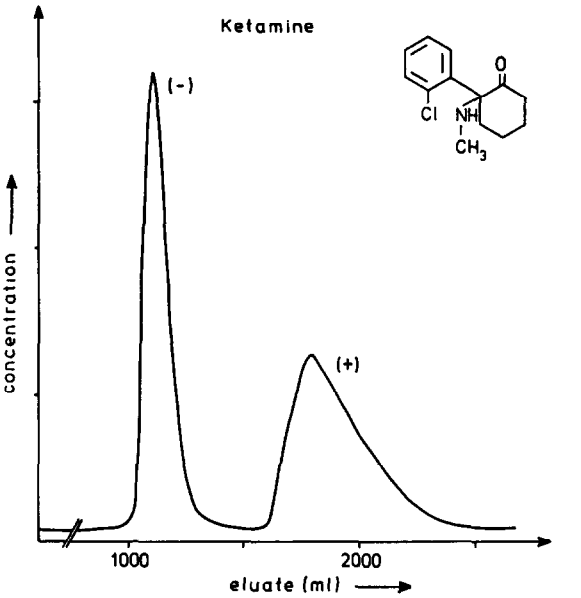


Fig. 7. Chromatographic separation of the enantiomers of ketamine (450 mg) on cellulose triacetate (CTA I). Column, 70 × 3.8 cm I.D.; mobile phase, ethanol–water (95:5). (Reprinted with permission from G. Blaschke, *J. Liq. Chromatogr.*, 9 (1986) 341, by courtesy of Marcel Dekker.)

or in bulk. Although a large number of analytical separations have already been reported and clearly demonstrate the potential of these CSPs [23,103–105], very few preparative applications have been published [106], probably because of the cost of these phases. The chromatographic materials are mechanically very stable and can easily be used under medium-pressure LC condi-

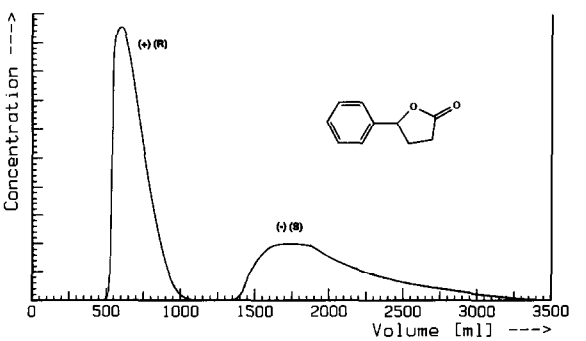


Fig. 8. Chromatographic separation of the enantiomers of 5 g of racemic γ-phenyl-γ-butyrolactone on cellulose triacetate (CTA I). Column, 70 × 5 cm I.D.; mobile phase, ethanol–water (95:5).

tions; the only limitation (apart from the high cost) is the same as for most cellulose derivatives and concerns the mobile phase because most of the solvents dissolve the polymeric phase.

Recently, benzoylcellulose derivatives were introduced by Francotte and co-workers as beads in the pure polymeric form [87,107–109]. These chiral phases have intrinsically the same chiral information as the corresponding sorbent coated on silica supports, but they exhibit a much higher loading capacity owing to the use of 100% of chiral material. The preparation procedure sparing the use of the very expensive macroporous silica support is also a substantial advantage. Both factors (high loading capacity and relatively low preparation costs) should be attractive for the further development of these new CSPs for preparative purposes.

The ability of these benzoylcellulose CSPs to resolve a wide range of compounds that are not separated on CTA, such as aliphatic alcohols, is very useful in view of the complementarity of the two CSPs (Fig. 10). The mechanism of interaction between aromatic alcohols and the silica-coated benzoylcellulose phase was investigated by Wainer *et al.* [103], but the proposed mechanism does not explain the interaction with other types of solutes [108].

3.4. Cellulose carbamate derivatives

The chromatographic properties of a number of cellulose phenylcarbamoyl derivatives (Fig. 11) coated on silica supports were investigated by Okamoto and co-workers [110,114,116–119] and some of these are also marketed by Daicel.

These CSPs can resolve some classes of racemates not resolved on the other cellulose derivatives, but they show the same limitations regarding costs and mobile phases. Only a few preparative separations have been reported so far (Fig. 12) and they are summarized in Table 6. Although no information is available on the pressure conditions, one would expect that these separations were performed under medium-pressure LC conditions. Owing to the presence of the polar –CONH– group in these derivatives, it is generally accepted that hydrogen bonding and dipole interactions are strongly involved in the complexation process. Therefore, these CSPs shows a good ability to resolve more polar compounds, even chiral amines or acids in their free form. With this type of cellulose-based CSPs, the presence of an amine (usually diethylamine) often improves the separation.

Optimization studies of the chromatographic conditions using this kind of CSP have recently been carried out, showing that mobile phase composition, flow-rate, loading and temperature can strongly influence the throughput of a preparative separation [39,46,48].

4. STATIONARY PHASES FROM SYNTHETIC CHIRAL POLYMERS

4.1. Poly(meth)acrylamides

Cross-linked, optically active polyacrylamides and polymethacrylamides have found successful applications as stationary phases for the separation of enantiomers. Thus, the acetates of racemic amino acid anilides (phenylalanine, phenylglycine, valine, 200–500 mg) can be separated on copolymers such as N-acroylphenylalanine cross-linked with N,N-dimethacroylhexamethylenediamine (100 × 2.5 m I.D. column) [120]. The physical properties of the polymeric CSPs are very dependent on the method of preparation. Blaschke and co-workers [26,79,121,122] reacted amines of optically pure configuration [(*S*)-1-phenylethylamine, (*S*)-1-cyclohexylethylamine] or esters of amino acids [(*S*)-phenylalanine ethyl ester] with methacrylic or acrylic anhydride or

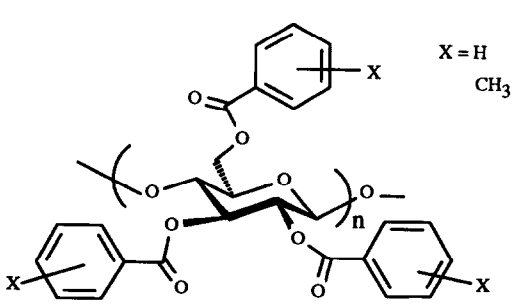


Fig. 9. Structure of benzoylcellulose.

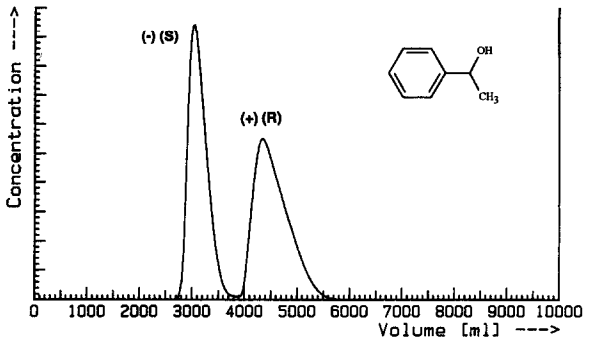


Fig. 10. Chromatographic resolution of 1 g of racemic phenylethanol on benzoylcellulose beads. Column, 75 × 5 cm I.D.; mobile phase, hexane–2-propanol (9:1); flow-rate, 60 ml/min.

the corresponding acid chlorides. Radical copolymerization of the (meth)acrylamide with ethylene diacrylate as a cross-linking agent affords the polymeric sorbent. The chromatographic separation of racemates, *i.e.*, the mechanism of separation and the chiral recognition, can be understood in terms of the differing fits of the antipodes in the asymmetric interaction sites of the cross-linked polymer and, hence, of the formation of diastereomeric complexes. Further stabilization results from the hydrogen-bonding interaction between the amide structure of the adsorbent and any amide or imide functions of the enantiomers to be separated.

These polyamide phases are polymeric gels (particle size 50–100 μm) which are deformed under pressure and are therefore suitable only for low-pressure chromatography. The degree of swelling depends on the composition of the mobile phase. Such polymers are versatile in their application [123–125]. They were used in the preparative enantiomer separation of various racemic pharmaceuticals [79,122]; usually the polymeric adsorbent is swollen in the eluent and then packed as a slurry. Preparative separations were mainly carried out using (*S*)-phenylalanine ethyl ester (I) or (*S*)-1-cyclohexylethylamine (II) as the chiral selector (Figs. 13 and 14 and Table 7). Loading experiments have been carried out with N-(2-methyl-1-phenylpropyl)acetamide (0.6–3.5 g) on poly[(*S*)-N-(1-phenylethyl)methacrylamide] (107 × 2.3 cm I.D. column) [123].

Optically active N-acryloylamide has also been anchored to the surface of silica gel in order to provide the properties required for a modern CSP, namely chemical and mechanical stability, good chromatographic performance and high enantioselectivity [45,79,122,126–128]. For that purpose, a carrier consisting of LiChrospher diol phase is esterified with methacrylic acid and suspended in a solution of the monomeric acrylamide or methacrylamide. Radical polymerization follows so that copolymerization of the monomer with the silica gel-immobilized methacryloyl groups yields a poly-N-acrylamide which is covalently bonded to the silica gel carrier. The proportion of polyamide by weight lies in the range of 10–20%. This type of sorbent is commercially available in the form of ChiraSpher® (Merck) {poly[(*S*)-N-(ethoxycarbonyl-2-phenylethyl)acrylamide]} in analytical and semi-preparative particle size distributions.

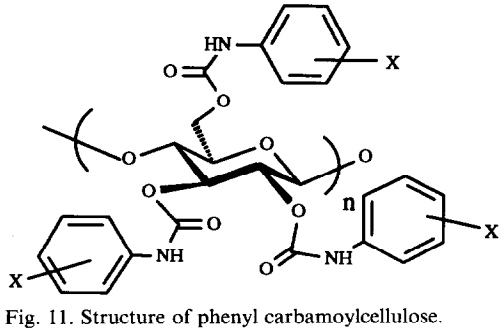


Fig. 11. Structure of phenyl carbamoylcellulose.

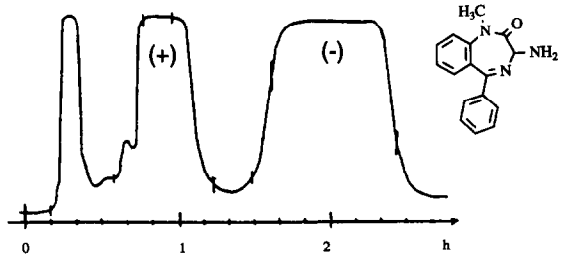
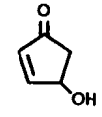
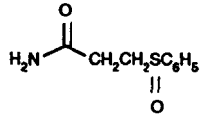
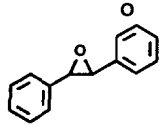
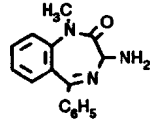
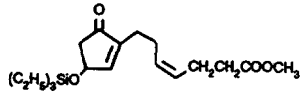
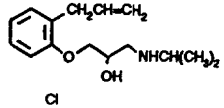
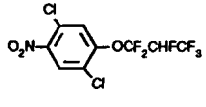


Fig. 12. Chromatographic resolution of 2 g of racemic benzodiazepine on Chiralcel OC. Column 20 × 4 cm I.D.; mobile phase, ethanol (anhydrous); flow-rate, 840 ml/h. (From ref. 112; chromatogram kindly provided by G. Dutruc-Rosset, Rhone-Poulenc Rorer.)

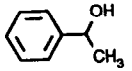
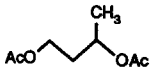
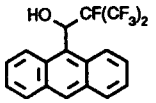
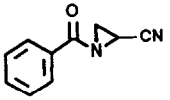
TABLE 6

CHIRAL SEPARATIONS ON BENZOYL- AND CARBAMOYLCELLULOSE

Structure	Sample mass (CSP)[a]	Column length × I.D. (cm)	Retention parameters (k'_1/k'_2 or ml)		α^b	Flow-rate (ml/h)	Purpose	Ref.
	– (OA)	55 × 7	–[c]	–	–	–	Application	102
	200 mg (OB)	50 × 2	220	670	5.3	594	Asymmetric synthesis	106
	8 g (OC)	50 × 10 +50 × 5	2.09/2.38		1.14	13 200	Application	111
	2 g (OC)	20 × 4	490	1100	–	840	Drug	112
	500 mg (OC)	50 × 2	–	–	–	1200	Drug intermediate	46
	150 mg (OD)	25 × 2	155	205	–	720	Drug	113
	200 mg (TBC)	75 × 5	–	–	–	720[d]	Insecticide intermediate	47

(Continued on p. 24)

TABLE 6 (*continued*)

Structure	Sample mass (CSP)[a]	Column length × I.D. (cm)	Retention parameters (k'_1/k'_2 or ml)		α^b	Flow-rate (ml/h)	Purpose	Ref.
	1 g (TBC)	75 × 5	3050	4330	1.62	3660	Chiral auxiliary	108
	2 g (TBC)	75 × 5	4670	7170	1.68	3660	Chiral auxiliary	108
	210 mg (MMBC)	75 × 5	3600	4850	1.46	720	NMR shift reagent	87
	170 mg (NMBC)	60 × 2.5	1160	1334	1.16	174	Mechanistic study	115

[a] OA: Chiralcel OA (Daicel Chemical Industries), cellulose triacetate II coated on silica gel. OB: Chiralcel OB (Daicel Chemical Industries), cellulose tribenzoate coated on silica gel. OC: Chiralcel OC (Daicel Chemical Industries), cellulose phenylcarbamate coated on silica gel. OD: Chiralcel OD (Daicel Chemical Industries), cellulose 3,5-dimethylphenylcarbamate coated on silica gel. TBC: tribenzoylcellulose beads. MMBC: *m*-methylbenzoylcellulose beads.

[b] Separation factor.

[c] Dashes: not given.

[d] With recycling.

The mass capacity is large on account of the tentacle-like structure of the surface. Separations that have already been carried out on polymeric gels can be transferred to ChiraSpher® phases without difficulty. The available choice of eluents ranges from non-polar through strongly polar organic solvents to buffered systems, *e.g.*, dioxane, ethanol, *n*-hexane, methanol, methyl *tert.*-butyl ether, 2-propanol, tetrahydrofuran, toluene. Gradient elution is also possible.

Preliminary runs with mixtures of solvents of various polarities have proved to be useful for the solution of separation problems. Suitable mixtures include *n*-hexane–dioxane (1:1), *n*-hexane–dichloromethane (1:1) and methanol–water (1:1).

Preparative chromatography is carried out by packing the sorbent (25 μm) in glass columns as a slurry in 2-propanol at a constant pressure or constant flow-rate and then rinsing with ethanol, dioxane and the eluent. In addition, the maintenance of selectivity on scale-up from analytical (particle size 5 μm) to preparative phases (particle size 25 μm) allows the use of HPLC as a pilot technique for optimization of overloading conditions that can be applied after a linear scale-up [45,129].

4.2. Cross-linked cyclodextrin phases

Cyclodextrins (Fig. 15) constitute another class of typical chiral hosts, forming stable inclusion complexes (usually 1:1 or 1:2) with a wide variety of molecules [137,138]. Owing to the inherent chirality of their building units, namely the α-1,4-linked glucose moiety, these cyclic oligosaccharides are chiral and the formation of diastereomeric complexes with the two enantiomers of racemic compounds can be very selective.

The inclusion occurs in the highly hydrophobic cavity of the cyclodextrin, which is generally represented as a truncated cone, acting in aqueous systems like a pocket for hydrophobic compounds. Even if cyclodextrin can also interact on its hydrophilic outside part (in this instance in a similar way to other polysaccharides such as amylose or cellulose), the most stereoselective interactions are those involving an inclusion in the hydrophobic cavity. For this reason it is clear that reversed-phase conditions are usually applied with cyclodextrin CSPs. Of course, the size of the cavity, which differs for α-, β- and γ-cyclodextrins, plays a determining role on the ability for complexing a defined molecule. It has been demonstrated that the presence of additives in the mobile phase can also strongly influence the success of the resolution [139] and must be optimized for each separation.

Applications using cyclodextrins as chiral

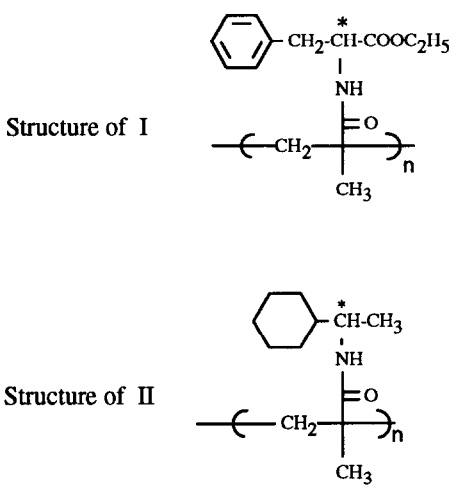


Fig. 13. Structure of poly[(*S*)-N-acryloylphenylalanine ethyl ester].

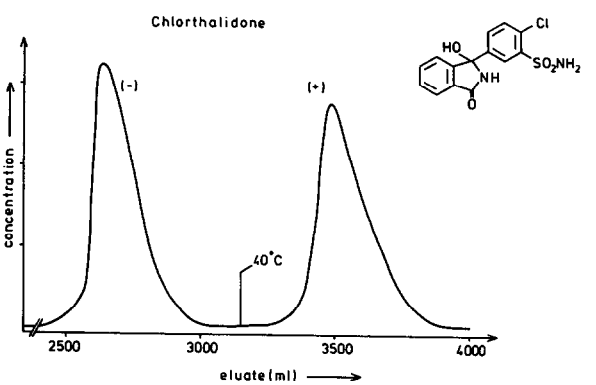
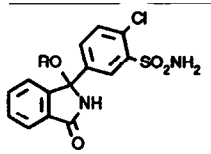
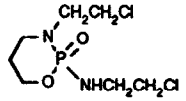
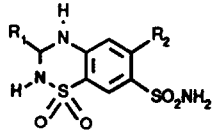
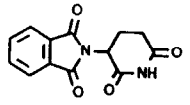


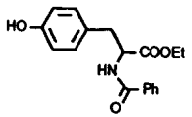
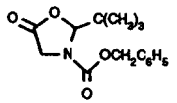
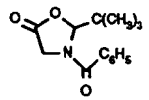
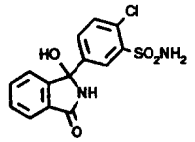
Fig. 14. Chromatographic separation of the enantiomers of chlorthalidone (530 mg) on poly(N-acryloylphenylalanine ethyl ester). Column, 36 × 3.2 cm I.D.; mobile phase, toluene–dioxane (1:1). (Reprinted with permission from G. Blaschke, *J. Liq. Chromatogr.*, 9 (1986) 341, by courtesy of Marcel Dekker.)

TABLE 7

CHIRAL SEPARATIONS ON POLY-[(*S*)-N-(ETHOXYCARBONYL-2-PHENYLETHYL)ACRYLAMIDE] (I), ON POLY-[(*S*)-N-(1-CYCLOHEXYLETHYL)METHACRYLAMIDE] (II) AND ON ChiraSpher® (ChS)

Et = Ethyl; Ph = phenyl.

Structure		Sample mass (CSP)	Column length × I.D. (cm)	Retention parameters (ml)		α^a	Flow-rate (ml/h)	Purpose	Ref.
	R = H	311 mg (I)	70 × 3.2	2900	3900	1.45	50	Application drug	130
	R = CH_3	299 mg (I)	70 × 3.2	1700	1870	1.17	50	Application	130
		1.55 g (I)	155 × 5	5000	6100	1.45	100	Pharmacological study	131, 132
 $R^2 = CF_3$ $R^1 = CH_2(CH_2)CH_3$		210 mg (I)	79 × 3.8	3800	4300	1.15	50	Thermal racemization	133
$R^2 = CF_3$ $R^1 = CH_2C_6H_5$		210 mg (I)	79 × 3.8	5200	5700	1.12	50	Thermal racemization	133
$R^2 = Cl$ $R^1 = CH_2CH(CH_3)_2$		253 mg (I)	79 × 3.8	–[b]	–	–	50	Thermal racemization	133
		505 mg (II)	62 × 2.5	–	–	–	24	Chiroptical properties	134

	302 mg (II)	65 × 2.5	1145	1290	–	–	Application	124
	5.3 g (ChS)	40 × 10	4864	6547	1.61[c]	19 200	Application	45, 135
	1.0 g (ChS)	40 × 10	7500	13 640	1.95[c]	19 200	Application	45, 135
	250 mg (ChS)	46 × 2.6	1440	1800	1.24	1080	Drug	136

[a] Separation factor.
[b] Dashes: not given.
[c] From analytical data.

hosts immobilized on a stationary phase were performed on polymers obtained by cross-linking of cyclodextrin with epichlorohydrin (β-CD-E) [140] or ethyleneglycol bis(epoxypropyl ether) (β-CD-P) [141,142] (Table 8). However, in all instances the efficiency and the loading capacity were relatively low. Moreover, the gel structure of these CSPs requires that only very low pressure can be applied and consequently only low flow-rates are usually used. The cyclodextrin CSPs introduced by Harada *et al.* [140] seem to have better mechanical properties, but only partial resolutions of various mandelic acid derivatives could be achieved.

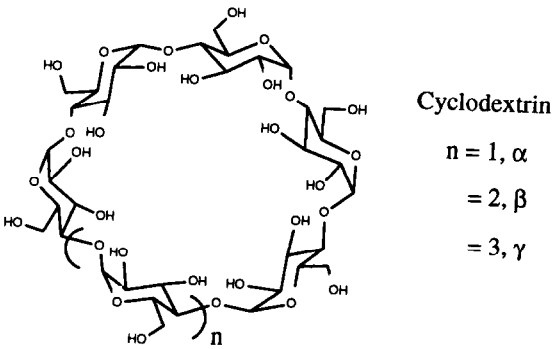


Fig. 15. Structure of cyclodextrin.

4.3. Ligand-exchange chromatography on polymeric phases

Ligand-exchange chromatography (LEC) is based on the reversible formation of complexes between metal ions (usually Cu^{2+} or Ni^{2+}) and chiral complexing agents carrying functional groups able to interact as ligands. Although chiral complexing agents such as hydroxy alcohols [143] and diamines [144,145] have also been reported, the mostly used complexing agents are α-amino acids chelating on the carboxylic and the amino group (Fig. 16).

CSPs of this type have been prepared by covalent bonding of the amino acid on organic polymeric and on inorganic silica supports. This section will focus on the polymer-supported CSPs. The preparation conditions of the phase not only can alter the mechanical properties of the CSP, but can also influence the resolution by participating in the complexation.

Various types of polymeric supports have been used for this purpose. More than 20 years ago, Bernauer [146] and Rogozhin and Davankov [47] independently reported on the first significant use of the ligand-exchange principle, introduced in 1961 by Helfferich [148], for the chromatographic separation of enantiomers. Most of the initial work was performed with cross-linked polystyrene as an inert supporting phase. Preparative resolutions have been reported by different research groups on L-proline cross-linked polystyrene-type CSPs (summarized in Table 9) and Jozefonvicz *et al.* [149] discussed the influence of various parameters such as the degree of cross-linking of the polymer, eluent composition, loading and flow-rate on the resolution. Nevertheless, the intensive investigations of Davankov *et al.* [150] in the field of LEC led to the introduction of an improved CSP using macronet isoporous styrene copolymers as a supporting material [151]. This sorbent exhibits better chromatographic performances and a higher exchange capacity than the previously developed CSPs based on cross-linked polystyrene. A wide range of racemic amino acids can be analytically resolved on these CSPs and even preparative resolutions (up to 20 g of racemic proline, Table 9) have been performed on an L-hydroxyproline CSP of this type [152,153]. The importance of various parameters (mobile phase, metal ion, pH, temperature, supporting phase, etc.) on the separation efficien-

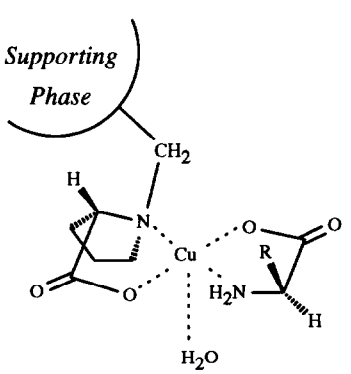
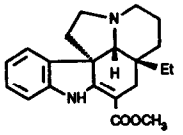
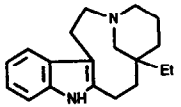


Fig. 16. Schematic representation of the Cu–ligand complexation between a proline stationary phase and an amino acid.

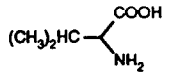
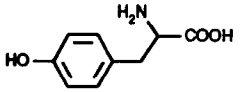
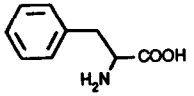
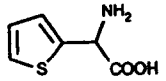
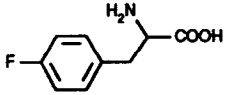
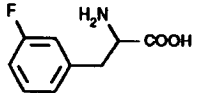
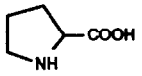
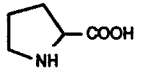
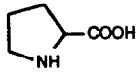
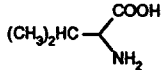
TABLE 8

CHIRAL SEPARATIONS ON POLYMERIC CYCLODEXTRIN CSPs

Structure (CSP)[a]	Sample mass	Column length × I.D. (cm)	Retention parameters (ml)		α^b	Purpose	Ref.
 (β-CD-P)	500 mg	90 × 5	8000	10 800	1.43[c]	Drug	141
 (β-CD-P)	200 mg	90 × 5	6600	7700	1.19[c]	Drug	141

[a] β-CD-P: β-cyclodextrin cross-linked with butane bisepoxide.
[b] Separation factor.
[c] From analytical data.

TABLE 9. CHIRAL SEPARATIONS ON POLYMERIC LEC CSPs

Structure	Sample mass (CSP)	Column length × I.D. (cm)	Retention parameters (ml)		α^a	Flow-rate (ml/h)	Purpose	Ref.
	10 g (Chirosolve, L-proline)	120 × 6	1760	3420	–[b]	400	Application	158
	300 mg (Chirosolve, L-proline)	44 × 2.5	–	–	–	240	Application	158
	200 mg (Chirosolve, L-proline)	44 × 2.5	–	–	–	240	Application	158
	200 mg (Chirosolve, L-proline)	44 × 2.5	–	–	–	240	Application	158
	250 mg (Chirosolve, D-pipecoline)	44 × 2.5	–	–	–	240	Application	158
	250 mg (Chirosolve, D-pipecoline)	44 × 2.5	–	–	–	240	Application	158
	500 mg (Polystyrene-L-proline)	47 × 0.9	–	–	–	7.5	Application	159
	162 mg (Polystyrene-L-proline)	80 × 0.8	60	100	–	20	Application	149
	20 g (Polystyrene-L-hydroxyproline)	–	–	–	3.95	–	Application	152
	6 g (Polystyrene-L-hydroxyproline)	–	–	–	1.52	–	Application	152

[a] Separation factor.
[b] Dashes: not given.

cy has been reviewed by Davankov *et al.* [150]. CSPs prepared by depositing (coating) of polystyrene containing amino acid residues on the surface of silica have also been developed but no preparative separations were reported [154].

In 1977 [155] packing materials from cross-linked polyacrylamide grafted with optically active amino acids were also introduced and the influence of the gel structure, the metal ion and the chiral graft were discussed [156]. The same research group showed that the polymer matrix can play an essential role in the stereoselection mechanism. Indeed, they pointed out that the same chiral selector under identical chromatographic conditions could lead to an inversion of the chiral recognition when fixed on a polystyrene or a polyacrylamide CSP. Further, the efficiency of the polyacrylamide-type CSP is improved by using a copolymer of acrylamide and vinylpyridine as a support [157]. By coating of silica (LiChroprep® Si 100, particle size 40–60 μm) with such a copolymer containing L-proline residues, a preparative material was prepared and various racemic amino acids were resolved (Table 9).

Recently, new ligand-exchanging resins based on highly cross-linked polyacrylamide have been introduced (Chirosolve; JPS Chimie, Bevais, Switzerland) and were especially recommended for medium-pressure LC preparative resolutions of amino acids and hydroxy acids [158] (Fig. 17). The chiral selectors used are D- or L-proline, -hydroxyproline, -phenylalanine, -valine and -pipecolinic acid. The performance description of these CSPs indicates chemical stability over a wide pH range (1–12) and good mechanical stability, allowing medium-pressure conditions (usually 15 bar), in addition to thermal stability up to 80°C. Recommendations are given for on-line demetallization in column chromatography for removing traces of metal ions. Some preparative applications are reported (200 mg–10 g) and they show the typical slow ligand exchange, as indicated by peak broadening [158].

Owing to the nature of the interactions, it is clear that this type of CSP is particularly appropriate for the resolution of racemic compounds carrying chelating functionalities such as amino acids, hydroxy acids and in a few instances amino alcohols. An advantage of the method is that derivatization of the solute, even those as polar as amino acids, is usually not required prior to chromatography and that very cheap mobile phases are used. The general applicability of LEC to the resolution of amino acids undoubtedly constitutes an attractive alternative for analytical investigations. From a practical point of view, the chromatographic method is probably less attractive than the various synthetic methods providing amino acids in high optical purities.

5. CHIRAL MODIFIED SILICA GELS

5.1. Cyclodextrin

By bonding cyclodextrin on silica supports, very efficient HPLC CSPs have been developed for analytical resolutions. An impressive number of analytical applications have appeared, mainly using the silica-bonded cyclodextrin CSPs developed by Haan and Armstrong [28], but to our knowledge, no preparative use has so far been reported. Nevertheless, the feasibility of preparative enantiomer separations by displacement chromatography has recently been demonstrated on an analytical column [160].

5.2. π-Acid and π-base phases

For the purpose of chromatographic racemate separation, optically active units can be attached to achiral silica gel carriers by means of ionic or covalent bonds. The enantioselective recognition leading to the formation of diastereoisomeric complexes can arise from hydrogen bonds, charge-transfer complexes and dipole and steric interactions. The best known chiral stationary phases of this type are the "Pirkle's phases", classifiable into π-acceptor and π-donor phases. Pirkle and co-workers, in particular, have carried out fundamental investigations yielding chromatographic data for the elucidation of the mechanism and the factors influencing chiral recognition, so that it is possible to make certain

predictions concerning the success of a separation [27,161–164].

The reciprocality concept introduced by Pirkle and co-workers [165–168] was the starting point for the development of chiral N-(3,5-dinitrobenzoyl)amino acid phases. The most frequently used π-acceptor phases are derived from the amino acids phenylglycine (DNBPG) or leucine (DNBLeu) covalently or ionically bonded to 3-aminopropylsilica gel [169–171] (Fig. 18 and Table 10). The CSPs are commercially available from Baker and Regis for the analytical or preparative separation of enantiomers. Other conventional chiral selectors [172] include valine, phenylalanine, tyrosine [42] and isoleucine (Table 11).

1,2-Diaminocyclohexane has also been used as a chiral selector after derivatization with 3,5-dinitrobenzoyl chloride (DACH–DNB) or with pentafluorobenzoyl chloride (DACH–PFB) [173]. One separation on the 100-mg scale on DACH–DNB has been reported [173].

For π-acceptor phases the substrate should exhibit high π-donating properties, *i.e.*, it should contain aromatic ring systems with alkyl, OR, NR_2 or SR substituents. In addition, the racemate should contain polar groups in the neighbourhood of the chiral centre in order to favour hydrogen bonds and dipole stacking. This frequently necessitates prechromatographic derivatization with achiral reagents (*e.g.*, the 2-naphthamides of amines, 1-naphthylcarbamates of alcohols).

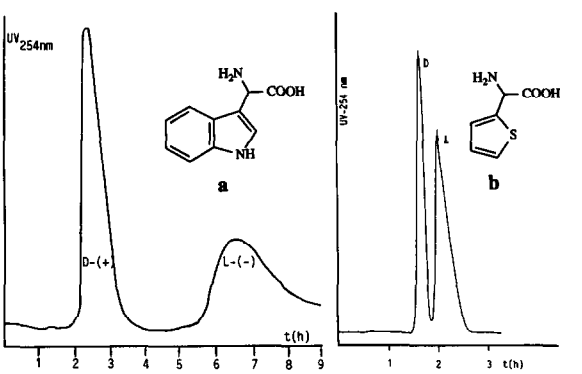
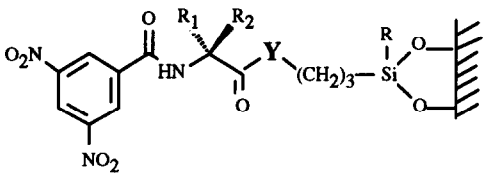


Fig. 17. Chromatographic resolution of (a) 500 mg of D,L-tryptophan (flow-rate, 3.7 ml/min) and (b) 175 mg of D,L-3-(2-thienyl)alanine (flow-rate, 2.5 ml/min) on Chirosolve-L-proline Cu. Column, 46 × 2.5 cm I.D.; mobile phase, 50 m*M* acetic acid–1.25 m*M* $Cu(OAc)_2$. (Chromatograms kindly provided by G. Jeanneret-Gris, JPS Chimie, Bevaix, Switzerland.)

2,2,2-Trifluoro-1-(9-anthryl)ethanol was the first stationary phase of the π-donor type [165,166,174]. The application of this reciprocality concept has led to the use of optically active sorbents with covalently bonded 5-arylhydantoins, N-(2-naphthyl)-2-amino acids, phosphine oxides, phthalides and 1-aryl-1-aminoalkanes as π-donor selectors for the chromatographic separation of π-acceptor racemates [172] (Fig. 19). Here, too, the samples are often derivatized to generate chiral recognition; the 3,5-dinitrobenzamides of amines and amino acids, the 3,5-dinitrophenylcarbamates of alcohols, the 3,5-dinitrophenylurea derivatives of amines and the 3,5-dinitroanilides of carboxylic acids are often used. Oi and co-workers [175,176] have developed commercial adsorbents with several chiral residues, but no preparative application has been reported so far. Chiral phases with both π-acceptor and π-donor properties have been investigated for their chromatographic selectivities with respect to, for example, N-(3,5-dinitrobenzoyl)-amines and N-(3,5-dinitrobenzoyl)amino acids [177,178].

In a few instances preparative separations have been performed and up to 1.5 g of bis-3,5-dinitrophenylcarbamates of vicinal diols have been separated on an (*S*)-N-(2-naphthyl)valine CSP under medium-pressure LC conditions [34] (Table 12).

These chemically modified silica gels are stable at high pressures and exhibit good chromatographic performance. Ionically or covalently bonded DNBPG and DNBLeu phases can differ significantly in their chromatographic behaviour [179–181]; however, the trend is towards covalently bonded adsorbents, particularly as the ionically bonded phases are only stable towards relatively apolar aprotic eluents. Nevertheless, they can be regenerated as required with the aid of chiral reagent solutions. In most instances binary eluent systems consisting of *n*-hexane and 2-pro-

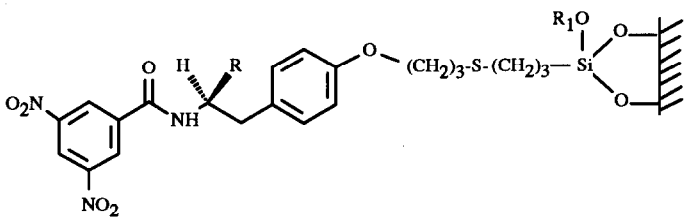


DNBPG: $R_1 = -C_6H_5$ $R_2 = -H$

DNBLeu: $R_1 = -H$ $R_2 = -CH_2CH(CH_3)_2$

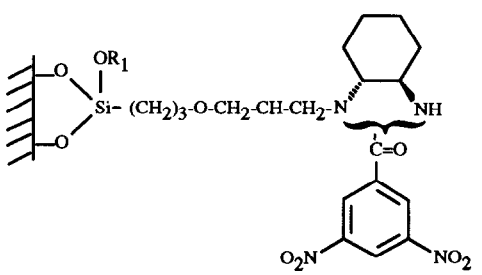
$Y = -O^{\ominus}, -NH_3^{\oplus}$; ionic phase

$Y = -NH-$; covalent phase



thio-DNB Tyr-E: $R = -COOCH_3$

thio-DNB Tyr-A: $R = -CONH(CH_2)_3CH_3$



DACH-DNB

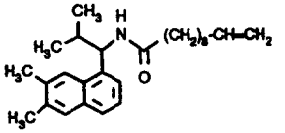
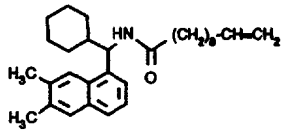
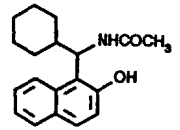
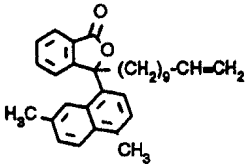
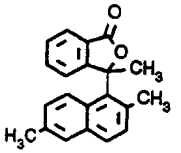
Fig. 18. Structure of the π-acceptor phases.

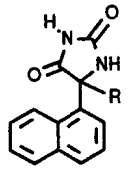
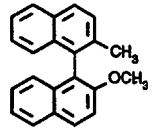
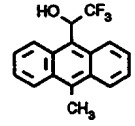
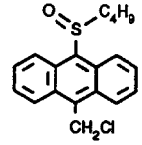
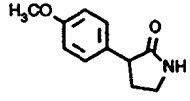
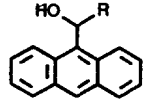
panol are used for normal-phase chromatography. The optimization of the selectivity and the resolution can be performed using *n*-hexane–alcohol–halogenated hydrocarbon mixtures [43,172,175]. The use of this type of CSPs under reversed-phase chromatographic conditions has been basically restricted to π-donor phases [166]. When samples with large differences in enantiomer ratio are to be analysed, the possibility exists of using covalent DNBPG phases with differing configurations of the chiral selector. This application is also of interest for preparative separations if the desired enantiomer is eluted first or the trace (impurity) enantiomer is to be removed gradually [182].

Numerous preparative separations on π-acceptor phases have been described and it is advantageous to employ the following optimization strategy [40,43,183,184]; starting from the analytical separation data, the chromatographic pa-

TABLE 10

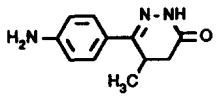
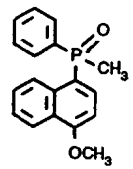
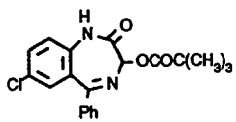
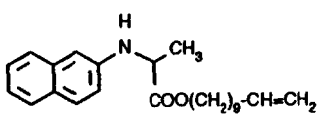
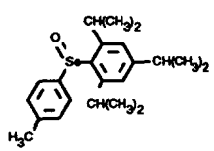
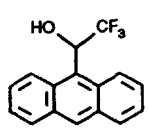
CHIRAL SEPARATIONS ON IONIC DINITROBENZOYLPHENYLGLYCINE (i-DNBPG), ON COVALENT DINITROBENZOYLPHENYLGLYCINE (co-DNBPG) AND ON COVALENT DINITROBENZOYLLEUCINE (DNBLeu)

Structure	Sample mass (CSP)	Column length × I.D. (cm)	Retention parameters (ml)		α^a	Flow-rate (ml/h)	Purpose	Ref.
	3.00 g (i-DNBG)	76 × 5	2900	5500	2.17	3600	Application	185, 186
	7.00 g (i-DNBG)	76 × 5	6350	12 300	2.23	3600	Application	185
	5.00 g (i-DNBG)	76 × 5	—[b]	—	1.55	3300	Application	185
	4.00 g (i-DNBG)	76 × 5	—	—	2.00	3300	Application	185, 187
	1.13 g (i-DNBG)	76 × 5	7900	12 900	1.68	3300	Application	188

 R = $(CH_2)_3CH{=}CH_2$	1.60 g (i-DNBG)	76 × 5	8200	11 900	1.55	3300	Application	188
R = CH_3	1.00 g (i-DNBG)	76 × 5	–	–	1.37	3300	Application	188
	1.00 g (i-DNBG)	76 × 5	5600	8600	1.86	3300	Application	188
	8.00 g (i.DNBG)	76 × 5	3800	5600	1.47	3300	Application	188
	3.00 g (i-DNBG)	76 × 5	–	–	1.24	3300	Application	188
	1.00 g (i-DNBG)	76 × 5	–	–	1.42	3300	Application	188
 R = CF_3	1.00 g	76 × 5	–	–	1.46	3300	Application	188
R = n-C_4H_9	1.00 g (i-DNBG)	76 × 5	–	–	1.63	3300	Application	188

(Continued on p. 36)

TABLE 10 (*continued*)

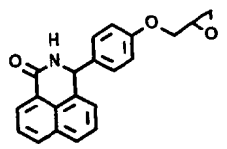
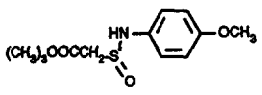
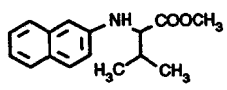
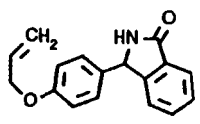
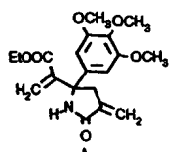
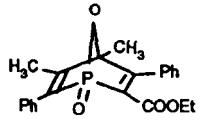
Structure	Sample mass (CSP)	Column length × I.D. (cm)	Retention parameters (ml)		α^a	Flow-rate (ml/h)	Purpose	Ref.
	1.00 g (i-DNBG)	–	–	–	–	–	Drug intermediate	189
	1.00 (co-DNBPG)	22 × 8	3733	5834	–	6720	Application	43
	200 mg (co-DNBPG)	15 × 4	–	–	>2.00	3000	Application	190
	100 g (co-DNBPG)	122 × 15	–	–	–	–	CSP precursor	191
	220 mg (co-DNBPG)	30 × 1.1	–	–	–	120	Chiroptical properties	192
	1.00 g (DNBLeu)	20 × 6	–	–	1.20	3000	Application	190

[a] Separation factor.

[b] Dashes: not given.

TABLE 11

CHIRAL SEPARATIONS ON COVALENT THIO-DNB Tyr-A AND THIO-DNB Tyr-E

Structure	Sample mass (CSP)	Column length × I.D. (cm)	Retention parameters (ml)		α[a]	Flow-rate (ml/h)	Purpose	Ref.
	300 mg (Tyr A)	26 × 4	2100	3200	–[b]	1800	CSP precursor	193
	200 mg (Tyr A)	26 × 4	483	777	1.83[c]	2520	Application	194
	1 g (Tyr A)	26 × 4	–	–	–	2520	Application	42
	300 mg (Tyr A)	26 × 4	630	840	–	2520	Application	42
	150 mg (Tyr A)	26 × 4	–	–	–	2526	Application	42
	150 mg (Tyr E)	26 × 4	1850	2380	1.24[c]	3000	Application	195

[a] Separation factor.
[b] Dashes: not given.
[c] From analytical data.

rameters, such as mobile phase and flow-rate, were optimized according to the criteria of preparative chromatography with respect to separation factor and resolution. This optimization was carried out with columns of analytical dimensions (25 × 0.46 cm I.D. or 25 × 1 cm I.D.) packed with the material used for preparative purposes. The same procedure was used for laying down the overload conditions; it is necessary to determine the maximum sample size and volume. Only when these data are available should the method be scaled up to the preparative level using a sorbent of the same selectivity and particle size. If the enantioselectivity is low ($1.05 < \alpha < 1.25$), it is preferable to use multiple (automatic) applications under analytical conditions. If the enantioselectivity α is between 1.25 and 2.0 a larger particle size (15–25 or 15–40 μm), semi-preparative pumps and column systems (analytical systems of greater column diameter or medium-pressure LC systems) are to be preferred. Here, too, the use of the recycling technique is recommended. Selectivities $\alpha > 2.0$ and higher resolution make it possible to apply flash chromatography with large particle sizes (>40 μm) [190]. Some preparative separations of enantiomers performed on the π-acid and π-base phases are illustrated in Figs. 20-22.

5.3. Ligand-exchange chromatography

A large number of new LEC CSPs prepared by chiral derivatization on the surface of silica using various approaches have appeared during the last decade [197] and numerous analytical resolutions have been reported. For example, Gübitz *et al.* [198] were able to resolve analytically nearly all common racemic amino acids on a CSP obtained by treating silica gel with 3-glycidoxypropyltrimethoxysilane and bonding L-pipecolic acid. The mechanical stability of these silica-based phases and the generally good chromatographic properties suggest that preparative applications should be possible and a baseline resolution of 60 mg of racemic α-methylphenylalanine on a preparative column using proline as a chiral selector has already been reported [199].

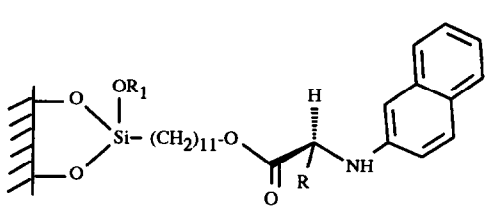


Fig. 19. Structure of the π-donor phases.

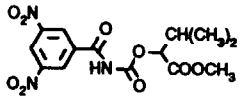
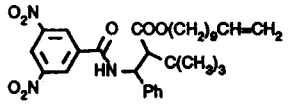
6. CONCLUSION

Owing to the increasing relevance of the preparation of enantiomers in an optically pure form, all methods meeting this aim have shown strong development. Among these methods, chromatographic separation on chiral stationary phases has proved to be very powerful and during the last 10 years numerous sorbents have appeared in the literature and on the market for analytical purposes. However, the method can also be used on a preparative scale to "produce" compounds in high optical purity. Compared with the number of analytical HPLC chiral columns there are at present fewer chiral stationary phases appropriate for separations on a preparative scale. This discrepancy arises from special requirements of the preparative materials, which should not only be efficient but also easy to prepare in large amounts, relatively cheap and chemically and physically stable. However, further developments of new phases for this purpose are to be expected.

The potential of the method has clearly been demonstrated by the number of applications on the various sorbents reported in this review (Tables 3–12), and the most often used stationary phase for preparative separations is undoubtedly cellulose triacetate (Table 5), which is relatively inexpensive and which shows wide applicability and a high loading capacity. This situation is clearly seen in Fig. 23, which represents the total number of (semi)preparative separations of enantiomers reported in the literature for 5 mg or more of injected racemate on the different CSPs. Fig. 23 shows that even though more efficient CSPs than microcrystalline cellulose triacetate (CTA I) have been developed in recent years, the low price and relatively broad application range

TABLE 12

CHIRAL SEPARATIONS ON COVALENT N-(2-NAPHTHYL)AMINO ACIDS (VALINE OR ALANINE)

Structure	Sample mass (CSP)	Column length × I.D. (cm)	Retention parameters (ml)		Flow-rate (ml/h)	Purpose	Ref.
	500 mg (valine)	91 × 2.5	880	1200	840	Application	7
	1.92 g (alanine)	76 × 5	1420	3070	1800	Application	196

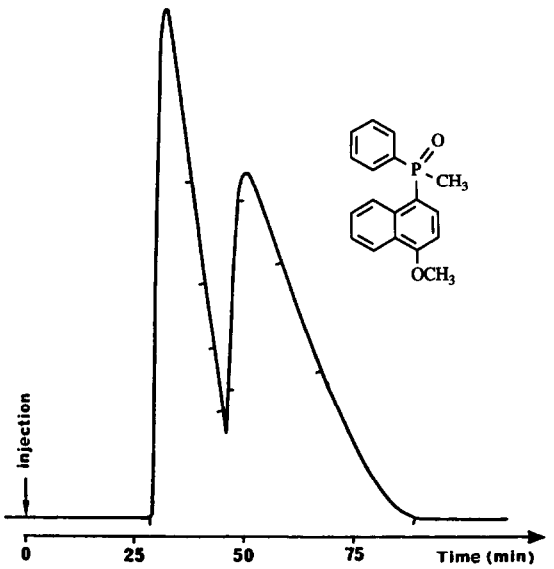


Fig. 20. Chromatographic resolution of 1 g of a racemic phosphine oxide derivative on (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine CSP; flow-rate, 112 ml/min. (Reprinted with permission from A. Tambuté *et al.*, *J. Chromatogr.*, 363 (1986) 81, by courtesy of Elsevier Science Publishers.)

of the latter are decisive factors for its acceptance for preparative purposes. It may also be suspected that an appreciable number of large-scale preparative separations have been performed on CTA I in industry, but that they have not been published.

Because of the efficiency of the method, the demand for preparative separations will certainly increase, notably under the influence of industry, which has recognized the usefulness of this approach to prepare enantiomers of new biologically active racemic compounds in amounts required for preliminary testing.

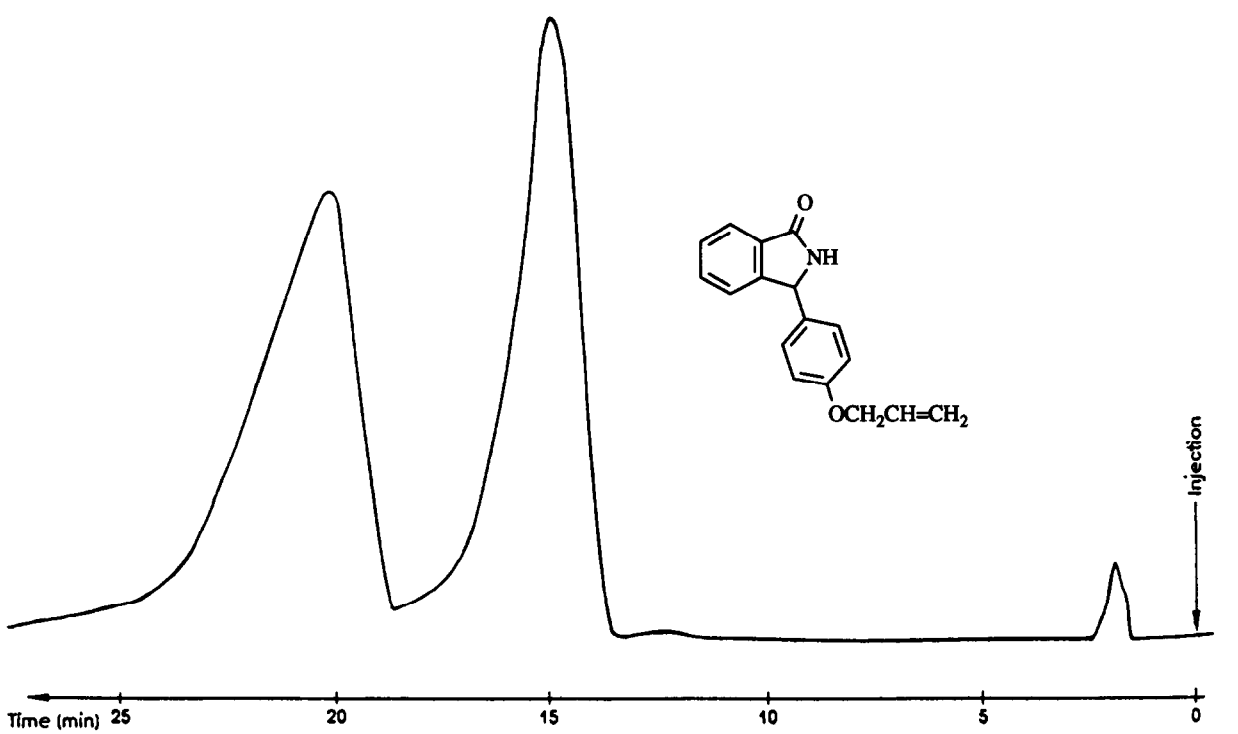


Fig. 21. Chromatographic resolution of 300 mg of a racemic lactam derivative on (*S*)-thio-N-(3,5-dinitrobenzoyl)tyrosine-*n*-butylamide; mobile phase, hexane–ethanol (9:1); flow-rate, 42 ml/min. (Reprinted with permission from M. Caude *et al.*, *J. Chromatogr.*, 550 (1991) 357, by courtesy of Elsevier Science Publishers.)

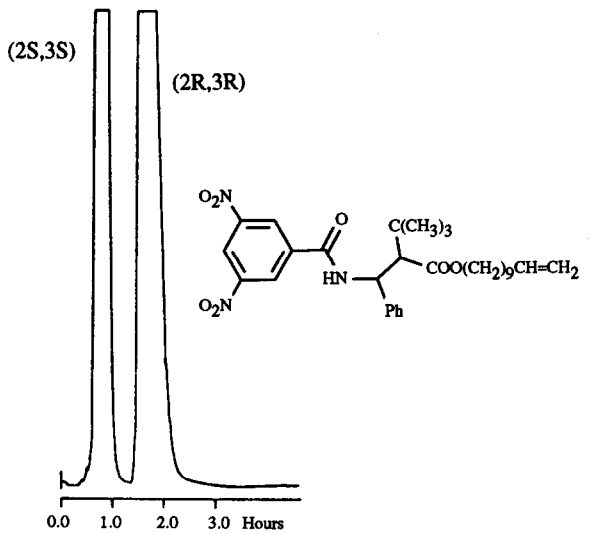


Fig. 22. Chromatographic resolution of 1.92 of racemic (*R*,*R*,*S*,*S*)-10-undecenyl N-3,5-dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethylethyl)propanoate on (*R*)-(+)-N-(2-naphthyl)-alanine-derived CSP; mobile phase, hexane–2-propanol (99:1); flow-rate, 30 ml/min. (Reprinted with permission from W. H. Pirkle and J. E. McCune, *J. Chromatogr.*, 441 (1988) 311, by courtesy of Elsevier Science Publishers.)

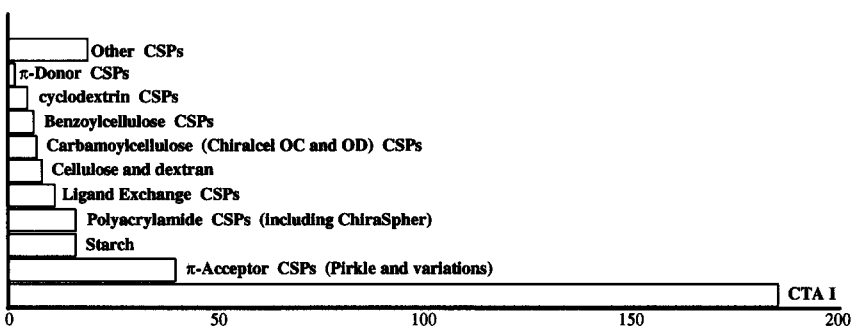


Fig. 23. Number of preparative and semi-preparative chromatographic resolutions performed on at least 5 mg of racemate on the different chiral stationary phases reported in the literature.

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