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APPLICATIONS AND LIMITATIONS OF COMMERCIALLY AVAILABLE CHIRAL STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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CONTENTS

1 INTRODUCTION

Interest in chiral separations by chromatographic methods has grown considerably in recent years, presumably for two reasons. In modern gas and liquid chromatography, the number of theoretical plates available for every-day separations has increased enormously; therefore, small chiral effects in a chromatographic system can be utilized to yield a good separation of enantiomers Moreover, a deeper insight into many biological processes has shown that chiral substances often have different effects on an organism, one isomer may be inactive or even harmful. This fact is of great interest in the design of novel pharmaceuticals and requires efficient methods for the separation of optical isomers

The chromatographic separation of enantiomers can be performed by various methods, but it is always necessary to use some kind of chiral discriminator or selector. Two different types of selectors can be distinguished: a chiral stationary phase (for a review of the state of the art in liquid chromatography, see ref. 1) or a chiral additive in the mobile phase, *e.g.*, as described in ref. 2. Another possibility is pre-column derivatization of the sample with chiral reagents to produce diastereomeric molecules which can be separated by non-chiral chromatographic methods (for an example, see ref 3).

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Of the numerous chiral stationary phases described in the literature for the liquid chromatographic separation of enantiomers, a considerable number are commercially available. In this paper we discuss the phases that are suitable for high-performance liquid chromatography (HPLC), *i.e.*, those with a mean particle diameter not larger than 10 μm. The range commercially available in autumn 1985 is surveyed. Although we have tried to achieve completeness, it is difficult as progress is very rapid in this field. Readers interested in the purchase of a chiral column for HPLC should ask the manufacturers for actual details.

The commercially available chiral stationary phases for HPLC use very different separation principles. We propose to classify them as follows:

(a) Chiral ligand-exchange chromatography.
(b) Chiral affinity chromatography.
(c) Chiral chromatography with helical polymers.
(d) Chiral chromatography with cavities.
(e) Chiral chromatography with "brush type" chemically bonded phases.

Using this classification, we discuss the commercially available chiral stationary phases based on data sheets and manuals from the manufacturers and on the scientific literature. The references cited are a selection from the vaste number of papers in this field and an attempt was made to represent the main workers in this area. Further searches in the literature for a certain type of chiral stationary phase can easily be done based on the information given in this paper.

2 LIGAND-EXCHANGE PHASES

Ligand-exchange chromatography for the separation of enantiomers was introduced in liquid chromatography by Davankov *et al.*[4,5]. They prepared various chiral stationary phases from chloromethylated polystyrene and amino acids. After loading the resin with Cu(II), Ni(II), Zn(II) or Cd(II), it was possible to separate racemates of amino acids.

Gübitz and co-workers[6,7] transferred this principle to silica-based stationary phases. They bonded L-proline or L-valine to silica via a 3-glycidoxypropyl spacer. After treatment with aqueous copper sulphate solution, the phases are ready for

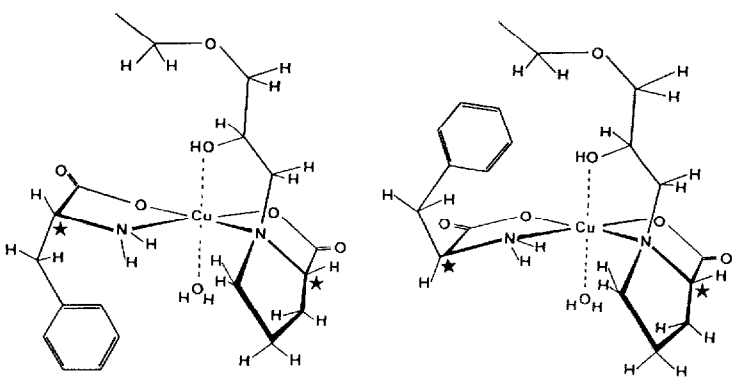


Fig. 1. Complex of D- (left) and L-phenylalanine (right) with copper-loaded L-proline stationary phase (From ref. 8, with permission.)

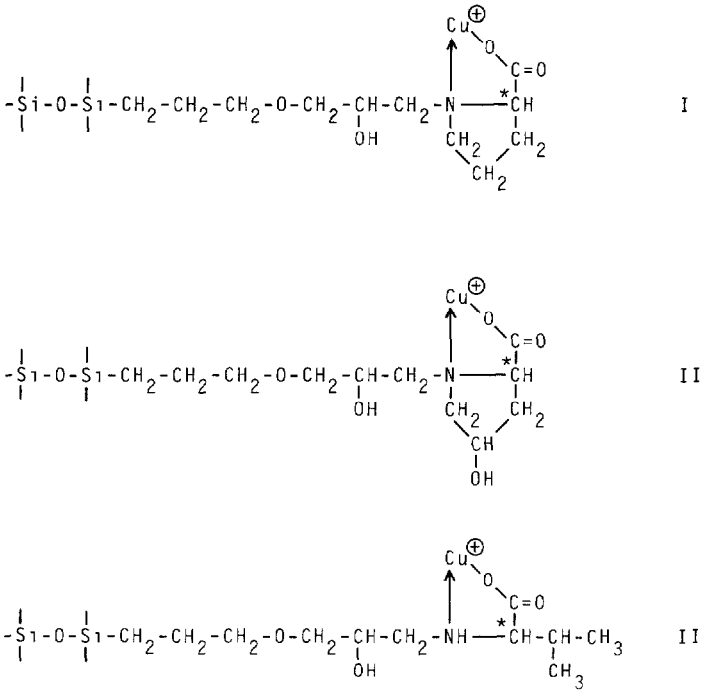


Fig 2 Structures of the commercially available proline–copper (I), hydroxyproline–copper (II) and valine–copper (III) phases.

enantioselective complex formation with amino acids, as shown in Fig 1[8]. These phases, whose structures are shown in Fig. 2, are commercially available from Serva as Chiral ProCu = Sil00Polyol and Chiral ValCu = Sil00Polyol, a further phase is Chiral HyproCu = Sil00Polyol with hydroxyproline as the amino acid Similar phases are sold by Daicel as Chiralpak WH and Chiralpak WM and by Macherey, Nagel & Co. as Nucleosil Chiral-1.

A successful separation into enantiomers can be expected if the sample molecule has two polar functional groups with the correct spacing, which can simultaneously act as ligands for the copper ion. Therefore, α-amino acids with their NH_2 and COO^- groups are very suitable for separations with these stationary phases. An example is shown in Fig. 3. Some β-amino alcohols and similar molecules could also be candidates for this method

The mobile phase is aqueous and should be approximately 0.5 m*M* in Cu(II) in order to prevent a loss of copper from the stationary phase. Increased temperatures (40–50°C) improve both the separation factor and the column efficiency[8]; this indicates that the separation is entropy controlled, a fact that is unique for the phases discussed in this paper. Samples with strong acids or bases should be avoided or derivatized.

Preparative separations are possible with these ligand-exchange phases and preparative-scale columns are available

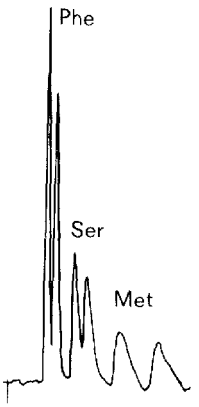


Fig 3 Separation of racemic amino acids on a chiral ligand-exchange phase (Serva). Column, 25 cm × 4 6 mm I D , stationary phase, Chiral ProCu = Si100Polyol (5 μm), mobile phase, 1 m*M* copper(II) sulphate in water, 1 ml/min, 100 bar, detector, UV (230 nm), sample, 20 μl with 1% of each amino acid

3. AFFINITY PHASES

Several proteins can undergo enantioselective interactions with a large number of pharmacologically active compounds. This effect was first used for chromatographic separations by Stewart and Doherty[9], who succeeded in resolving of D- and L-tryptophan on bovine serum albumin (BSA) bound to agarose. Allenmark *et al.*[10] made the method accessible to HPLC by binding BSA to HPLC-grade silica. Another protein phase was developed by Hermansson[11], who used α_1-acid glycoprotein (α_1-AGP, orosomucoid) coated and cross-linked on silica. The BSA column is available from Macherey, Nagel & Co. as Resolvosil and the α_1-AGP column from LKB as EnantioPac.

The separation mechanism of protein columns is not known, although there is no doubt that it is based on principles of bioaffinity. It includes hydrophobic interactions (similar to a true reversed phase), interactions of polar groups and steric effects[12]. Both phases are excellently suited for the separation of pharmaceuticals into enantiomers and often show high separation factors. Resolvosil is recommended for aromatic amino acids, amino acid derivatives, aromatic sulphoxides, coumarin derivatives, benzoin and benzoin derivatives. The various samples successfully separated on EnantioPac include important drugs such as ephedrine, disopyramide and methadone.

Protein columns are demanding in that the chiral separation depends on the chromatographic conditions, such as pH, ionic strength, organic modifier concentration (charged or uncharged) and temperature[10–14] Therefore, the optimum combination of these parameters has to be determined for each separation problem. Recommended values are pH 5–9, ionic strength 0–500 m*M*, organic modifiers up to 5% (1- or 2-propanol) or 0–10 m*M* (N,N-dimethyloctylamine, *tert.*-butylammonium bromide, octanoic acid) and temperature up to 35°C.

The selectivity of affinity phases is often excellent (see Figs 4 and 5). Unfortunately, the column efficiencies are low and, as the load of protein on the silica is

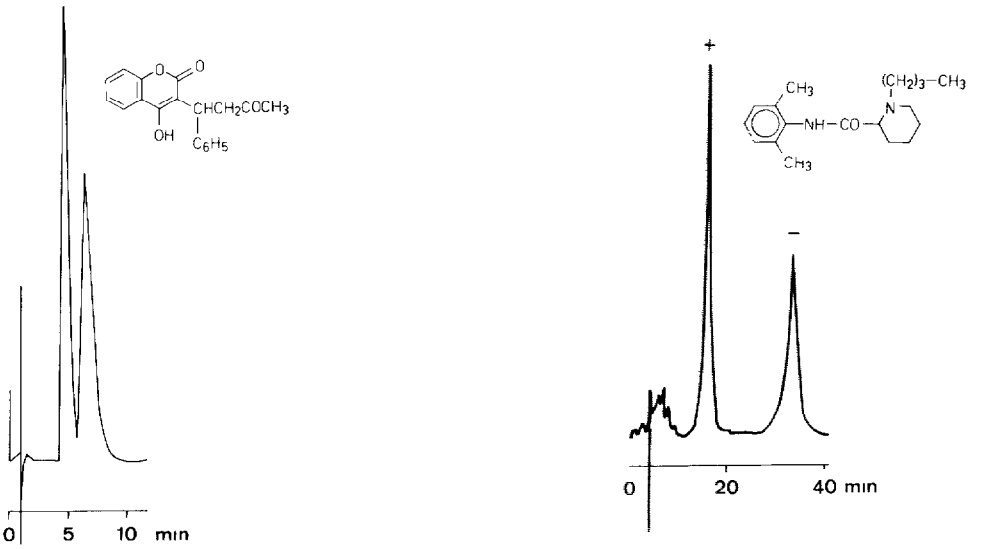


Fig 4 Separation of racemic warfarin on a bovine serum albumin phase (Macherey, Nagel & Co.) Column, 15 cm × 4 mm I D , stationary phase, Resolvosil (10 μm), mobile phase, 0 05 *M* phosphate buffer (pH 6 79)–1-propanol (97 3), 2 ml/min, detector, UV (225 nm), sample, 10 μl, 50 μM

Fig 5 Separation of racemic bupivacaine on an α_1-acid glycoprotein phase (LKB) Column, 10 cm × 4 mm I D , stationary phase, EnantioPac (10 μm), mobile phase, phosphate buffer, μ = 0 02 + 0 1 *M* sodium chloride (pH 7 2)–2-propanol (96:4), 0 3 ml min, temperature, 35°C, detector, UV (215 nm)

low, the sample capacity is not more than 1–2 nmol per injection. Preparative separations are impossible. Biological samples need to be analysed with great care, and a reversed-phase pre-column[15] or even a column switching technique may be necessary. For some drugs a pre-column derivatization may be needed in order to obtain a good resolution; this was reported for amino-alcohols (which include some β-blockers) which were derivatized with phosgene to obtain oxazolidones[16].

4 HELICAL POLYMER PHASES

Polymers with a helical structure are able to separate enantiomers by steric effects. Optical antipodes are retained differently if their attachment between the layers of the helix is different and therefore a chiral effect is noticeable.

4.1 Cellulose derivatives

Cellulose may be used for HPLC separations of enantiomers, *e g.*, tryptophan, as reported by Gübitz *et al.*[17]. However, it seems that cellulose derivatives have a greater resolving capacity. At present, underivatized cellulose of HPLC grade is not commercially available.

Derivatization of the hydroxy groups does not destroy the helical structure of cellulose. Cellulose triacetate was first used by Hesse and Hagel[18] for chiral separations. It was introduced in HPLC by Mannschreck and co-workers[19,20]. This sta-

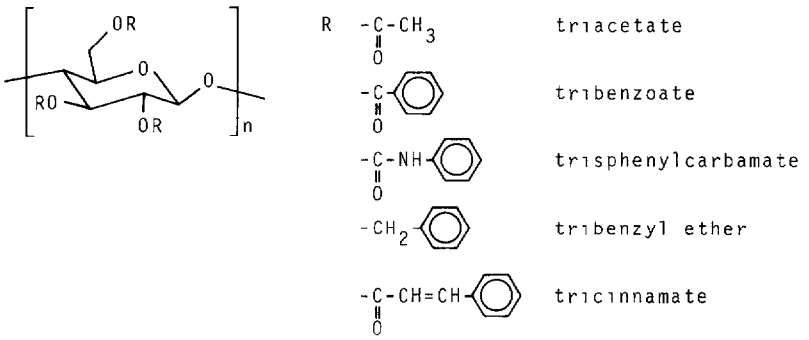


Fig 6 Structures of derivatized cellulose

tionary phase (pure cellulose triacetate) is available from Macherey, Nagel & Co. as Cellulose CEL-AC-40 XF. The maximum pressure drop is limited to 80 bar. The standard eluent is 96% ethanol.

Recently some new cellulose phases have been developed by Okamoto's group[21] These five different cellulose derivatives are all adsorbed on macroporous silica and are available from Daicel: Chiralcel OA is cellulose triacetate, Chiralcel OB is cellulose tribenzoate, Chiralcel OC is cellulose trisphenylcarbamate, Chiralcel OE is cellulose tribenzyl ether and Chiralcel OK is cellulose tricinnamate (Fig. 6). The properties of these phases are described as follows[21]:

Triacetate: for many racemates, especially effective for substrates with a phosphorus atom at an asymmetric centre. In general, low separation factors.

Tribenzoate· for racemates with carbonyl group(s) in the neighbourhood of an asymmetric centre.

Trisphenylcarbamate: for polar racemates, sensitive to the molecular geometry of the substrates (Fig 7).

Tribenzyl ether effective with protic solvents as mobile phases

Tricinnamate for many aromatic racemates and barbiturates High retention times.

Samples with strong acids or bases should be avoided. Good results are ob-

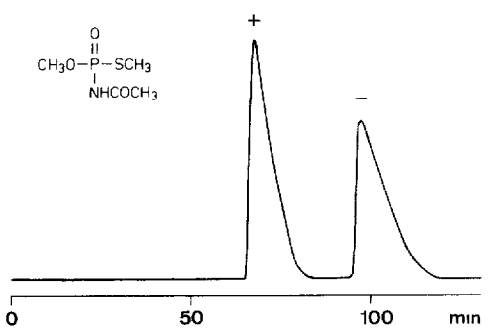


Fig 7. Separation of racemic oltran on a cellulose trisphenylcarbamate phase (Daicel) Column, 25 cm × 4 6 mm I.D , stationary phase, Chiralcel OC (10 μm), mobile phase, hexane–2-propanol (9 1), 0 5 ml/min, detector, UV (220 nm).

tained, if carboxylic acids are converted into phenyl esters, amines into benzoic acid amides and alcohols into benzoic acid esters.

The separation factor is strongly influenced by the type of mobile phase[20,21]. Although it is possible to use polar and non-polar eluents, Daicel recommends a limitation to the following solvents: hexane (also used as a storage solvent), hexane–2-propanol, methanol, ethanol, methanol–water and ethanol–water.

As these stationary phases are prone to irreversible adsorption, biological samples should not be injected without a pre-column. Preparative separations are possible[22]. However, because the Daicel phases, which are coated on silica, are very expensive, an alternative is to use the bulk material from Macherey, Nagel & Co (also available in larger particle diameters) or from Merck (cellulose triacetate, 15–25 or 25–40 μm) for these purposes.

4.2. Poly(triphenylmethyl methacrylate)

The polymerization of triphenylmethyl methacrylate in the presence of a chiral anion catalyst yields an isotactic polymer whose chirality is caused by its helical structure This phase was first synthesized by Okamoto *et al.*[23] and also used for liquid chromatography[24]. The chemical structure of poly(triphenylmethyl methacrylate) is shown in Fig 8 Stationary phases for HPLC were polymerized in the presence of a (+)-6-benzylsparteine–butyllithium complex and coated on 10 μm silica[25]. They are available from Daicel as Chiralpak OT(+), which is (+)-poly(triphenylmethyl methacrylate), and Chiralpak OP(+), which is poly(2-pyridyldiphenylmethyl methacrylate).

These phases show an extraordinary selectivity for many enantiomers. Many samples can be successfully separated if they possess a rigid non-planar structure (often with a C_2 symmetry axis), *e.g.*, *trans*-disubstituted cyclic molecules with six-, four- or three-membered rings. An example for the latter class of compounds is *trans*-stilbene oxide, whose separation factor on Chiralpak OT(+) is 5.2[25]. For the separation of *cis*- and *trans*-phenothrin, an insecticidal pyrethroid, into the four possible isomers, see Fig. 9.

The recommended mobile phases for the Chiralpak OT(+) and OP(+) columns are hexane (also as a storage solvent), hexane–2-propanol and methanol. Chiralpak OP(+) may also be used with methanol–water. For Chiralpak OT(+) the temperature should be below 15°C (down to 0°C) because the separation is better at low temperatures. The OP(+) column is used at room temperature

Samples with strong acids or bases should be avoided. Good results are ob-

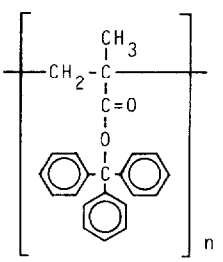


Fig. 8 Structure of poly(triphenylmethyl methacrylate)

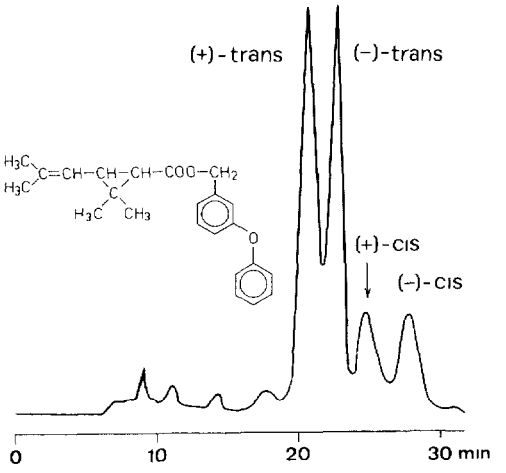


Fig 9 Separation of racemic *cis*- and *trans*-phenothrin (3-phenoxybenzyl chrysanthemate) on a poly(triphenylmethyl methacrylate) phase (Daicel) Column, 25 cm × 4 6 mm I D., stationary phase, Chiralpak OT(+) (10 μm), mobile phase, methanol, 0 5 ml/min, detector. UV (254 nm)

tained if carboxylic acids are converted into phenyl esters, amines into benzoic acid amides and alcohols into benzoic acid esters.

5. CAVITY PHASES

Cyclodextrins are cyclic oligoglucose molecules (Fig. 10). Their structure is

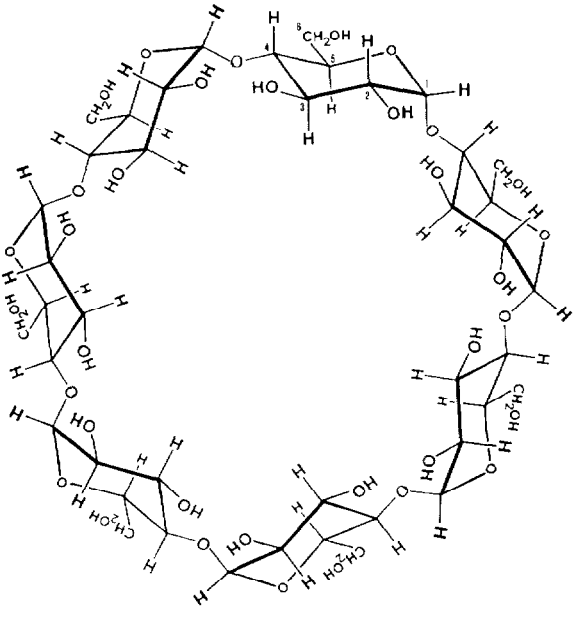


Fig 10 Structure of β-cyclodextrin

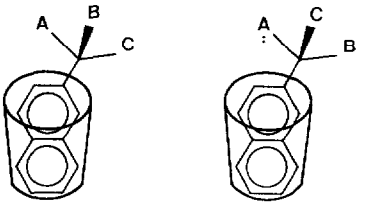


Fig 11 Model of the chiral recognition by cyclodextrins Whereas the aromatic part of the molecule dips into the cavity, the three substituents can interact with the clockwise oriented 2-hydroxy groups on the rim

unique in that it resembles a truncated cone with both ends open. The larger opening of the cone is rimmed with the secondary 2-hydroxy groups of the glucose units, all rotated to the right, and the smaller opening is rimmed with the more polar primary hydroxy groups. The interior of the cyclodextrin cavity contains no hydroxy groups and therefore is relatively hydrophobic. If a chiral molecule fits exactly into the cavity with its less polar (preferably aromatic) side, a separation into the enantiomers can be expected (Fig. 11) Small molecules that are completely enveloped by the cyclodextrin cannot be separated.

This effect was first used for the non-chromatographic separation of racemates (via inclusion complexes) by Cramer and Dietsche[26]. Polymerized cyclodextrin can act as a stationary phase for classical column liquid chromatography[27] Armstrong and co-workers[28,29] developed a material suitable for HPLC with the cyclodextrin ring bound to silica via a 6- to 10-atom spacer.

Various HPLC-grade cyclodextrins are available from Astec. Cyclobond I is β-cyclodextrin with seven glucose units (cavity I.D = 8 Å) which can separate a variety of enantiomers: dansyl amino acids, β-naphthylamide and β-naphthyl ester derivatives of amino acids, barbiturates[30] and metallocenes[31]. The separation of racemic hexobarbital is shown in Fig. 12 Cyclobond II is γ-cyclodextrin with eight glucose units (cavity I.D. = 9 5 Å); however, no chiral separations are known with this stationary phase. Cyclobond III is α-cyclodextrin with six glucose units (cavity I.D. = 5.7 Å); little is known about this new phase, which is suitable for small molecules only. α- and β-cyclodextrin for HPLC will also be available from Serva in 1986.

Cyclodextrin phases are used like reversed phases. Suitable eluents are mixtures

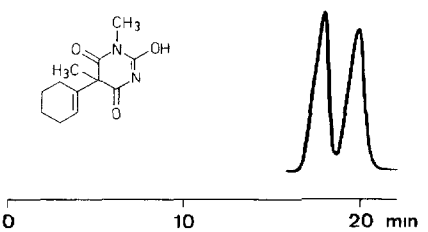


Fig 12 Separation of racemic hexobarbital on a β-cyclodextrin phase Column, 10 cm × 4 6 mm I D , stationary phase, Cyclobond I (5 μm), mobile phase, water–methanol (8 2), 1 ml/min, temperature, 22°C, detector, UV (254 nm) (From ref 30, with permission)

of water with methanol, ethanol or acetonitrile (with less polar mobile phases the chiral separation effect is lost). Gradient elution is possible, and also the regeneration of the column with absolute methanol, ethanol or acetonitrile. Column switching with reversed phases could be very interesting.

Cyclodextrin phases are cheaper than many other chiral phases. Preparative separations are possible and preparative-scale columns are available.

6 "BRUSH TYPE" PHASES

The hydroxy groups of the silica surface are accessible for chemical derivatization. The most widely used phase of this type is $C_{18}H_{37}$-modified silica, the well known RP-18 and ODS phases. As one can imagine that the organic groups are directed away from the silica network, this structure is often called a "brush". Chiral "brush type" phases for HPLC were first used by Mikeš *et al.*[32] for the separation of racemic helicenes. Later, the groups of Pirkle and of Ôi synthesized a large number of "brush type" phases, some of which are commercially available

6.1. π-acceptor phases

The first commercially available chiral "brush type" phase for HPLC was ionically bound N-(3,5-dinitrobenzoyl)phenylglycine (phase I in Fig 13). It was synthesized by Pirkle *et al* [33] by pumping a tetrahydrofuran solution of (*R*)-N-(3,5-dinitro-

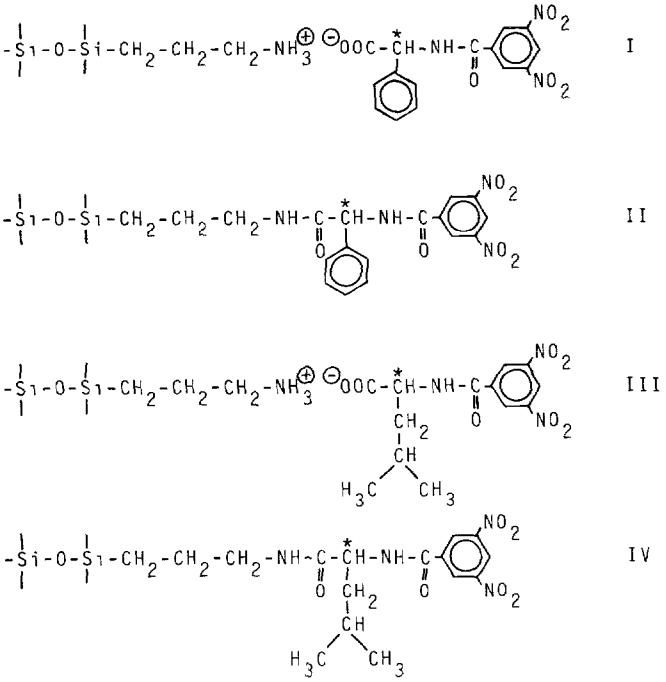


Fig 13 Structures of commercially available "brush-type" π-acceptor phases I = Ionic N-(3,5-dinitrobenzoyl)phenylglycine. II = covalent N-(3,5-dinitrobenzoyl)phenylglycine, III = ionic N-(3,5-dinitrobenzoyl)leucine, IV = covalent N-(3.5-dinitrobenzoyl)leucine

benzoyl)phenylglycine through a pre-packed column of aminopropyl-silica Soon it was clear that this phase can separate the enantiomers of a wide range of classes of compounds[33,34] Other dinitrobenzoyl (DNB) phases followed. covalent DNB-phenylglycine (phase II in Fig 13), ionic DNB-leucine (phase III) and covalent DNB-leucine (phase IV).

All these phases are commercially available. Regis offers Ionic D-Phenyl Glycine, Ionic L-Leucine, Covalent D-Phenyl Glycine, Covalent L-Phenyl Glycine, Covalent D,L-Phenyl Glycine (a racemic column) and Covalent L-Leucine, all from the micro to the preparative scale (D- or D,L-leucine phases on request) Baker offers Bakerbond Chiral Ionic DNBPG, Bakerbond Chiral Covalent DNBPG and Bakerbond Chiral Covalent DNBLeu. Serva offers Chiral DNBPG-C = Sil00Polyol, Chiral DNBDL-C = Sil00Polyol (D-leucine) and Chiral DNBLL-C = Sil00Polyol (L-leucine); all these phases are covalent. Sumitomo offers Sumipax OA-2000 and Sumipax OA-2000A, which are ionic and covalent DNB-phenylglycine, respectively.

Although a lot of work has been done in this field, the separation mechanism is not understood in all instances. However, there is no doubt that charge-transfer interactions, hydrogen-bonding interactions, so-called "dipole stacking" interactions[35] and steric effects are involved. As the dinitrobenzoyl group is a π-acceptor, possible samples for all these phases should possess a π-donor group (*e.g.*, an aromatic ring with an alkyl, OR, NR_2 or SR substituent). Moreover, the sample needs to be able to act as a donor or acceptor for hydrogen bonds or to enter into dipole-stacking processes. Many possible classes of compounds have been indicated[34,36]. Others published later include benzodiazepinones[37] (Fig. 14) and N-acylated heterocyclic amines[38]. If necessary, the sample can be derivatized; this was described for the determination of D- and L-propanolol in human serum as oxazolidones[39].

It often cannot be predicted which of the four phases (ionic and covalent DNB-phenylglycine and DNB-leucine) will give the best results in a particular separation problem. The enantiomeric selectivities in some instances differ markedly[40]. An important restriction is the fact that the ionic phases cannot withstand mobile phases more polar than 20% propanol in hexane With the covalent phases all

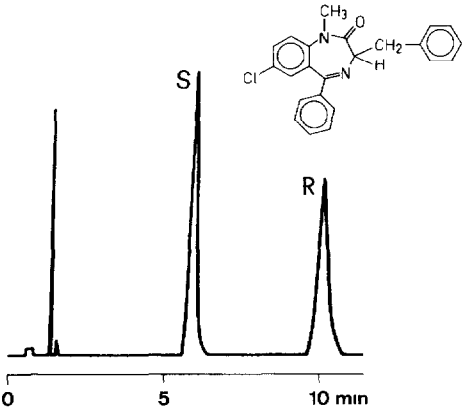


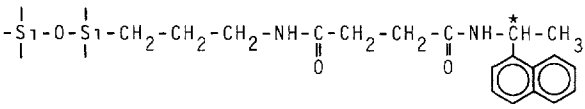
Fig 14 Separation of racemic 3-benzyldiazepam on a dinitrobenzoylleucine phase Column, 25 cm × 4 6 mm I D , stationary phase, covalent (*S*)-N-(3,5-dinitrobenzoyl)leucine (5 μm), mobile phase, hexane–2-propanol (90 10), 2 ml/min, detector, UV (254 nm) (From ref. 37, with permission)

eluents, even water, are allowed (this is explicitly declared by all manufacturers with the exception of Baker). However, aqueous mobile phases should not be used as storage liquids and should be replaced with methanol With a covalent DNB column, the user is free to optimize the separation via the composition of the mobile phase[41].

If the active part of the stationary phase of a ionic DNB column has been washed out by a too polar mobile phase, regeneration is possible. Regeneration reagents or ready-to-use solutions are sold by Aldrich, Baker, Regis and Sigma.

The covalent DNB-phenylglycine phase is available from Regis in three types. D-, L- and DL-(racemic). The D- and L-columns give reversed elution orders. This is convenient when a small amount of one enantiomer has to be quantified in the presence of the other. In these instances it is better to use the column which elutes the small peak first. The racemic column gives identical separations to the chiral ones except that the chiral resolution is not present, a valuable aid in the detection of enantiomers in complex mixtures. Chiral and racemic columns can be coupled to influence the extent of chiral separation[42].

Preparative separations are possible[37,43] and preparative-scale columns (with HPLC-grade phases as well as with larger particle sizes) are available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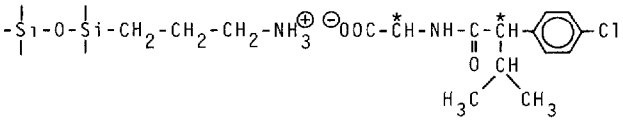
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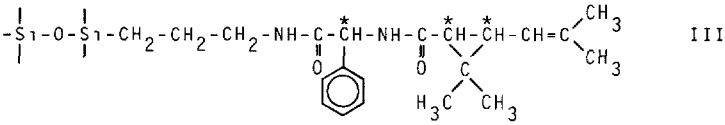
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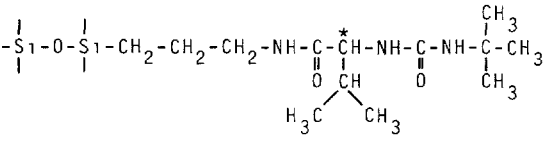
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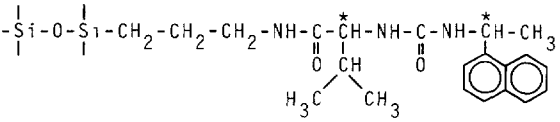
V

Fig 15 Structures of commercially available "brush type" phases of Ôi *et al* I = Sumipax OA-1000 and OA-1000A, II = Sumipax OA-2100; III = Sumipax OA-2200, IV = Sumipax OA-3000, V = Sumipax OA-4000 and OA-4100

6.2. Other phases

Several "brush type" chiral stationary phases for HPLC were synthesized by Ôi *et al.*[44] and are available from Sumitomo. Their structures are shown in Fig. 15. Sumipax OA-1000 and Sumipax OA-1000A are (*S*)-1-(α-naphthyl)ethylamine covalently bound as amide (I in Fig. 15)[45]. Only the OA-1000A phase is suited for use with aqueous mobile phases because any water-soluble impurities have been removed during the manufacturing process. Sumipax OA-2100 is ionic N-(*S*)-2-(4-chlorophenyl)isovaleroyl-(*R*)-phenylglycine (II)[46], Sumipax OA-2200 is covalent (N-(1*R*,3*R*)-*trans*-chrysanthemoyl-(*R*)-phenylglycine (III)[47], Sumipax OA-3000 is covalent N-*tert*-butylaminocarbonyl-(*S*)-valine (IV)[48], Sumipax OA-4000 is covalent N-(*S*)-1-(α-naphthyl)ethylaminocarbonyl-(*S*)-valine and Sumipax OA-4100 is the optical antipode with both asymmetric centres in the *R* configuration (V)[49].

As these phases were introduced in 1984, little is known about their properties. Their recommended uses are as follows

OA-1000 and OA-1000A. these are π-donor phases, so the samples need to have a π-acceptor group The columns are suitable for the separation of amines and amino acids as 3,5-dinitrobenzoyl derivatives and of carboxylic acids as 3,5-dinitroanilide derivatives. The OA-1000A column is for aqueous mobile phases.

OA-2100· this is a phase with two asymmetric carbon atoms and a weak π-donor. It is suitable for amines, amino acid esters or amides as 3,5-dinitrobenzoyl derivatives and for carboxylic acids as 3,5-dinitroanilide derivatives

OA-2200: this is an extraordinary phase in that it has three asymmetric carbon

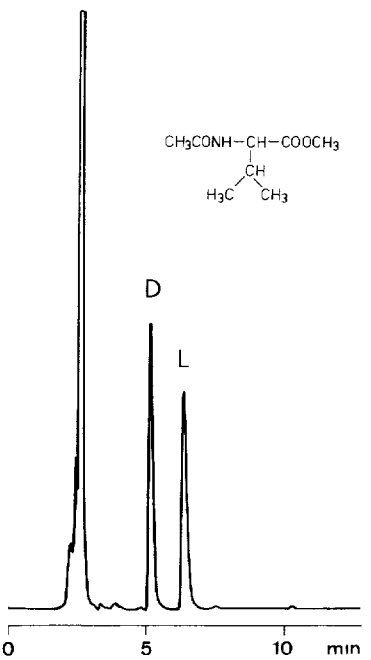

Fig. 16 Separation of racemic N-acetylvaline methyl ester on an N-*tert*-butylaminocarbonyl-(*S*)-valine phase (Sumitomo) Column, 25 cm × 4 mm I D , stationary phase, Sumipax OA-3000 (5 μm); mobile phase, *n*-hexane–1,2-dichloroethane–ethanol (50 15 1) 1 ml/min; detector, UV (230 nm).

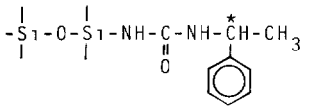

Fig. 17 Structure of commercially available Supelcosil LC-(R)-Urea phase

atoms. It is suitable for amino acid esters as 3,5-dinitrobenzoyl derivatives It was possible to separate some fungicidic alcohols into enantiomers[47].

OA-3000. this is a urea phase as it contains the NHCONH group Therefore, it is suitable for dipole-stacking processes and the samples need not necessarily have a π-acceptor group Possible samples are amino acids and oxy acids as N-acetyl-O-ester derivatives (Fig. 16) and as 3,5-dinitrophenylurethane-O-ester derivatives.

OA-4000 and OA-4100: these phases are very promising because here a π-donor group is combined with the urea group Therefore, the possible interactions are charge-transfer and dipole-stacking processes Moreover, the phases contain two asymmetric carbon atoms, which seems to improve the enantioselectivity[44]. These phases are recommended for many amines, carboxylic acids, amino acids, oxy acids, alcohols, amino alcohols and esters, even without derivatization. All kinds of amides, carbamates and ureas are possible samples, with or even without π-acceptor groups, as these compounds could undergo dipole-stacking with the phases. OA-4000 and OA-4100 are optical antipodes and therefore show a reversed elution order for chiral samples

Preparative separations are possible and preparative-scale columns are available.

A similar urea-type phase was developed by Supelco[50], an (*R*)-(1-phenyl)ethylurea silica called Supelcosil LC-(R)-Urea (Fig. 17). In comparison with the OA-4000 series from Sumitomo, it seems that the π-donor/dipole-stacking combination is realized in a less effective manner as the π-donor is phenyl instead of naphthyl. Supelcosil LC-(R)-Urea can separate, *e.g.*, racemic PTH-amino acids (see Fig 18).

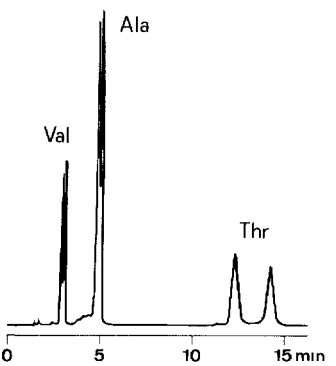

Fig 18 Separation of racemic PTH-amino acids on a phenylethylurea phase (Supelco) Column, 25 cm × 4.6 mm I.D , stationary phase, Supelcosil LC-(R)-Urea (5 μm); mobile phase, *n*-hexane–1,2-dichloroethane–ethanol (50 10 1), 2 ml/min, temperature, ambient, detector, UV (254 nm), sample, 5 μl mobile phase solution with 2 5 μg of each isomer

7 CONCLUSIONS

The range of commercial chiral stationary phases for HPLC is now very broad and it is difficult to decide which phase could solve a given separation problem. Of course, it is not recommended to buy a column with which aqueous mobile phases cannot be used if the sample is soluble only in water Often the chemical structure of the sample can give a hint of a suitable type of chiral phase. Such structure elements include charge-transfer groups or rigid spatial arrangements. Using the possibility of achiral derivatization, many samples can be adapted to the various chiral stationary phases. This is especially true for amino acids. In other instances the analyst has to search the literature on chiral separations for a similar molecule or has to make an attempt at random Many companies lend their columns so that the consumer can check the possibilities of a certain phase for his separation problem.

All common detectors can be used with chiral stationary phases. However, as the polarimeter, utilized as a HPLC detector, only gives a signal if a large amount of a chiral sample is injected, its coupling with columns of low loadability seems not to be practicable. This is especially true for affinity phases.

8 LIST OF COMMERCIALLY AVAILABLE CHIRAL STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Commercially available chiral stationary phases are listed in Table 1

9 LIST OF MANUFACTURERS

Aldrich

Aldrich Chemical Co Inc., P O. Box 355, Milwaukee, WI 53201, U S.A.

In Europe· Aldrich-Chemie Gesellschaft mbH & Co. KG, D-7924 Steinheim, F.R G.

Sells regeneration reagents for ionic DNB phases

Astec

Advanced Separation Technologies Inc., 37 Leslie Court, P.O. Box 297, Whippany, NJ 07981, U.S.A.

In Europe Paul Bucher, Analytik und Biotechnologie, Schützengraben 7, CH-4051 Basle, Switzerland.

Baker

J T. Baker Research Products, 222 Red School Lane, Phillipsburg, NJ 08865, U.S A.

In Europe. Baker Chemikalien, Postfach 1661, D-6080 Gross Gerau, F.R.G.

Daicel

Daicel Chemical Industries, Ltd., 8-1, Kasumigaseki 3-chome, Chiyoda-ku, Tokyo 100, Japan.

In Europe· Daicel (Europa) GmbH, Königsallee 92a, D-4000 Düsseldorf 1, F.R.G.

TABLE 1

COMMERCIALLY AVAILABLE CHIRAL STATIONARY PHASES

Manufacturer	*Name of phase*	*Class**	*Chiral element***
Astec	Cyclobond I	Cavity	β-Cyclodextrin
Baker	Bakerbond Chiral Ionic DNBPG	"Brush", π-acceptor	(*R*)-DNB-phenylglycine
	Bakerbond Chiral Covalent DNBPG	"Brush", π-acceptor	(*R*)-DNB-phenylglycine
	Bakerbond Chiral Covalent DNBLeu	"Brush", π-acceptor	(*S*)-DNB-leucine
Daicel	Chiralcel OA	Helical	Cellulose triacetate
	Chiralcel OB	Helical	Cellulose tribenzoate
	Chiralcel OC	Helical	Cellulose trisphenylcarbamate
	Chiralcel OE	Helical	Cellulose tribenzyl ether
	Chiralcel OK	Helical	Cellulose tricinnamate
	Chiralpak OT(+)	Helical	Poly(triphenylmethyl methacrylate)
	Chiralpak OP(+)	Helical	Poly(2-pyridyldiphenylmethyl methacrylate)
	Chiralpak WH	Ligand exchange	Proline–copper
	Chiralpak WM	Ligand exchange	Amino acid–copper
LKB	EnantioPac	Affinity	α_1-Acid glycoprotein
Macherey, Nagel & Co	Cellulose CEL-AC-40 XF***	Helical	Cellulose triacetate
	Nucleosil Chiral-1	Ligand exchange	Hydroxyproline–copper
	Resolvosil	Affinity	Bovine serum albumin

Regis	Ionic D-Phenyl Glycine (Pirkle Type 1A)	"Brush", π-acceptor	(*R*)-DNB-phenylglycine
	Covalent D-Phenyl Glycine	"Brush", π-acceptor	(*R*)-DNB-phenylglycine
	Covalent L-Phenyl Glycine	"Brush", π-acceptor	(*S*)-DNB-phenylglycine
	Covalent D,L-Phenyl Glycine	"Brush", π-acceptor	(*R*,*S*)-DNB-phenylglycine
	Ionic L-Leucine	"Brush", π-acceptor	(*S*)-DNB-leucine
	Covalent L-Leucine	"Brush", π-acceptor	(*S*)-DNB-leucine
Serva	Chiral BDex = Sil00Polyol	Cavity	β-Cyclodextrin
	Chiral DNBPG-C = Sil00Polyol	"Brush", π-acceptor	(*R*)-DNB-phenylglycine (covalent)
	Chiral DNBLL-C = Sil00Polyol	"Brush", π-acceptor	(*S*)-DNB-leucine (covalent)
	Chiral DNBDL-C = Sil00Polyol	"Brush", π-acceptor	(*R*)-DNB-leucine (covalent)
	Chiral HyproCu = Sil00Polyol	Ligand exchange	Hydroxyproline–copper
	Chiral ProCu = Sil00Polyol	Ligand exchange	Proline–copper
	Chiral ValCu = Sil00Polyol	Ligand exchange	Valine–copper
Sumitomo	Sumipax OA-1000	"Brush", π-donor	α-Naphthylethylamide
	Sumipax OA-1000A	"Brush", π-donor	α-Naphthylethylamide
	Sumipax OA-2000	"Brush", π-acceptor	(*R*)-DNB-phenylglycine (ionic)
	Sumipax OA-2000A	"Brush", π-acceptor	(*R*)-DNB-phenylglycine (covalent)
	Sumipax OA-2100	"Brush", π-donor	Chlorophenylisovaleroylphenylglycine
	Sumipax OA-2200	"Brush"	Chrysanthemoylphenylglycine
	Sumipax OA-3000	"Brush", urea type	*tert*-Butylaminocarbonyl valine
	Sumipax OA-4000	"Brush", urea, π-donor	(*S*),(*S*)-α-Naphthylethylaminocarbonylvaline
	Sumipax OA-4100	"Brush", urea, π-donor	(*R*),(*R*)-α-Naphthylethylaminocarbonylvaline
Supelco	Supelcosil LC-(R)-Urea	"Brush", urea type	Phenylethylurea

* "Brush" type phases always include dipole and hydrogen bonding interactions

** DNB = 3,5-dinitrobenzoyl

*** This cellulose triacetate from Macherey, Nagel & Co. is the only phase in this table that is not based on silica, this 7-μm material is pure cellulose triacetate.

In U S.A.: Daicel (USA), Inc., 611 West 6th Street, Room 2152, Los Angeles, CA 90017, U.S.A.

LKB

LKB-Produkter AB, Box 305, S-16126 Bromma, Sweden

In U.S.A · LKB Instruments Ltd., 9319 Gaither Road, Gaithersburg, MD 20877, U.S.A.

Macherey, Nagel & Co

Macherey, Nagel & Co., Neumann-Neander-Strasse 6–8, Postfach 307, D-5160 Düren, F.R.G

In U.S.A.· Alltech Associates, 2051 Waukegan Road, Deerfield, IL 60015, U.S.A.

Merck

E. Merck, Frankfurter Strasse 250, D-6100 Darmstadt 1, F.R.G.

In U.S.A EM Science, 111 Woodcrest Road, P.O. Box 5018, Cherry Hill, NJ 08034, U.S.A

Sells cellulose triacetate for medium-pressure LC

Regis

Regis Chemical Company, 8210 Austin Avenue, Morton Grove, IL 60053, U.S.A.

In Europe· Promochem GmbH, Postfach 1246, D-4230 Wesel, F R.G

Serva

Serva Feinbiochemica, Carl-Benz-Strasse 7, Postfach 105260, D-6900 Heidelberg 1, F.R.G.

In U.S.A.: Serva Fine Biochemicals Inc., P.O Box A, Garden City Park, Long Island, NY 11040, U.S.A

Sigma

Sigma Chemical Company, P O. Box 14508, St Louis, MO 63178, U.S.A.

In Europe: Sigma Chemie GmbH, Am Bahnsteig 7, D-8028 Taufkirchen, F.R.G.

Sells regeneration reagents for ionic DNB columns.

Sumitomo

Sumitomo Chemical, Sumitomo Building, 5-15, Kitahama, Higashi-ku, Osaka 541, Japan.

In Europe: Sumitomo Düsseldorf Representative Office, Nordstern Versicherungs-Gebäude, Georg-Glock-Strasse 14, D-4000 Düsseldorf 30, F.R G.

In U.S.A.: Sumitomo Chemical America, Inc , 345 Park Avenue, New York, NY 10154, U.S.A.

Supelco

Supelco Inc , Supelco Park, Bellefonte, PA 16823, U.S.A.

In Europe Supelco SA, Chemin du Lavasson 2, CH-1196 Gland, Switzerland.

10 SUMMARY

This review surveys commercially available chiral stationary phases for HPLC. It is a guide to help the practitioner choose a stationary phase for a particular separation problem. It gives some information about the recommended eluents and also about the compatibility with achiral phases for column switching. Special hints and possibilities for preparative separations are mentioned.

REFERENCES

1 G. Gubitz, *GIT Fachz. Lab., Suppl. Chromatogr.*, 4/85 (1985) 6
2 C. Pettersson, *J. Chromatogr.*, 316 (1984) 553
3 R. H. Buck and K. Krummen, *J. Chromatogr.*, 315 (1984) 279
4 V. A. Davankov, S. V. Rogozhin, A. V. Semechkin and T. P. Sachkova, *J. Chromatogr.*, 82 (1973) 359
5 V. A. Davankov, *Adv. Chromatogr.*, 18 (1980) 139
6 G. Gubitz, W. Jellenz, G. Lofler and W. Santi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 145.
7 G. Gubitz, W. Jellenz and W. Santi, *J. Liq. Chromatogr.* 4 (1981) 701
8 G. Gubitz, W. Jellenz and W. Santi, *J. Chromatogr.*, 203 (1981) 377
9 K. K. Stewart and R. F. Doherty, *Proc. Natl. Acad. Sci. USA*, 70 (1973) 2850
10 S. Allenmark, B. Bomgren and H. Borén, *J. Chromatogr.*, 264 (1983) 63
11 J. Hermansson, *J. Chromatogr.*, 269 (1983) 71
12 S. Allenmark, B. Bomgren and H. Borén, *J. Chromatogr.*, 316 (1984) 617
13 J. Hermansson, *J. Chromatogr.*, 298 (1984) 67
14 G. Schill, I. W. Wainer and S. A. Barkan, *J. Liq. Chromatogr.*, 9 (1986) 641
15 J. Hermansson and M. Eriksson, paper presented at the *9th International Symposium on Column Liquid Chromatography, Edinburgh, 1985*
16 J. Hermansson, *J. Chromatogr.*, 325 (1985) 379
17 G. Gubitz, W. Jellenz and D. Schonleber, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 31
18 G. Hesse and R. Hagel, *Chromatographia*, 6 (1973) 277
19 K. R. Lindner and A. Mannschreck, *J. Chromatogr.*, 193 (1980) 308
20 H. Koller, K.-H. Rimbock and A. Mannschreck, *J. Chromatogr.*, 282 (1983) 89
21 A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi and Y. Toga, *Chromatographia*, 19 (1984) 280
22 K.-H. Rimbock, F. Kastner and A. Mannschreck, *J. Chromatogr.*, 329 (1985) 307
23 Y. Okamoto, K. Suzuki, K. Ohta, K. Hatada and H. Yuki, *J. Am. Chem. Soc.*, 101 (1979) 4763
24 H. Yuki, Y. Okamoto and I. Okamoto, *J. Am. Chem. Soc.*, 102 (1980) 6356
25 Y. Okamoto, S. Honda, I. Okamoto, H. Yuki, S. Murata, R. Noyori and H. Takaya, *J. Am. Chem. Soc.*, 103 (1981) 6971
26 F. Cramer and W. Dietsche, *Chem. Ber.*, 92 (1958) 378
27 J. L. Hoffman, *Anal. Biochem.*, 33 (1970) 209
28 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411
29 T. J. Ward and D. W. Armstrong, *J. Liq. Chromatogr.*, 9 (1986) 407
30 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237
31 D. W. Armstrong, W. DeMond and B. P. Czech, *Anal. Chem.*, 57 (1985) 481
32 F. Mikeš, G. Boshart and E. Gil-Av, *J. Chromatogr.*, 122 (1976) 205
33 W. H. Pirkle, J. M. Finn, J. L. Schreiner and B. C. Hamper, *J. Am. Chem. Soc.*, 103 (1981) 3964
34 W. H. Pirkle, J. M. Finn, B. C. Hamper, J. Schreiner and J. R. Pribish, in E. L. Eliel and S. Otsuka (Editors), *Asymmetric Reactions and Processes in Chemistry (ACS Symposium Series*, No. 185), American Chemical Society, Washington, DC, 1982, Ch. 18, p. 245
35 W. H. Pirkle, C. J. Welch and M. H. Hyun, *J. Org. Chem.*, 48 (1983) 5022
36 I. W. Wainer and T. D. Doyle, *LC, Liq. Chromatogr. HPLC Mag.*, 2 (1984) 88
37 W. H. Pirkle and A. Tsipouras, *J. Chromatogr.*, 291 (1984) 291

38 W H Pirkle, C J Welch, G S Mahler, A I. Meyers. L M Fuentes and M Boes. *J Org Chem*, 49 (1984) 2504
39 I. W Wainer, T D Doyle. K H Donn and J R Powell. *J Chromatogr*, 306 (1984) 405
40 Z -Y Yang, S Barkan, C Brunner. J D Weber. T D Doyle and I W Wainer, *J Chromatogr*, 324 (1985) 444
41 M Zief, L J Crane and J Horvath, *J Liq Chromatogr*, 7 (1984) 709
42 R Däppen and W H Pirkle, paper presented at the *Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, New Orleans, LA, 1985*, in press
43 W H Pirkle and J M Finn, *J Org Chem*, 47 (1982) 4037
44 N Ôi, *Bunseki Kagaku*, 33 (1984) E401
45 N Ôi, M Nagase and T Doi, *J Chromatogr.*, 257 (1983) 111
46 N Ôi, M Nagase, Y Inda and T Doi, *J Chromatogr*, 265 (1983) 111
47 N Ôi, M Nagase, Y Inda and T Doi, *J Chromatogr*, 259 (1983) 487
48 N Ôi and H Kithara, *J Chromatogr*, 285 (1984) 198
49 N Ôi and H Kithara, *Abstracts of the 49th Biannual Meeting of the Chemical Society of Japan*, I (1984) 386
50 D J Gisch, paper presented at the *9th International Symposium on Column Liquid Chromatography, Edinburgh, 1985*