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Review

Enantiomer separation by gas chromatography on chiral stationary phases

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ABSTRACT

The analytical separation of enantiomers by capillary gas chromatography on chiral stationary phases (CSPs) is reviewed. Inherent principles and methodological advances are described. Three principal CSPs tailored for hydrogen bonding, coordination and inclusion are currently employed. Cyclodextrin derivatives have proved to be the most versatile and universal CSPs in gas chromatography. Anchoring the CSPs to a polysiloxane backbone leads to Chirasil-type stationary phases with improved temperature stability, efficiency and lifetimes. Immobilization of Chirasil-type stationary phases allows enantiomer separations also in capillary supercritical fluid chromatography and capillary electrochromatography. Thermodynamic parameters of enantioselectivity in gas chromatography, kinetic studies of enantiomerization and methods of enantiomer labelling are discussed.

CONTENTS

1. INTRODUCTION

The unambiguous determination of enantiomeric compositions and absolute configurations is an important analytical task in the synthesis, characterization and use of chiral compounds (optical isomers, enantiomers) such as chiral research chemicals, intermediates, auxiliaries, metabolites, precursors, drugs, pesticides, fungicides, herbicides, pheromones, flavours and fragrants. As the insight into chirality–activity relationships steadily improves and, as a consequence, legislation of chiral compounds becomes more stringent, the development of precise methods for the determination of enantiomeric purities up to, and in some cases higher

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than, an enantiomeric excess (e.e.) of 99% is of great importance. In turn, the advancing state of the art of contemporary enantiomer analysis will enable regulatory authorities to set high standards for the evaluation of chiral bioactive compounds.

The difficulty in determining e.e. arises from the fact that enantiomers have (apart from their inherent chiroptics) *identical* properties in a *non-chiral* environment (assuming ideal conditions). Methods of distinguishing enantiomers must, therefore, rely on their chiroptical characteristics (polarimetry, circular dichroism, optical rotatory dispersion) or must employ a *chiral* auxiliary resolving agent in the spirit of Pasteur's resolution principles via diastereomer formation (crystallography) or association (NMR, chromatography).

Because of the high efficiency, sensitivity and speed, chiral separation by high-resolution capillary gas chromatography represents a versatile method for enantiomer analysis. However, the prerequisite of the method is the volatility, thermal stability and resolvability of the chiral analyte, restricting its universal use. To this end, capillary supercritical fluid chromatography and capillary electrochromatography represent important complementary methods applicable also to non-volatile molecules.

In this account, inherent principles and methods of enantiomer separation by gas chromatography are discussed. For relevant applications of the method the reader is referred to previous reviews [1–9].

2. METHODOLOGY

The separation of enantiomers by gas chromatography can be performed in two modes: (1) *indirect method:* off-column conversion of enantiomers into diastereomeric derivatives by complete chemical reaction with an *enantiomerically pure* resolving agent and subsequent gas chromatographic separation of the diastereomers on a conventional *non-chiral* stationary phase, and (2) *direct method:* gas chromatographic separation of the enantiomers on a *chiral* stationary phase containing a resolving agent of high (but not necessarily complete) enantiomeric purity.

While method 1 involves the formation of diastereomers before separation, method 2 involves the rapid and reversible diastereomeric association between the chiral stationary phase (CSP) (also termed chiral selector) and the racemic or non-racemic analyte (selectand). As diastereomers display different physical properties, discrimination by incomplete recovery, decomposition and losses may occur during work-up, isolation and sample handling in method 1. In gas chromatography, such fractionation may in principle arise in split injection techniques and the original enantiomeric composition may be falsified by differing detector responses to diastereomers. Consequently, method 2 is preferred whenever possible for the determination of e.e. This approach requires an efficient selector–selectand system displaying chiral recognition. Fortunately, by employing high-resolution capillary columns, the efficiency is mostly high enough to resolve racemates having a difference of the free enthalpy (Gibbs free energy), $-\Delta_{R,S}(\Delta G)$, (see below) of diastereomeric association as little as 0.1 kJ/mol. With method 2 no discrimination of the enantiomers is expected at any time during the analytical procedure (in the absence of non-linear effects in enantiomerically enriched mixtures (the EE effect) [10]), and e.e. of the analyte determined by method 2 is independent of the enantiomeric purity of the chiral stationary phase. A low enantiomeric purity of the chiral auxiliary, however, results in small separation factors $\alpha = k'_R/k'_S$ (where k' = capacity factor and R refers arbitrarily to the second-eluted enantiomer and S to the first-eluted enantiomer) and approaches unity when the chiral selector in the stationary phase is racemic. Method 2 is especially useful for e.e. determination when no sample derivatization is required, *e.g.*, in headspace analysis by which the volatile enantiomers can be analysed directly with minute amounts of vapour. Owing to the enormous separation power of gas chromatography in the high-resolution capillary column mode of operation, contaminants and impurities are usually separated from the analytes and the simultaneous analysis of the enantiomers of different compounds (*e.g.*, all proteinogenic amino acids) is feasible in one analytical run (*c.f.*, Fig. 1. [11]).

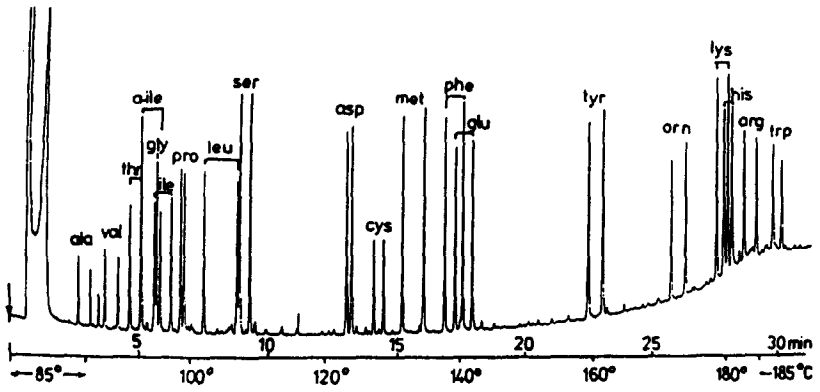

Fig. 1. Simultaneous enantiomer separation of twenty proteinogenic amino acids as N,O,S-pentafluoropropanoate isopropyl ester (histidine as N^{im}-ethoxycarbonyl) derivatives by gas chromatography on Chirasil-Val (4) between 84 and 185°C at 0.35 bar (gauge) hydrogen. Column: 50 m × 0.27 mm I.D. glass capillary. D-Enantiomers are eluted before L-enantiomers [11].

Established ancillary techniques such as multidimensional chromatography (in series-coupled column operation), use of interfacing and coupled methods (GC–MS) can readily be adapted also in chiral separations. The sensitivity can be extended to the picogram level by GC–MS or GC–electron-capture detection (ECD). GC–MS with selected-ion monitoring (SIM) can detect trace amounts of enantiomers in complex matrices.

Only a few investigations have been performed on (semi)preparative enantiomer separations in gas chromatography with packed columns (*cf.*, citations in refs. 12–15). It should be noted that recovery is simple and enantiomerically pure compounds can be obtained also on enantiomerically impure CSPs. The separation factor for the enantiomers to be isolated should be $\alpha > 1.3$.

The success of a chiral separation can be verified by control experiments. Unequivocal criteria of enantiomer separation are the occurrence of peak coalescence (first kind) when the corresponding racemic stationary phase is used and of peak inversion (first kind) when CSPs of opposite configuration are applied for enantiomerically enriched analytes [16,17].

The enantioselectivity $-\Delta_{R,S}(\Delta G)$ observed in gas chromatography arises from the diastereomeric association between the enantiomers of the selectand and the chiral selector. Because an (achiral) detection device responds equally to enantiomers, irrespective of their molecular configuration, the comparison of relative peak areas provides an unambiguous measure of the enantiomeric ratio from which e.e. can be calculated, provided that the detector is employed in its linear range. Flame ionization detection (FID) is considered to provide a linear response over several orders of magnitude.

The assignment of absolute configurations is another important application in enantiomer analysis. Unequivocal proof of absolute configuration can be obtained by co-injection of a sample with established stereochemistry. The correlation of absolute configuration with the elution order of structurally related enantiomers, *e.g.*, members of homologous series of compounds, may lead to serious pitfalls as many scenarios of peak inversions are feasible.

3. CLASSIFICATION OF CHIRAL STATIONARY PHASES

Enantiomer separation by gas chromatography is mainly performed on three types of chiral stationary phases (CSPs) [18]: (1) on chiral amino acid derivatives via hydrogen bonding [1,2,4,11,19], (2) on chiral metal coordination compounds via complexation [20–22] and (3) on cyclodextrin derivatives via (*inter alia*) inclusion [7,8,23].

Initially, all chiral selectors were used as involatile neat liquids or as solutions in squalane or polysiloxanes. Subsequently, a number of chiral selectors were chemically linked to polysiloxanes (Chirasil-type stationary phases). This strategy was first exemplified by the synthesis of Chirasil-Val [24] and related phases [25,26]. This approach has recently been extended to complexation gas chromatography by the synthesis of Chirasil-Metal [27] and to inclusion gas chromatography by the synthesis of Chirasil-Dex [28–31]. The chemically bonded chiral polymers combine the selectivity of the CSP with the efficiency of polysiloxanes, thus affording high-resolution capillary columns with an extended range of operating temperature. Moreover, Chirasil stationary phases can be immobilized on the inner wall of fused-silica capillary columns by moderate [32–34] or extensive cross-linking [31]. Immobilization of polysiloxane CSPs is a prerequisite for their use in chiral supercritical fluid chromatography (SFC) [35,36] and capillary electrochromatography (cEC) [37,38] for analysis of involatile and/or thermally labile enantiomers.

3.1. *Enantioselective gas chromatography using chiral amino acid derivatives*

The first successful separation of racemic N-trifluoroacetylamino acid esters on glass capillary columns coated with involatile N-trifluoroacetyl-L-isoleucine lauryl ester (**1**) (*cf.*, Fig. 2) was achieved by Gil-Av *et al.* in 1966 [39]. A semi-preparative version of this methodology was reported later [40]. The great potential of this fundamental approach has greatly stimulated research on enantiomer separation not only in GC but also in other chromatographic techniques such as HPLC.

Numerous hydrogen-bonding chiral phases have subsequently been investigated by several groups [41,42]. It was recognized that in the dipeptide phase **2** (*cf.*, Fig. 2) the C-terminal amino acid was not essential to chiral recognition whereas the additional amide function was important for additional hydrogen bonding and, therefore, the second chiral centre was removed by preparing the diamide **3**, *e.g.*, derived from valine [43]. This chiral selector was subsequently coupled by Frank *et al.* [24] via the amino function to a statistical copolymer of dimethylsiloxane and (2-carboxypropyl) methylsiloxane of appropriate viscosity. The resulting polymeric CSP Chirasil-Val **4** exhibits excellent gas chromatographic properties (efficiency, enantioselectivity) for the enantiomer separation of a number of classes of chiral compounds undergoing hydrogen bonding over a broad temperature range. Chirasil-Val is commercially available in both enantiomeric forms (Chrompack, Middelburg, Netherlands). The simultaneous enantiomer separation of all proteinogenic amino acids in less than 25 min is illustrated in Fig. 1 [11]. A straightforward approach to polymeric CSPs is based on the modification of cyanoalkyl-substituted polysiloxanes (XE-60, OV-225) [25,26]. For instance, the diamide **3** was chemically linked to the polysiloxane to give (L)-**5** and diasteriomeric (L,*R* and L,*S*)-**6**, which found many applications in enantiomer analysis as described by König [5].

Enantiomer separation by hydrogen-bonding CSPs generally requires derivatization of the analyte in order to increase volatility and/or to introduce suitable functions for additional hydrogen-bonding association. Versatile derivatization agents are isocyanates and phosgene [44,45].

3.2. *Enantioselective gas chromatography using chiral metal coordination compounds*

Another principle of chiral recognition based on coordination was introduced in gas chromatography by Schurig [16], who in 1977 employed the chiral metal coordination compound dicarbonylrhodium(I)-3-trifluoroacetyl-(1*R*)-camphorate (**7**) (*cf.*, Fig. 3) for the enantiomer separation of 3-methylcyclopentene. The scope of enantiomer separation by complexation gas chromatography was later extended to oxygen-, nitrogen- and sulphur-functionalized compounds [46] using chiral ketoenolate bis-chelates of divalent transition metal ions derived from terpene ketones such as camphor, menthone, carvone, pulegone and others [47]. Thus, manganese(II)- and nickel(II)-[3-(heptafluorobutanoyl)-(1*R*)-

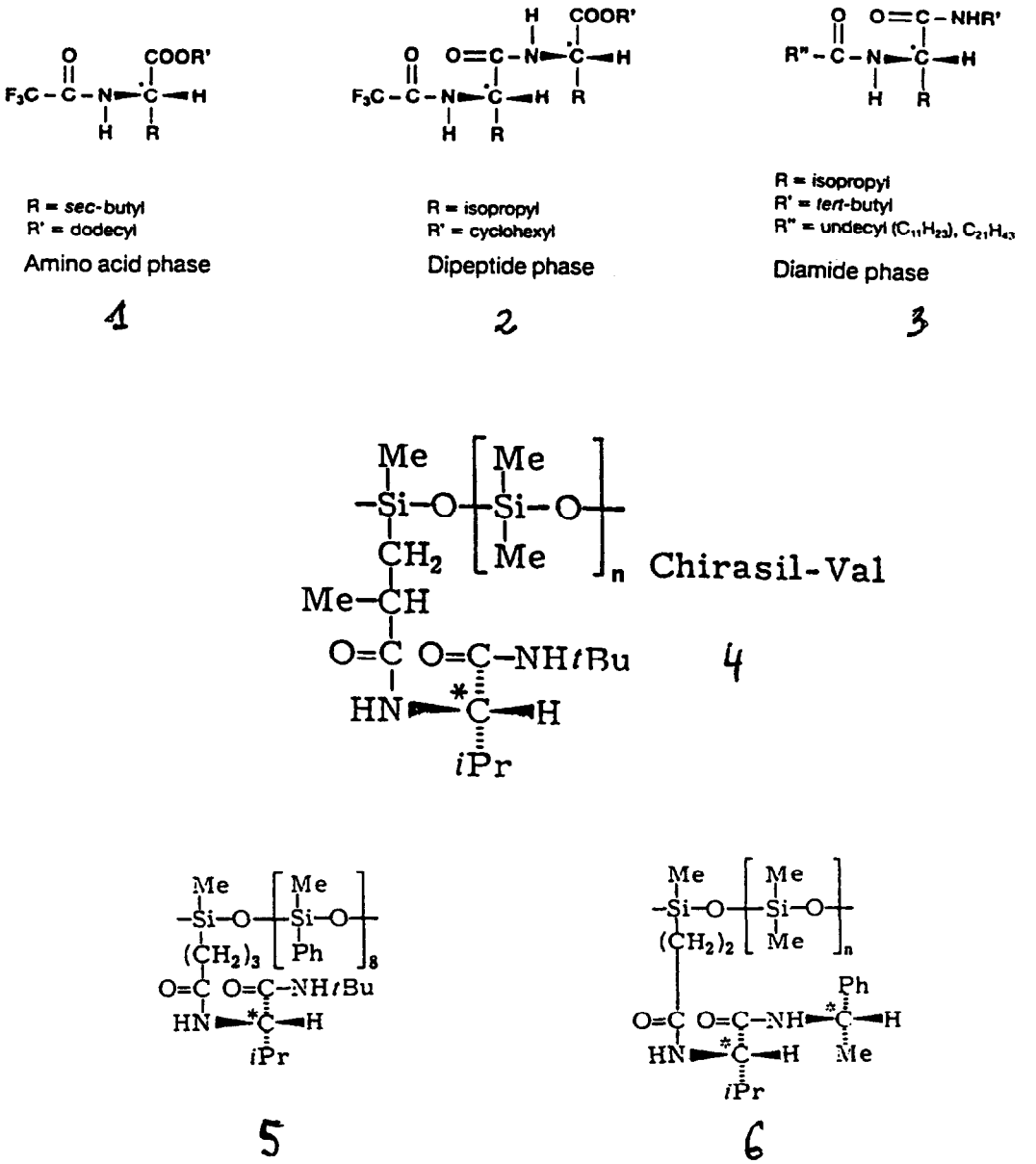

Fig. 2. Hydrogen bonding-type chiral stationary phases.

camphorate] (**8** and **9**) (*cf.*, Fig. 3) proved to be versatile CSPs for the separation of underivatized cyclic ethers, esters, acetals, aldehydes, ketones and alcohols, among them many pheromones [21], essential oils [48], metabolites [49] and products of catalytic asymmetric synthesis [50].

In Fig. 4 the enantiomer separation of simple aliphatic oxiranes, belonging to the smallest chiral molecules, by complexation gas chromatography is illustrated.

Complexation gas chromatography is well suited for the determination of thermodynamic parameters of enantioselectivity, for kinetic studies of enantiomerization and for unusual enantioselective phenomena occurring during

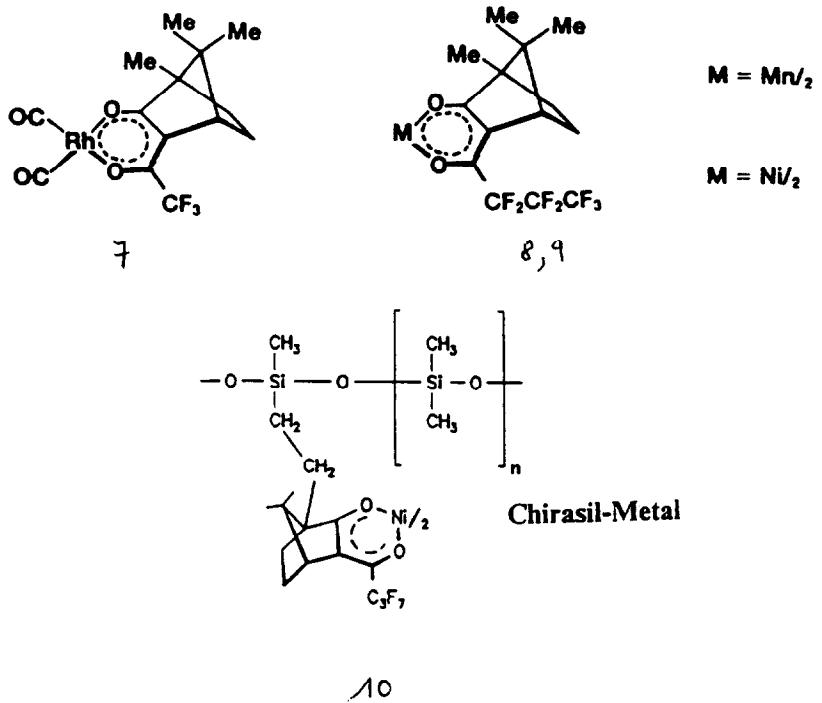

Fig. 3. Coordination-type chiral stationary phases.

enantiomer separation [50]. (Semi)preparative enantiomer separations have also been carried out by complexation gas chromatography [48,51,52].

A limiting factor of the coordination-type CSPs **8** and **9** is the low temperature range of operation (25–120°C). The thermostability has recently been increased by the preparation of immobilized polymeric CSPs (Chirasil-Metal) (**10**) (*cf.*, Fig. 3) [27]. In Fig. 5 the rapid gas chromatographic enantiomer separation of cyclic ethers on Chirasil-Nickel (**10**) in less than 20 s is shown.

Chirasil-Metal can also be applied with supercritical carbon dioxide as mobile phase (capillary SFC) [53]. Fig. 6 compares the enantiomer separation of 1-phenylethanol on Chirasil-Nickel (**10**) by complexation gas chromatography and complexation supercritical fluid chromatography. In the latter method the use of a lower separation temperature improves the selectivity considerably.

3.3. Enantioselective gas chromatography using cyclodextrin derivatives

The first enantiomer separation using an inclusion-type CSP in gas chromatography was reported in 1983 for α- and β-pinene and *cis*- and *trans*-pinane on packed columns containing native α-cyclodextrin in formamide [54]. This method was also utilized for preparative enantiomer separations [55]. Later it was recognized that alkylated cyclodextrins (CDs) can be employed in high-resolution capillary columns for enantiomer analysis. Thus, neat permethylated β-cyclodextrin [heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin] (**11**) (*cf.*, Fig. 7) was used above its melting point [56] and down to 76°C [57]. Per-*n*-pentylated and 3-acyl-2,6-*n*-pentylated CDs are viscous liquids even at room temperature. The CD derivatives **13–18** were used in the undiluted form for the separation of enantiomers of many classes of compounds on deactivated Pyrex glass capillary columns by König and co-workers in

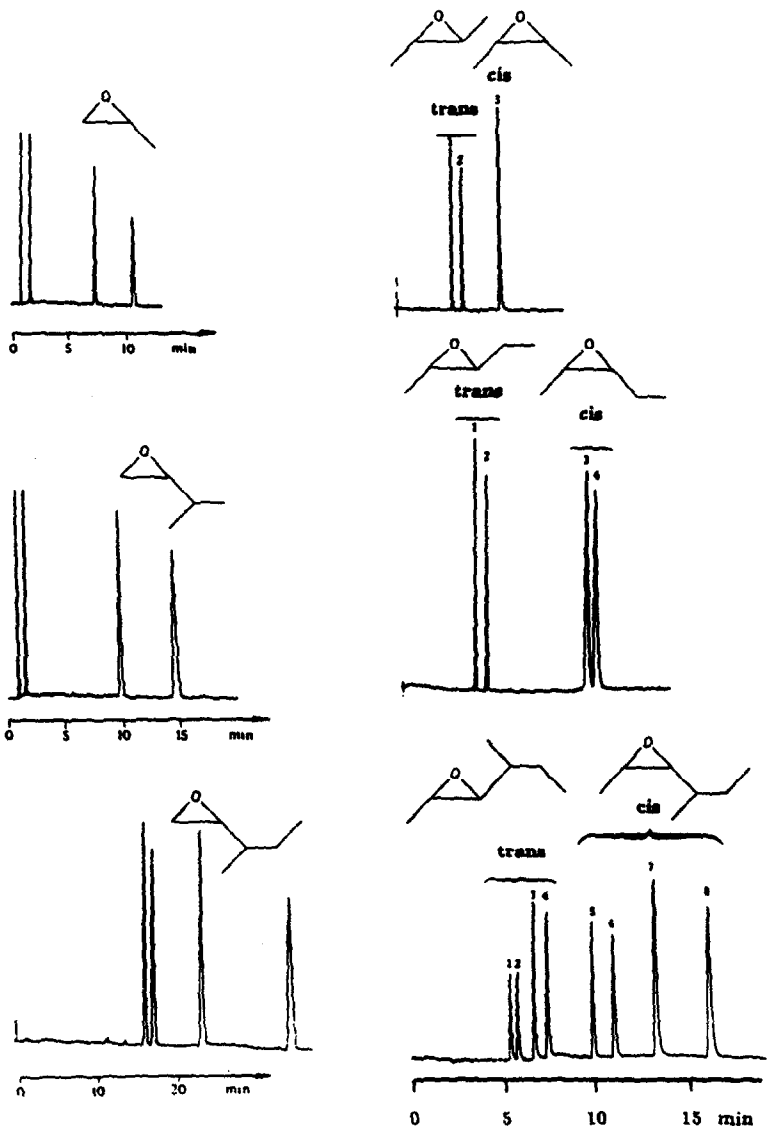

Fig. 4. Enantiomer separation of aliphatic oxiranes by complexation gas chromatography on nickel(II) bis[3-(heptafluorobutanoyl)-(1*S*)-10-ethylidenecamphorate] (0.125 molal in OV-101) between 70 and 90°C. Column: 25 m × 0.25 mm I.D. glass capillary [50].

1988 [58–61]. The more polar CD derivatives containing hydroxypropyl, free hydroxy or trifluoroacetyl groups (**19–24**), developed by Armstrong and co-workers [62–64], were coated on fused-silica capillary columns.

In Figs. 8 and 9, representative gas chromatograms employing CD derivatives are shown.

A different approach was introduced in 1987 by Schurig and Nowotny [65]. In order to combine the enantioselectivity of CDs with the excellent coating properties and efficiency of polysiloxanes, alkylated CDs were dissolved in moderately polar silicones such as OV-1701. Thus, the CD derivatives can be employed for

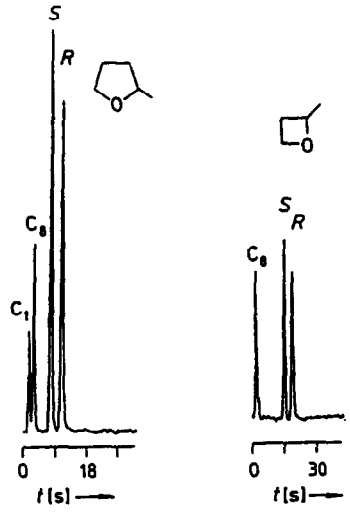

Fig. 5. Enantiomer separation of 2-methyltetrahydrofuran and 2-methyloxetane by complexation gas chromatography on immobilized Chirasil-Nickel (**10**) at 115°C and 2.0 bar (gauge) nitrogen (left) and 140°C and 2.0 bar (gauge) nitrogen (right). Column: 1.5 m × 0.05 mm I.D. fused-silica capillary, film thickness 0.25 μm [27].

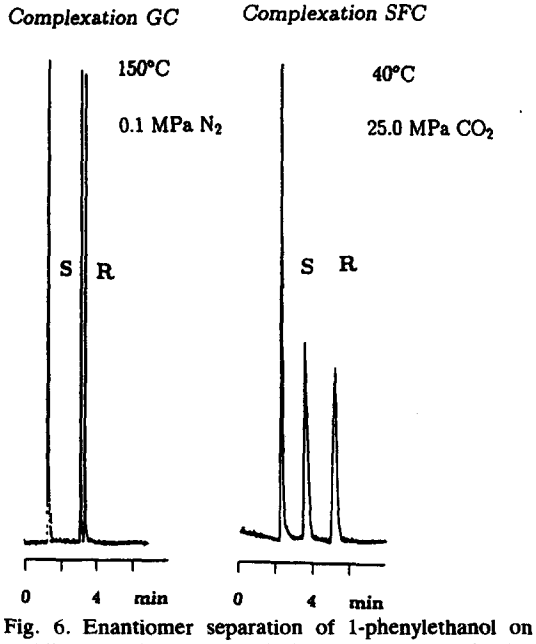

Fig. 6. Enantiomer separation of 1-phenylethanol on immobilized Chirasil-Nickel (**10**) (0.25 μm) by (left) complexation gas chromatography at 150°C and 1.0 bar (gauge) nitrogen with a 25 m × 0.25 mm I.D. fused-silica capillary column and (right) complexation supercritical fluid chromatography at 40°C and 0.883 g/ml (25.0 MPa) carbon dioxide with a 2 m × 0.05 mm I.D. fused-silica capillary column, film thickness 0.25 μm [53].

gas chromatographic enantiomer separations irrespective of their melting points and phase transitions. Fused-silica capillary columns coated with heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (**11**) in OV-1701, commercially available from leading column manufacturers (*e.g.*, Chrompack) and **12** in OV-1701 show a very broad range of applicability in enantiomer separations of various classes of compounds [66,67]. The resolution of racemic unfunctionalized saturated hydrocarbons is illustrated in Fig. 10.

A chiral test mixture, devised to probe the efficiency, enantioselectivity and inertness of permethyl-β-cyclodextrin–OV-1701 capillary columns, covers the whole polarity spectrum ranging from highly polar racemates (an underivatized carboxylic acid, amine, diol and alcohol) to apolar analytes (hydrocarbons), as shown in Fig. 11 [68].

Many other CD derivatives have been described which are suitable for enantiomer separation by gas chromatography [69–71]. Recently, *n*-pentylated CDs, introduced by König and coworkers [72–75], were also diluted in OV-1701 and are commercially available (Macherey–Nagel, Düren, Germany) (*cf.*, Fig. 12C).

The presence of three hydroxyl groups which can be regioselectively alkylated and acylated offers an enormous number of possible α-, β-, and γ-cyclodextrin derivatives which are not always readily accessible and may require tedious purification steps. It has been demonstrated, however, that fused-silica columns coated with the simplest CD, *i.e.*, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin diluted in OV-1701 [65], can resolve an appreciable number of volatile racemates between 25 and 250°C [76].

A new class of CSP has obtained by chemically linking the CD derivatives to the polysiloxane backbone [28,29]. In analogy with Chirasil-Val (**4**), this new polymeric CSP has been named Chirasil-Dex (**24**) (*cf.*, Fig. 13) [29]. In the synthesis, care has been taken to link the CD to the polymer by only one 6-O-octamethylene spacing group [30].

Fused-silica columns coated with Chirasil-Dex (available from Chrompack International) have the following advantages: use of a non-polar polysiloxane matrix (in which CD derivatives

Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin	11
Heptakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-β-cyclodextrin	12
Hexakis(2,3,6-tri-*O*-n-pentyl)-α-cyclodextrin	13
Hexakis(3-*O*-acetyl-2,6-di-*O*-n-pentyl)-α-cyclodextrin	14
Heptakis(2,3,6-tri-*O*-n-pentyl)-β-cyclodextrin	15
Heptakis(3-*O*-acetyl-2,6-di-*O*-n-pentyl)-β-cyclodextrin	16
Octakis(2,3,6-tri-*O*-n-pentyl)-γ-cyclodextrin	17
Octakis(3-*O*-butanoyl-2,6-di-*O*-n-pentyl)-γ-cyclodextrin	18
Hexakis(2-*O*-(*S*)-2-hydroxypropyl-3,6-di-*O*-methyl)-α-cyclodextrin (PMHP-α-CD)	19
Heptakis(2-*O*-(*S*)-2-hydroxypropyl-3,6-di-*O*-methyl)-β-cyclodextrin (PMHP-β-CD)	20
Hexakis(2,6-di-*O*-n-pentyl)-α-cyclodextrin (Dipentyl-α-CD)	21
Heptakis(2,6-di-*O*-n-pentyl)-β-cyclodextrin (Dipentyl-β-CD)	22
Heptakis(3-*O*-trifluoroacetyl-2,6-di-*O*-n-pentyl)-β-cyclodextrin (DPTFA-β-CD)	23

Fig. 7. Representative cyclodextrin-type chiral stationary phases.

cannot be physically diluted), resulting in low elution temperatures for polar analytes; a high degree of inertness, allowing analyses of polar compounds without prior derivatization; a higher CD concentration, resulting in increased separation factors, α; long-term stability with absence of droplet formation leading to breakdown of efficiency; immobilization by cross-linking and/or surface bonding; and compatibility with all injection techniques.

The immobilization of the resulting Chirasil-Dex CSPs on the fused-silica surface by thermal treatment represents a refinement of the methodology [30,34]. These CSPs show superior per-

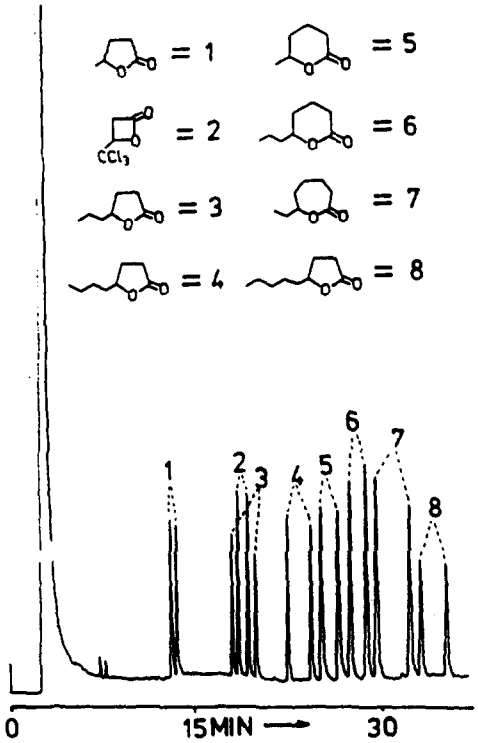

Fig. 8. Enantiomer separation of lactones by gas chromatography on octakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin (**18**) (undiluted) at 150°C and 1 bar (gauge) hydrogen. Column: 50 m × 0.25 mm I.D. Pyrex glass capillary [61].

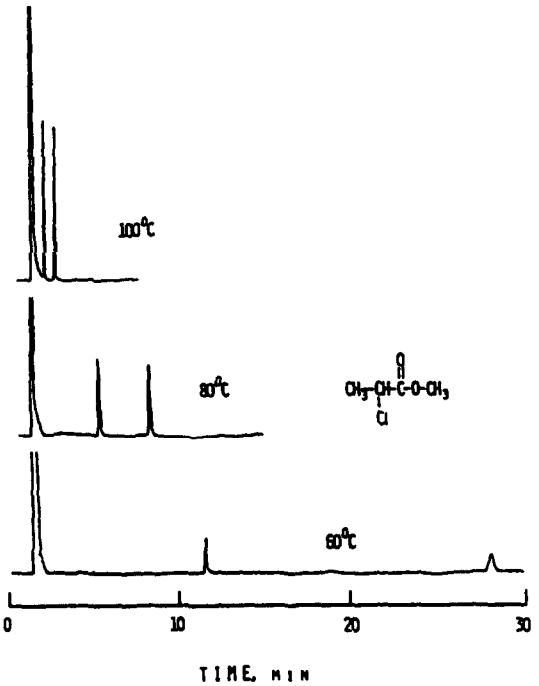

Fig. 9. Enantiomer separation of methyl 2-chloropropanoate by gas chromatography on heptakis(3-O-trifluoroacetyl-2,6-di-O-*n*-pentyl)-β-cyclodextrin (**23**) (undiluted) at three temperatures. Column: 20 m × 0.25 mm I.D. non-deactivated fused-silica capillary [64].

formance compared with the dissolved system [30,77]. Recently, Chirasil-Dex-TFA (**25**) has been introduced as a polysiloxane-bonded and immobilized 2,6-permethylated-3-trifluoroacetylated β-CD [78]. Representative gas chromatograms illustrating enantiomer separations of organochlorines on **25** are shown in Fig. 12A and B. Stable atropisomeric polychlorinated biphenyls (PCBs) are preferentially separated on **24** (*cf.*, Fig. 12D) [79]. Immobilized CSPs are preferred when electron-capture detection (ECD) is used because of the very low bleeding rate. Organochlorines have previously been separated with CDs dissolved in polysiloxanes (*cf.*, Fig. 12C) (see also ref. 80).

The immobilization of Chirasil-Dex CSPs **24** and **25** and the resulting compatibility with solvent intake and stability against polar solvents was the prerequisite for its used for enantiomer separation of involatile compounds by SFC [34,82] and (cEC) [37,38]. Highly cross-linked CSPs have also been prepared recently and used for GC, SFC and cEC [31]. Enantioselective gas chromatography involving CDs have been used for important coupled techniques including parallel column operation [72], GC coupled on-line with an isotope ratio mass spectrometer [83], GC–MS-SIM [84] and multi-dimensional techniques (MDGC–MS) [85].

The separation factors α for enantiomers separated on CD-derived CSPs are generally low. This feature is compensated for by the high efficiency of capillary columns. Small separation factors are even useful in terms of analysis times and in reduction of overlapping in multi-component matrices. Unfortunately, CDs are readily available only in the all-D-configuration form. Hence, peak switching by inverting the chirality of the CSP [leading to peak inversion (first

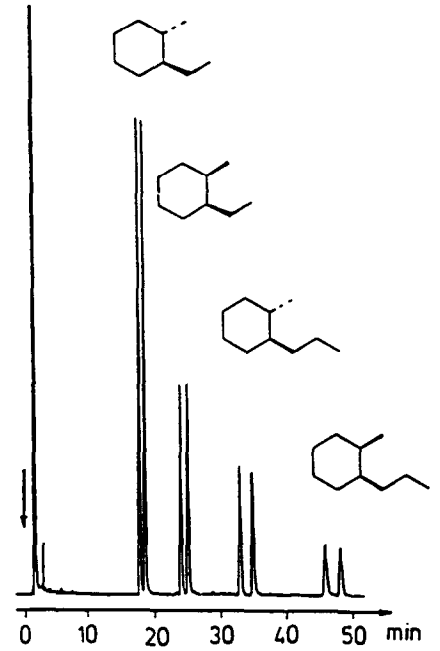

Fig. 10. Enantiomer separation of *trans*- and *cis*-1-ethyl-2-methylcyclohexane and of *trans*- and *cis*-1-methyl-2-*n*-propylcyclohexane by gas chromatography on heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (**11**) (10%, w/w, in OV-1701) at 50°C and 0.7 bar (gauge) helium. Column 25 m × 0.25 mm I.D. fused-silica capillary, film thickness 0.25 μm [67].

kind)], important in the analysis of enantiomeric impurities which should preferentially be eluted before the main component [17], is not feasible with CDs. In some instances, however, a change of the CD selector may lead to an accidental change in the elution order of the enantiomers [62].

The rationalization of chiral recognition involving CD derivatives is difficult as almost all classes of chiral compounds are susceptible to enantiomer separation on a certain CD-derived CSP, often with no logical dependence on molecular shape, size and functionalities. Obviously, multimodal recognition processes take place, which may involve *inter alia* inclusion, hydrogen bonding, dipole–dipole interactions and other forces. As enantiomer separations have also been observed with amylose derivatives, inclusion is not a prerequisite for chiral recognition using carbohydrates [86,87]. Mechanistic investigations, some of which include molecular modelling studies, have been advanced [7,64,88–90]. Unusual temperature-dependent peak broadening effects and/or peak inversions have also been observed [80,91–93].

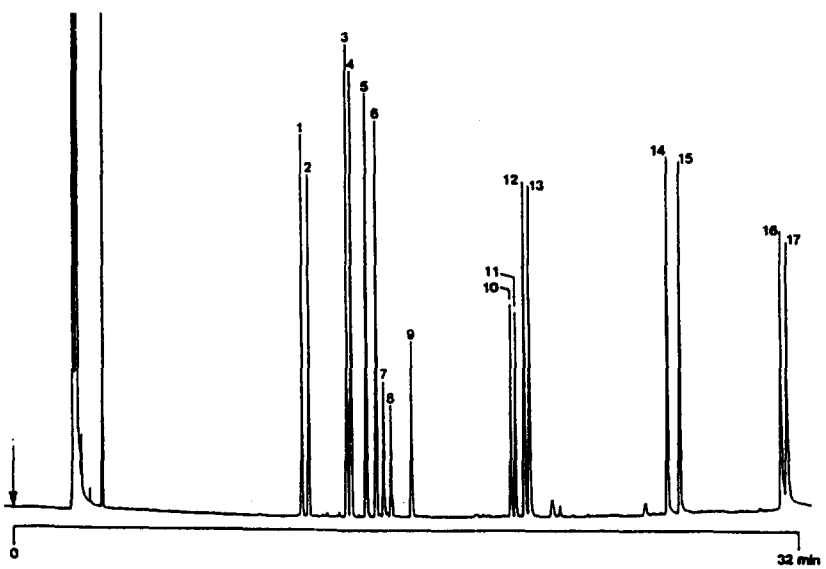

Fig. 11. Enantiomer separation of α-pinene (1, 2), *trans*-pinane (3, 4), *cis*-pinane (5, 6), 2,3-butanediol (*rac*) (7, 8), 2,3-butanediol (*meso*) (9), γ-valerolactone (10, 11), 1-phenylethylamine (12, 13), 1-phenylethanol (14, 15) and 2-ethylhexanoic acid (16, 17) (Schurig test mixture) by gas chromatography on heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (**11**) (10%, w/w, in OV-1701) at 50°C and 0.7 bar (gauge) helium. Column: 50 m × 0.25 mm I.D. fused-silica capillary, film thickness 0.25 μm. Courtesy Chrompack International, Middelburg, Netherlands.

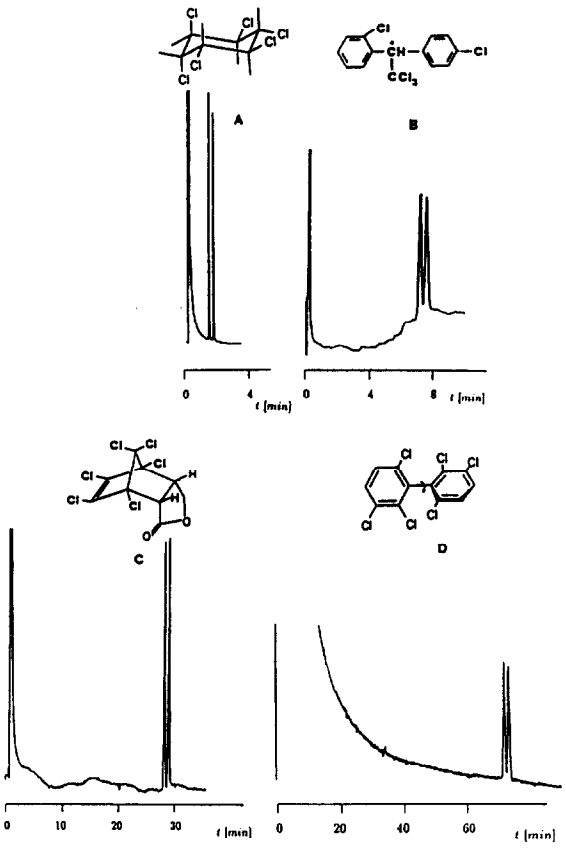

Fig. 12. Enantiomer separation of organochlorines by gas chromatography [81]. (A) α-Hexachlorocyclohexane (α-HCH). Column: 10 m × 0.25 mm I.D. fused-silica capillary coated with Chirasil-Dex-TFA (**25**) [78], film thickness 0.25 μm; 170°C, 0.6 bar (gauge) hydrogen. (B) 1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane. Conditions as in (A). (C) Endosulfan lactone (1,7,8,9,10,10-hexachloro-4-oxa-tricyclo[5.2.1.$0^{2,6}$]dec-8-enone). Column: 25 m × 0.25 mm I.D. fused-silica capillary coated with octakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin (**18**) [61] (40%, w/w), film thickness 0.25 μm; 190°C, 1.0 bar (gauge) hydrogen. (D) Atropisomeric 2,2′,3,3′,6,6′-hexachlorobiphenyl [79]. Column: 25 m × 0.25 mm I.D. fused-silica capillary coated with Chirasil-Dex (**24**), film thickness 0.25 μm; 170°C, 1.0 bar (gauge) hydrogen.

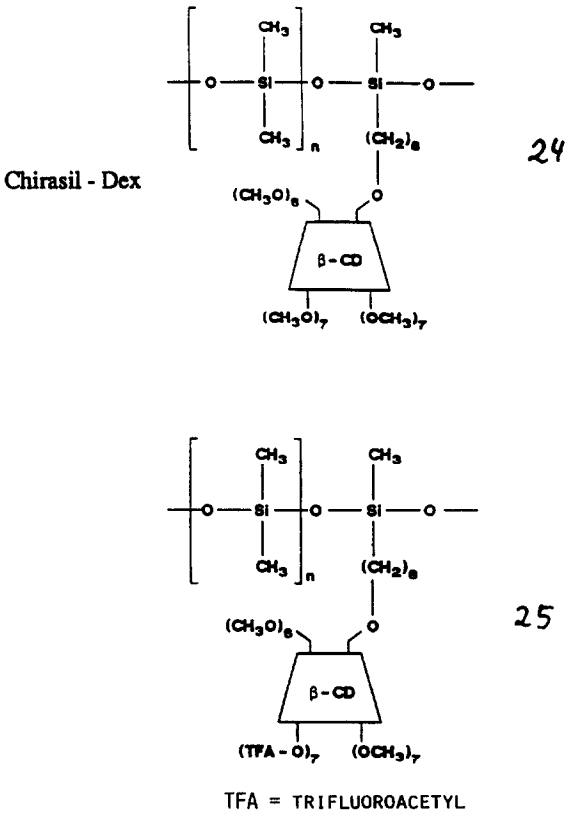

Fig. 13. Chirasil-Dex-type chiral stationary phases.

4. THERMODYNAMICS OF ENANTIOSELECTIVITY

It should be recognized that enantiomer separations by chromatography are caused by the difference in the free enthalpy (Gibbs energy) $-\Delta_{R,S}(\Delta G)$ of the diastereomeric association equilibria between chiral selector and selectand and is thus thermodynamic in nature. An important prerequisite is, however, a fast and reversible association equilibrium (fast kinetics).

The chemical association equilibrium in the stationary phase is described by K. For enantiomer separation, the Gibbs–Helmholtz equation is written as follows [94,95]:

$$-\Delta_{R,S}(\Delta G) = RT \ln \frac{K_R}{K_S} = -\Delta_{R,S}(\Delta H) + T\Delta_{R,S}(\Delta S) \quad (1)$$

where R refers arbitrarily to the second-eluted enantiomer and S to the first-eluted enantiomer For a 1:1 molecular association, the quantities $\Delta_{R,S}(\Delta S)$ and $\Delta_{R,S}(\Delta H)$ usually display an opposing, temperature-dependent effect on $-\Delta_{R,S}(\Delta G)$. At the isoenantioselective temperature T_{isoenant},

$$T_{\text{isoenant}} = \frac{\Delta_{R,S}(\Delta H)}{\Delta_{R,S}(\Delta S)} \quad (2)$$

peak coalescence (second kind) occurs [$\Delta_{R,S}(\Delta G) = 0$ ($K_R = K_S$; no enantiomer separation)]. Above $T_{isoenant}$, the sign of enantioselectivity changes, leading to peak inversion (second kind). Below the coalescence temperature, the sign of enantioselectivity $\Delta_{R,S}(\Delta G)$ is governed by $-\Delta_{R,S}(\Delta H)$ and above it by $\Delta_{R,S}(\Delta S)$. Evidence for the reversal of enantioselectivity has been found in gas chromatographic enantiomer separations [50,80,96,97,98]. In gas chromatography, even at high temperatures, enantioselectivity is usually dominated by enthalpy control and the separation factors α increase with decreasing temperature.

For undiluted CSPs the quantity $-\Delta_{R,S}(\Delta G)$ can easily be obtained from the separation factor α_{undil} according to the equation [99]

$$-\Delta_{R,S}(\Delta G) = RT \ln \frac{K_R}{K_S} = RT \ln \alpha_{undil} \quad (3)$$

Although occasionally used, eqn. 3 is not valid for diluted CSPs, because α_{dil} is concentration dependent, whereas $-\Delta_{R,S}(\Delta G)$ is strictly independent of concentration. With diluted CSPs, the solubility of the chiral selectand in the solvent, which is non-enantioselective, must be separated from its enantioselective association with the chiral selector by the concept of the retention increase R' (or chemical capacity factor). R' is experimentally accessible from relative retention data r (column with selector in solvent) and r_0 (reference column without selector in solvent) [47,95] according to the equation

$$R' = K \cdot m = \frac{r - r_0}{r_0} = \text{associated/non-associated} \quad (4)$$

where m = molality (mol/kg solvent, a temperature-independent quantity) of the selector in the polysiloxane (dissolved or polymer-bonded) and K = association constant. R' represents the fraction of associated to non-associated selectand in the stationary phase.

Thus, R' is a measure of the strength of the selector–selectand association (for a constant molality m). It follows for diluted CSPs that the quantity $-\Delta_{R,S}(\Delta G)$ can easily be obtained from the differences in retention increases R'_R and R'_S of the enantiomers according to the equation [95]

$$-\Delta_{R,S}(\Delta G) = RT \ln \frac{K_R}{K_S} = RT \ln \frac{R'_R}{R'_S} \quad (5)$$

Whereas $-\Delta_{R,S}(\Delta G)$ is independent of the molality m of the CSP, it can be shown that the separation factor α_{dil} is concentration dependent in diluted CSPs [95]:

$$\alpha_{dil} = \frac{K_R m + 1}{K_S m + 1} = \frac{R'_R + 1}{R'_S + 1} \quad (6)$$

It should be noted that according to eqn. 6 only in the case of very strong selector–selectand associations ($R' \gg 1$) may $-\Delta_{R,S}(\Delta G)$ in diluted systems be approximated from eqn. 3. Eqn. 6 has experimentally been verified for CSPs containing heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (**11**) [30]. It is a direct consequence of eqn. 6 that the α *vs.* CD concentration curves level off at higher mass percentages. The optimum is already reached at low CD concentrations when the chemical association is strong (*i.e.*, large K or R'). Plots of α *vs.* m (for convenience expressed as mass percentages, w/w) are shown in Fig. 14 and a practical example is depicted in Fig. 15.

The experimental data imply that in most instances no further improvement in selectivity (as expressed by the separation factor α) is achieved above a CD mass percentage of approximately 30% (for **11**) or approximately 50% (for CD derivatives with high molecular masses, *e.g.*, those containing *n*-pentyl groups). Thus, undiluted CDs [23] are recommended only for very weakly interacting analytes.

5. ENANTIOMERIZATION

The configurational integrity of the enantiomers during the gas chromatographic process of separation is essential for a correct enantiomer analysis. When enantiomers are prone to inversion of configuration, characteristic peak profiles are obtained which are recognized by the appearance of a plateau between the terminal peaks of the enantiomers [46]. By peak form analysis, activation parameters of enantiomeriza-

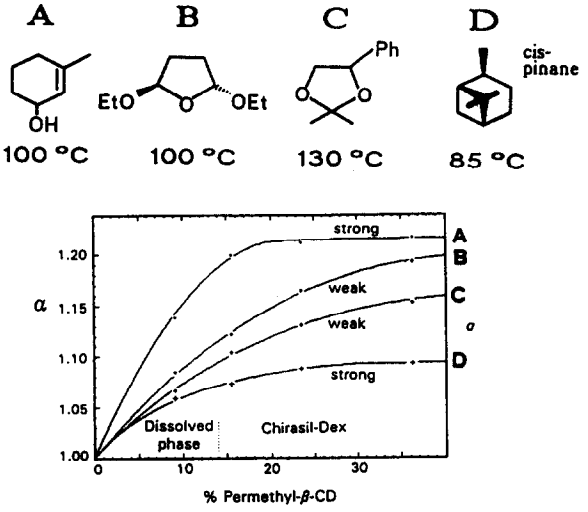

Fig. 14. Plots of separation factors α *vs.* CD mass percentage for four racemates exhibiting strong or weak selector–selectand association. (A) 1-Methylcyclohex-1-en-3-ol; (B) *trans*-2,5-ethoxy-tetrahydrofuran; (C) 2,2-dimethyl-4-phenyl-1,3-dioxolane; (D) *cis*-pinane. Columns: 25 m × 0.25 mm I.D. fused-silica capillaries coated with **11** in OV-1701 and different specimens of Chirasil-Dex (**24**), film thickness 0.25 μm; 1.0 bar (gauge) hydrogen [30].

tion ($\Delta G^{\ddagger}$) can be determined by dynamic gas chromatography based on the process depicted in Fig. 16 [100]. If enantiomerization is fast on the chromatographic time scale, peak coalescence (third kind) will arise.

The interconverting enantiomers have a different energy in the stationary phase due to enantioselective partition constants (gas–liquid) $K_{cR} > K_{cS}$ (*cf.*, Fig. 16) responsible for enantiomer separation. Hence, the interconversion rates, $k^{s}_{S\rightarrow R} > k^{s}_{R\rightarrow S}$, are different according to the equation [100]

$$\frac{K_{cR}}{K_{cS}} = \frac{k^{s}_{S\rightarrow R}}{k^{s}_{R\rightarrow S}} \qquad (7)$$

While the first-eluted enantiomer S is transformed at a higher rate into the second-eluted enantiomer R ($k^{s}_{S\rightarrow R} > k^{s}_{R\rightarrow S}$), the second-eluted enantiomer R has a longer residence time in the column ($K_{cR} > K_{cS}$). Thus, enrichment and depletion of R (and *vice versa* that of S) cancel each other and no overall deracemization occurs, in agreement with the principle of microscopic

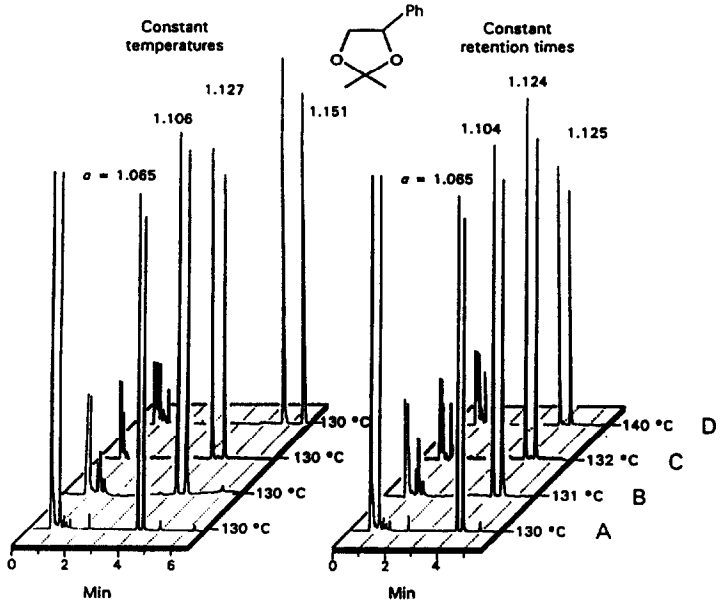

Fig. 15. Dependence of the separation factor α of 2,2-dimethyl-4-phenyl-1,3-dioxolane on the mass percentages of the CD in Chirasil-Dex (**24**) at constant temperatures (left) and at constant retention times (right). (A), (B), (C) and (D) = 9, 16, 24 and 36%, respectively. Column: 25 m × 0.25 mm (I.D.) fused-silica capillary coated with Chirasil-Dex (**24**) (not immobilized), film thickness 0.25 μm [30].

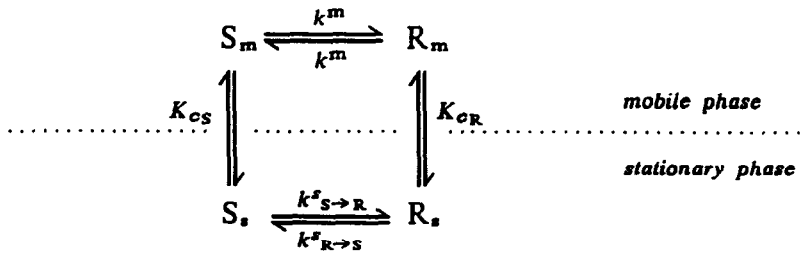

Fig. 16. Cyclic process involving enantiomerization [100].

reversibility (*cf.*, Fig. 16). Examples featuring enantiomerization have been described in complexation gas chromatography for 1-chloro-2,2-dimethylaziridine [46,100], 1,6-dioxa[4.4]spirononane [46], phenyloxirane and isopropenyloxirane [50] and homofuran [101] (*cf.*, Fig. 17) and in inclusion gas chromatography for diaziridines [102] and atropisomeric biphenyls [103]. Perfect agreement between calculated and experimental chromatograms was obtained [100–102]. Only minute amounts of the racemic compound suffice for the determination of kinetic data of inversion.

6. THE METHOD OF ENANTIOMER LABELLING

The addition of a known amount of an antipodic or racemic compound is an elegant procedure for introducing an internal standard for the determination of enantiomeric compounds in a complex matrix [104–106]. In the absence of

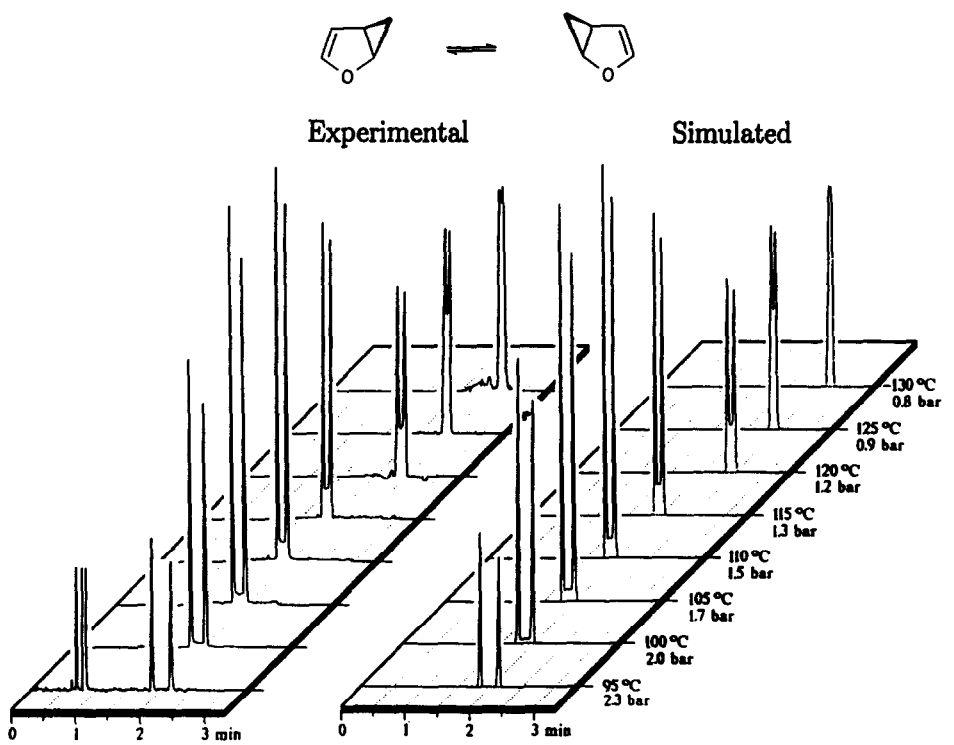

Fig. 17. Elution profiles of homofuran at different temperatures and inlet pressures (N_2). Left, experimental gas chromatograms [column: 10 m × 0.1 mm I.D. fused-silica capillary coated with Chirasil-Nickel (**10**), film thickness 0.25 μm]; right, simulated chromatograms [101].

non-linear effects in enantiomerically enriched mixtures (the EE effect [10]), the enantiomeric ratios are not influenced by sample manipulations (derivatization, dilution, injection, detection, chemical and physical losses). The method of enantiomer labelling presupposes a precise knowledge of the enantiomeric purities of the sample and the standards.

The following relationships apply when defining [107]:

$A = [R]/[S]$ before addition of the label

$B = [R']/[S']$ after addition of the label

C = amount of label in mg

(i) for enantiomer labelling with enantiomerically pure enantiomer R:

$$R\,(\text{mg}) = (AC)/(B - A)$$

$$S\,(\text{mg}) = R\,(\text{mg})/A$$

(ii) for enantiomer labelling with racemate:

$$R\,(\text{mg}) = \{(1 - B)/[(B/A) - 1]\}C/2$$

$$S\,(\text{mg}) = [(1 - B)/(B - A)]C/2$$

The method of enantiomer labelling is useful, *e.g.*, in enzymatic reactions where the amounts of products are too small to be isolated [108]. The combination of enantiomer labelling and isotopic labelling has also been carried out with GC–chemical ionization MS-SIM [109].

7. SELECTION OF CHIRAL STATIONARY PHASES FOR GAS CHROMATOGRAPHY

Since the introduction of chiral capillary gas chromatography, more than 25 years have passed. Nevertheless, the topic still constitutes a very active field and interesting new discoveries are made. As far as new CSPs are concerned, the last few years have witnessed surprising achievements with CD-derived selectors. However, there is no universal CSP available and selection is still governed by trial and error. Apart from remarkable exceptions (*cf.*, Fig. 9) [12,64,88], the separation factors α are generally low with CD derivatives and therefore the column efficiency must be very high. The film thickness employed is usually $d_f = 0.25$ μm. The α values are temperature dependent and are not relevant in temperature-programmed runs. Unfortunately, the resolution R_S is not always quoted in the literature, although it is important for assessing efficiency and selectivity at the same time. In a recent collection of separation factors α, obtained by capillary GC on cyclodextrin CSPs, the important information on efficiency and retention at a given selectivity α was not given [110]. Important information is available from the Chirbase data bank for GC (and HPLC) [111].

In general, the potential user is advised to consult published chromatograms because they give the best clue to properties such as selectivity, efficiency, capacity factor, retention, baseline stability and sensitivity. Information on long-term stability of columns is, unfortunately, not readily available. Chiral test mixtures [68,71,112] should be used to compare columns from different sources. A list of commercially available CD-derived CSPs for GC has been compiled recently [85].

Because of the very wide range of classes of compounds that are resolvable on CD derivatives, this type of chiral selector should be considered first when facing a problem in enantiomer analysis. If the separation factor α is too low or zero, if the use of both enantiomeric forms of the CSP is required (*e.g.*, for peak switching) or if a cyclodextrin-derived CSP is not readily available, hydrogen bonding-type or coordination-type chiral selectors should be considered. Tables collecting enantiomers that have been separated on amino acid selectors are available [4,94,113]. Enantiomers separated by complexation gas chromatography have been listed [6]. The Chirbase data bank may also be consulted [111].

It should be remembered that new enantioselective partitioning systems are expected to be found continuously and what seems to be an ideal CSP in gas chromatography at the present time might be superseded by superior chiral selectors in the near future.

Established column dimensions for gas chromatographic enantiomer separations are 10–25

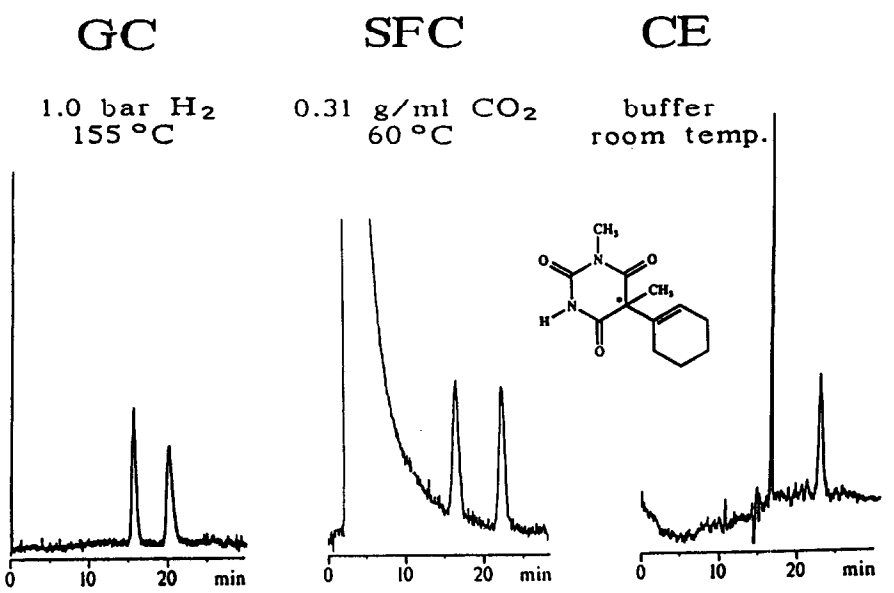

Fig. 18. Enantiomer separation of hexobarbital by gas chromatography (left), supercritical fluid chromatography (middle) and electrochromatography (right). Column: 1 m × 0.05 mm I.D. fused-silica capillary coated wtih Chirasil-Dex (**24**) (immobilized), film thickness 0.25 μm.

m × 0.25 mm I.D. However, column miniaturization may have important merits in terms of shorter analysis times [27,114] (*cf.*, Fig. 5), improvement of detection limits and for unified enantioselective capillary chromatography [115]. The last term implies that one individual column can be used for enantiomer analysis by capillary gas chromatography and supercritical fluid chromatography (*cf.*, Fig. 6) and, additionally, by capillary electrochromatography (cEC). In Fig. 18 the enantiomer separation of hexobarbital on the same 1 m × 0.05 mm I.D. fused-silica capillary column by GC, SFC and cEC is shown [115]. In this example, immobilized Chirasil-Dex is compatible with fluid mobile phases such as supercritical carbon dioxide [27] and an aqueous buffer [37,38].

8. ACKNOWLEDGEMENTS

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