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Review

Separation of enantiomers by gas chromatography

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Abstract

The separation of enantiomers by gas chromatography is performed on chiral stationary phases (CSPs) via hydrogen bonding, coordination and inclusion. Thus, typical chiral selectors are amino acid derivatives, terpene-derived metal coordination compounds and modified cyclodextrins. In Chirasil-type stationary phases the chiral selector is anchored to a polysiloxane backbone improving gas chromatographic performance. The present review article describes the state-of-the-art, scope and limitations, applications and mechanistic considerations at the advent of the millennium incorporating 16 figures and 168 references.

Keywords: Reviews; Enantiomer separation; Chiral stationary phases, GC

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1. Introduction and historical perspective

The separation of enantiomers by gas chromatography (GC) on a chiral stationary phase (CSP) was discovered by Gil-Av et al. at the Weizmann Institute of Science, Israel, in 1966 [1]. The situation prevailing at the outset of enantioselective GC in the late 1960s is illustrated by Gil-Av in retrospect [2]:

> *When we started this work in* 1964, *this topic was in a 'state of frustration'. Nobody believed it could be done. In fact, people were convinced that there could not possibly be a large enough difference in the interaction between the* D- *and* L-*solute with an asymmetric solvent. This was the feeling people had, even those known as unorthodox thinkers. This view had also some experimental basis, because a number of communications had been published, in which it was claimed that such resolutions could be effected, but nobody was able to reproduce these results, and some of them were shown to be definitely wrong.*

The solution to the problem is described by Gil-Av as follows [2]:

> *When we started our work with B. Feibush – who had the courage to accept this problem for his Ph.D. thesis – we based ourselves on the following two ideas. First of all, nature can do it, enzymes differentiate between enantiomers. Therefore we thought*: *let us have a system which has some of the properties, at least in a rudimentary fashion, of an enzyme. In other words, we decided to try phases with –CO– and –NH– functions grouped around an asymmetric centre, i.e., derivatives of α-amino acids. Secondly, we reasoned that we had to amplify the effect, because we expected it to be very small. This meant the use of long capillaries. As stainless steel is expensive, and we expected a need for many columns we decided to start by building a capillary drawing machine, and by acquiring the delicate skills of drawing long capillaries and coating them . . . the idea worked.*

[1].

In 1967 Gil-Av and Feibush pioneered also the use of a packed column for the semi-preparative separation of amino acid enantiomers by GC and they utilised an (off-column) chiroptical detection device to prove that they had indeed separated enantiomers [3].

While at the beginning of this work only selected chiral compounds could be separated into enantiomers, the situation is almost reversed 30 years later. According to the Chirbase data bank (cf. Section 11), for almost every racemic compound of a variety of different classes of compounds, ranging from apolar to polar, an appropriate chiral stationary phase is available and 22 000 separations of enantiomers involving 5500 basic chiral compounds, documented in 2200 publications, have been performed by GC before the advent of the new millennium [4].

2. Scope of separation of enantiomers by gas chromatography

High efficiency, sensitivity and speed of separation are important advantages of separation of enantiomers by high-resolution capillary gas chromatography (HRC-GC). Due to the enormous separation power of capillary gas chromatography, contaminants and impurities are usually separated from the analytes and the simultaneous analysis of multicomponent mixtures of enantiomers (e.g., all proteinogenic amino acids, cf. Fig. 1) is possible in one analytical run. Established ancillary techniques such as multidimensional gas chromatography (in series-coupled column operation), interfacing and coupling methods (gas chromatography–mass spectrometry, GC–MS) are amenable to chiral analysis. Employing the GC–MS selected ion monitoring (SIM) mode, trace amounts of enantiomers present in complex matrices can easily be detected. The universal flame ionisation detector is linear over five-orders of magnitude and detectability can further be extended to the picogram level by GC–MS, electron-capture detection (ECD) or element-specific detection.

The main application of the separation of enantiomers by gas chromatography is concerned with the precise determination of enantiomeric compositions of chiral research chemicals, intermediates, aux-

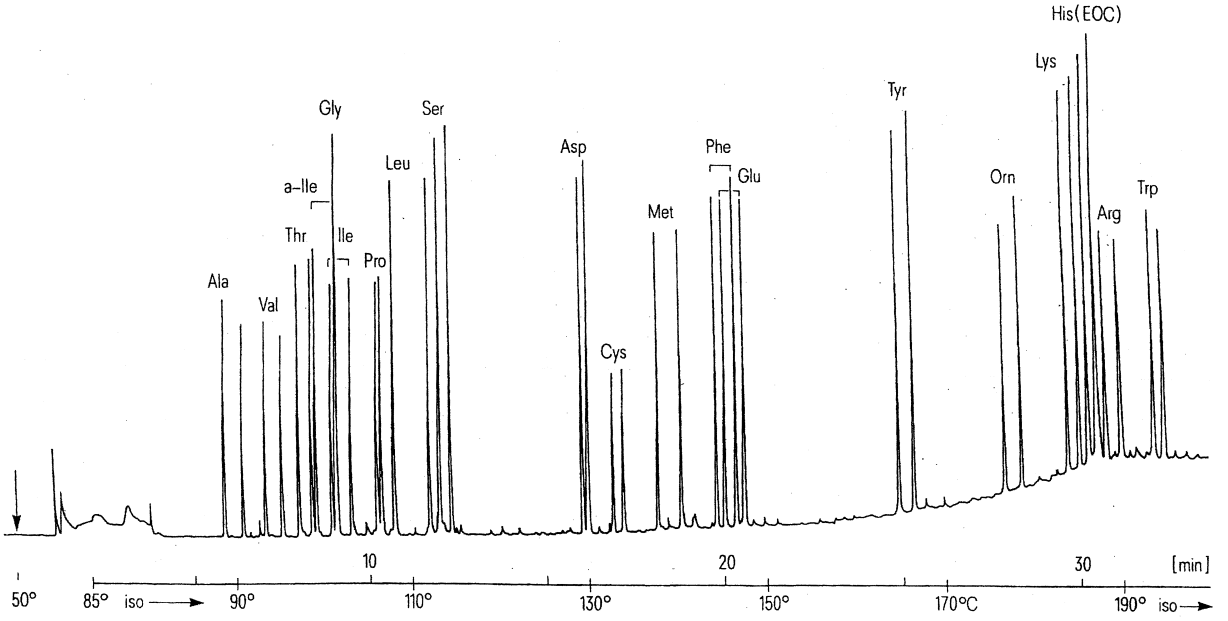


Fig. 1. Simultaneous separation of the enantiomers of 20 proteinogenic amino acids as *N,O,S*-pentafluoropropanoyl *O*-2-propyl esters (histidine as N^{im}-ethoxycarbonyl) on 0.13 μm Chirasil-Val **4** at 4 kPa hydrogen. Temperature program: 50°C, 5°C/min to 85°C, 3 min isothermal, 4°C/min to 190°C. Column: 20 m×0.3 mm I.D. glass capillary. D-Enantiomers are eluted before L-enantiomers (reproduced with permission from Refs. [12,13]).

iliaries, metabolites, precursors, drugs, pesticides, fungicides, herbicides, pheromones, flavours and fragrances. As the insight into chirality–activity relationships steadily improves and, as a consequence, legislation of chiral compounds becomes more stringent, the development of reliable methods for the determination of the enantiomeric excess (ee) up to ee=99.9% is of great importance {% ee= $100(R-S)/(R+S)$ with R the major enantiomer, S the minor enantiomer [5]}. This goal is readily met by enantioselective gas chromatography. Enantioselective gas chromatography is especially suited for chiral analysis when no sample derivatisation is required, e.g., by headspace analysis of flavours and pheromones whereby volatile enantiomers are directly analysed from the vapour phase of the sample matrix. In contrast to liquid chromatographic methods, the delicate choice of solvents, modifiers and gradient elution systems is absent in gas chromatography.

Prerequisites for the use of gas chromatography however are volatility, thermal stability and resolvability of the chiral analyte, restricting its exclusive use.

3. Classification of chiral stationary phases for gas chromatography

Three principal CSPs, distinguishable by the mode of selector–selectand interaction, i.e., hydrogen bonding, coordination and inclusion, have thoroughly been investigated [6–9]:

- Separation of enantiomers on chiral amino acid derivatives via hydrogen-bonding [1–3,10–15]
- Separation of enantiomers on chiral metal coordination compounds via complexation [16,17]
- Separation of enantiomers on cyclodextrin derivatives via (inter alia) inclusion [18–22]

Initially, the chiral selectors were used as involatile neat liquids or as solutions in squalane or polysiloxanes, respectively. Subsequently, in an effort to combine chemical selectivity with chromatographic efficiency, the chiral selectors were chemically

linked to polysiloxanes. The resulting chiral polysiloxanes (Chirasil-type) CSPs possessing improved temperature stability, efficiency and robustness were introduced 1977 by Frank, Nicholson and Bayer using the valine diamide selector of Feibush [23] (Chirasil-Val) [10–12] and the approach was later extended to metal coordination compounds (Chirasil-Metal) [24], modified cyclodextrins (Chirasil-Dex) [24,25] and a chiral crown ether (Chirasil-Crown) [26,27]. Immobilisation of Chirasil-type stationary phases on the inner column wall furnishes versatile chemically bonded chiral stationary phases (CB-CSPs).

3.1. Chiral stationary phases based on hydrogen-bonding

The separation of enantiomers of *N*-trifluoroacetyl amino acid alkyl esters was achieved on *N*-trifluoroacetyl-L-isoleucine lauryl ester **1** (cf. Scheme 1) using a glass capillary column [1] and on *N*-trifluoroacetyl-L-valyl-L-valine cyclohexyl ester **2** (cf. Scheme 1) using a packed column [3]. In subsequent trials with a multitude of CSPs it was recognised that in the dipeptide phase **2** (cf. Scheme 1) the C-terminal amino acid was not critical for chiral recognition while the additional amide function was essential for additional hydrogen bonding. As a consequence, the C-terminal amino acid was abandoned and replaced by a sterically hindered amine yielding the diamide **3** derived from valine [23]. This chiral selector was subsequently coupled via the amino function to a statistical copolymer of dimethylsiloxane and (2-carboxypropyl)methylsiloxane to yield Chirasil-Val **4** [10,11]. The temperature-programmed simultaneous separation of enantiomers of all proteinogenic amino acids on Chirasil-Val **4** in less than 25 min is illustrated in Fig. 1.

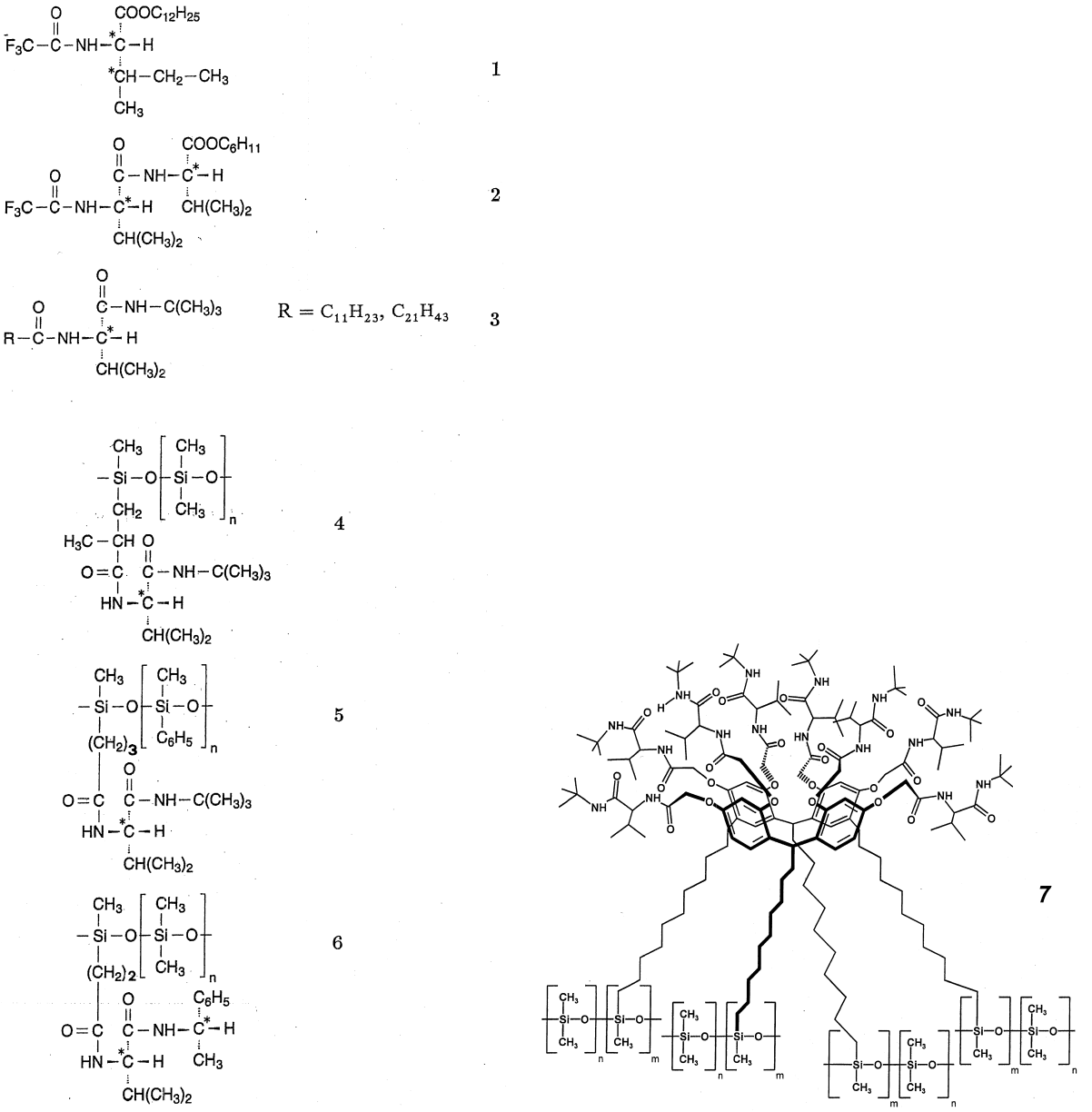
The commercial availability of Chirasil-Val **4** in both enantiomeric forms (L- and D-ChirasilVal) allows peak switching scenarios which are useful in trace enantiomer analysis. In Fig. 2 as little as 0.04% D-enantiomer in L-leucine (as *N*-trifluoroacetyl *O*-methyl ester) was detected on each of the enantiomeric CSPs (cf. Fig. 2) [28]. This important advance offered the possibility of detecting minor racemisation upon peptide hydrolysis and/or amino acid derivatisation and to prove enhancement of enantiomeric purity by crystallisation of amino acids [29] (and references cited therein).

A straightforward approach to polymeric CSPs is based on the modification of cyanoalkyl-substituted polysiloxanes (XE-60, OV-225) [15,30–32]. For instance the diamide **3** was chemically linked to the polysiloxane to give (L)-5 [30,31]. The epimeric CSPs (L,*R* and L,*S*)-6 [15,32] contain two chiral centres which may either enhance enantioselectivity (matched-case) or compensate enantioselectivity (mismatched-case).

Koppenhoefer et al. modified the chiral backbone in Chirasil-Val **4** by variation of the loading and polarity and by the introduction of rigid spacers [33–35]. In Chirasil-Val **4** the chiral moieties are statistically distributed along the polymer chain. A more ordered Chirasil-type CSP has been obtained by block condensation of 1,5-bis(diethylamino)hexamethyltrisiloxane and 2′,2′,2′-trifluoroethyl-(3-dichloromethylsilyl)-2-methylpropionate followed by nucleophilic displacement of the functionalised polysiloxane with chiral amines and amino acids [36]. The immobilisation of the CSPs by thermal [36] and radical-mediated cross-linking [37,38] has been studied and the extent of radical-induced racemisation was determined.

A highly ordered supramolecular structure has recently been prepared by linking chiral L-valine *tert*.-butylamide moieties to the eight hydroxy groups of a resorc[4]arene basked-type structure obtained from resorcinol and 1-undecanal. The calixarene was subsequently chemically linked via four spacer units to a dimethylpolysiloxane to give Chirasil-Calix **7** (cf. Scheme 1) [39]. Surprisingly, the ordered cyclic arrangement of the chiral selectors, juxtaposed in close proximity to each other, did not markedly improve enantioselectivity as compared to Chirasil-Val **4** and aromatic amino acid derivatives did not appear to exhibit any beneficial effect due to supramolecular inclusion. The synthesis of thiacalix[4]arenes with pendant chiral amines and their application as CSP for the separation of enantiomers of derivatised amino acids, alcohols and amines has also been described recently [40,41].

There have been many efforts to unravel the mechanism of chiral recognition in hydrogen-bonding selector–selectand systems [42] (and references cited therein). More recently, Gil-Av and co-workers studied the retention behaviour of enantiomers on the



Scheme 1. Hydrogen-bonding type chiral stationary phases.

binary mixture of *N*-lauroyl-L-valine *tert*.-butylamide in squalane as a function of self-association of the selector in the oligomeric pleated sheet β-structure of the diamide [43,44].

On L-amino acid-derived selectors D-amino acid derivatives usually elute before the L-enantiomers [14,15]. The assignment of absolute configurations (cf. Section 5) may however be obscured by the possibility of a temperature-dependent inversion of the elution order of the enantiomers [45,46] due to enthalpy/entropy compensation [47] or due to multimodal chiral recognition mechanisms.

Separation of enantiomers by hydrogen-bonding CSPs usually requires derivatisation of the analyte (e.g., amino acids) in order to increase volatility and/or to introduce suitable functions for additional

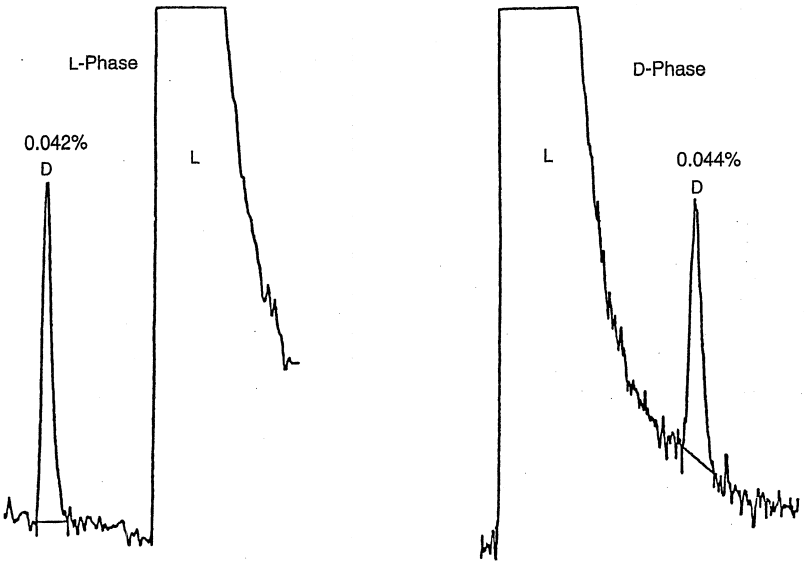


Fig. 2. Trace analysis of enantiomers of D-*N*-trifluoroacetyl-leucine *O*-methyl ester in L-enantiomer on L- and D-Chirasil-Val **4**. Left; column: 20 m×0.25 mm I.D. fused-silica capillary, 95°C, 0.3 bar hydrogen (split 100:1). Right; column: 25 m×0.25 mm I.D. fused-silica capillary, 110°C, 0.4 bar hydrogen, split 100:1 (reproduced with permission from Ref. [28]).

hydrogen-bonding association as well as for refined detection purposes (ECD) [14,15].

3.2. Chiral stationary phases based on metal coordination

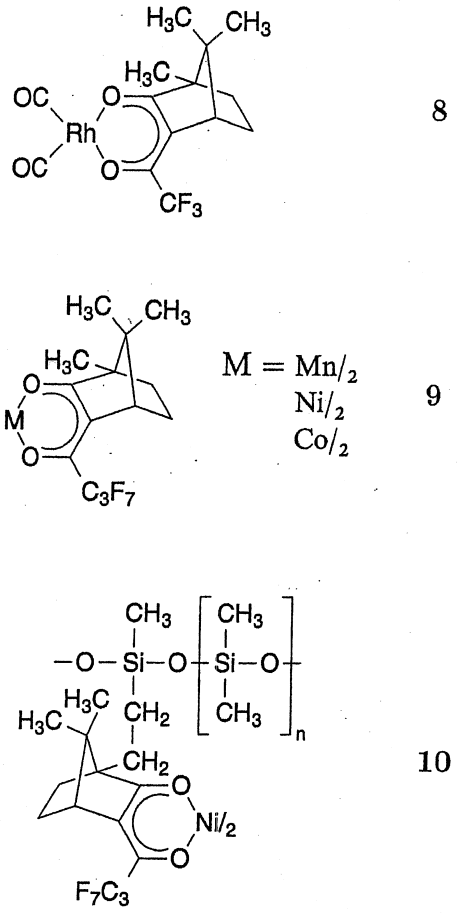
The chiral metal coordination compound dicarbonyl rhodium(I)-3-trifiuoroacetyl-(1*R*)-camphorate **8** (cf. Scheme 2) dissolved in squalane was used for the separation of the enantiomers of the chiral olefin 3-methylcyclopentene by complexation gas chromatography in 1977 [48] following earlier unsuccessful experiments which started in 1969 [49]. The separation of the enantiomers was corroborated by employing both enantiomeric selectors obtained from 1*R*- and 1*S*-camphor, respectively, resulting in peak inversion and by employing the racemic selector resulting in peak coalescence (during the course of these investigations it was found unexpectedly that racemic **8** forms dichroic red–green crystals while the pure enantiomers of **8** form yellow crystals, this being the first case of chirodichroism reported [50]).

The scope of separation of enantiomers by complexation gas chromatography was later extended to chiral oxygen-, nitrogen- and sulfur-containing compounds using various chiral 1,3-diketonate *bis* chelates of manganese(II), cobalt(II) and nickel(II) derived from perfluoroacylated terpene-ketones such as camphor **9**, 3- and 4-pinanone, thujone, nopinone, menthone, isomenthone, carvone and pulegone [16,51]. The simultaneous separation of the eight stereoisomers of *sec*-butyloxirane, containing three chiral centres, is shown in Fig. 3 [7,52].

A limiting factor of coordination-type CSPs **8** and **9** is the low temperature range of operation (25–120°C). The thermostability was therefore increased by the preparation of immobilised polymeric CSPs (Chirasil-Nickel) **10** (cf. Scheme 2, the 1*S*-configuration is depicted) [24]. Chirasil-Nickel **10** has also been used as CSP in supercritical fluid chromatography with carbon dioxide as mobile phase [53,54].

Before the advent of modified cyclodextrins, complexation gas chromatography represented a very useful tool for chiral analysis of volatile non-hydrogen-bonding compounds such as pheromones [55], flavours and fragrances [55] and educts and products of enzymatic reactions such as oxiranes [56].

Enantioselective complexation gas chromatography was also extensively investigated in terms of inherent principles of chiral recognition. Thus, four enantioselective processes [52,57] and six peak coalescence phenomena [57,58] were distinguished



Scheme 2. Coordination type chiral stationary phases.

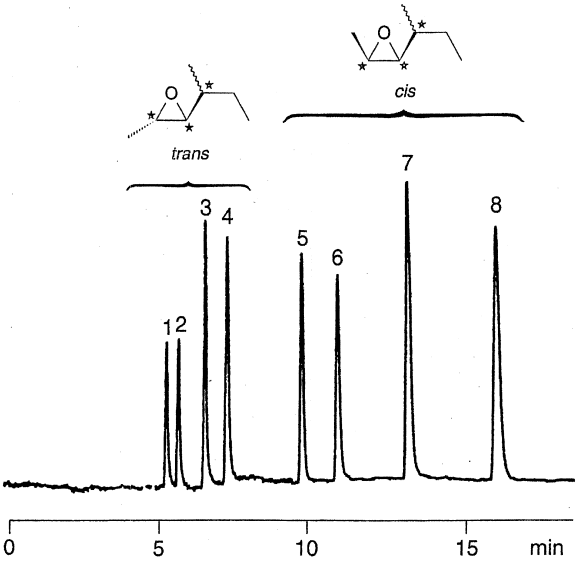


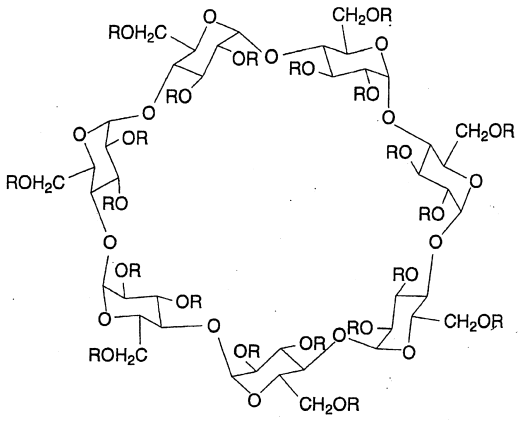
Fig. 3. Separation of eight stereoisomers of *sec*-butyloxirane by complexation gas chromatography on nickel(II) bis[3-(heptafluorobutanoyl)-10-ethylidene)-(1*S*)-camphorate] [a derivative of **9**] (0.125 molal in OV-101) at 90°C. Column: 25 m×0.25 mm I.D. glass capillary (reproduced with permission from Refs. [7,52]). Bold and dotted lines in the formulas represent *cis*/*trans*-isomerism and do not imply absolute configurations.

in enantioselective complexation gas chromatography. Moreover, the existence of the isoenantioselective temperature as a result of enthalpy/entropy compensation [47] has been verified [52,57,59] (cf. Section 4) and the first instance of a peak profile featuring plateau formation due to enantiomerisation (cf. Section 7) was detected for 1-chloro-2,2-dimethylaziridine in complexation gas chromatography [16,60].

3.3. Chiral stationary phases based on (inter alia) inclusion

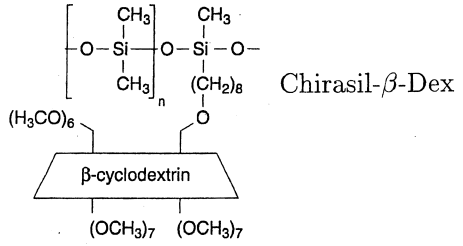
The separation of enantiomers of the apolar racemic hydrocarbons α- and β-pinene on packed columns coated with a mixture of native α-cyclodextrin and formamide was demonstrated by Kościelski et al. in 1983 [61,62]. Despite a large separation factor α, the columns had a limited life time and efficiency was poor. In another pioneering advance, Juvancz et al. [63,64] and later Venema and Tolsma [65] demonstrated that undiluted permethylated β-cyclodextrin [heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin, **11**] (cf. Scheme 3) can be employed in capillary columns for high-resolution separation of enantiomers at high temperatures or at ambient temperatures in a supercooled state, respectively [65]. Subsequently, to overcome the problems associated with the high melting point of permethylated cyclodextrin, two different strategies were pursued:

(i) Schurig and Nowotny dissolved permethylated β-cyclodextrin in moderately polar polysiloxanes (e.g., OV-1701) [66,67]. By diluting the chiral selector, the inherent enantioselectivity of the modified cyclodextrin was combined with the unique gas chromatographic properties of polysiloxanes. Moreover, any modified cyclodextrin which can be dis-



$R = CH_3$
heptakis(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin **11**

$R = C_5H_{11}$, heptakis(2,3,6-tri-*O*-pentyl)-*β*-cyclodextrin **14**



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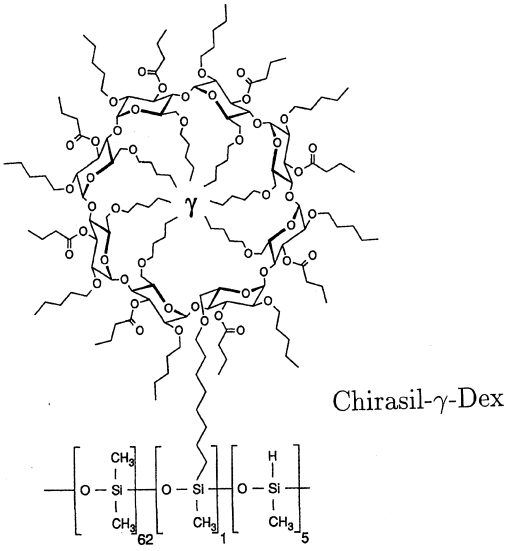

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Scheme 3. Inclusion type chiral stationary phases. Depending on the reaction conditions the octamethylene spacer in **25** and **26** may also orginate from the 2-position.

solved may be employed for gas chromatographic separation of enantiomers irrespective of its melting point or any phase transition it may undergo at a certain temperature. The simultaneous separation of enantiomers of different classes of compounds used as a test mixture [68] is depicted in Fig. 4.

The method of dilution is nowadays employed routinely and columns thus prepared are commercially available from most vendors of gas-chromatographic equipment.

(ii) König and co-workers found that per-*n*-pentylated cyclodextrins are liquid at room temperature. The related cyclodextrin derivatives (trade name Lipodex) listed below can thus be applied in the undiluted form for the separation of enantiomers of many classes of compounds employing deactivated glass or fused-silica capillary columns [20,69–72]:

hexakis(2,3,6-tri-O-*n*-pentyl)-α-cyclodextrin **12**

hexakis(3-*O*-acetyl-2,6-di-*O*-*n*-pentyl)-α-cyclodextrin **13**

heptakis(2,3,6-tri-*O*-*n*-pentyl)-β-cyclodextrin **14**

heptakis(3-*O*-acetyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin **15**

octakis(2,3,6-tri-*O*-*n*-pentyl)-γ-cyclodextrin **16**

octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin **17**

The presence of three hydroxyl groups which can be regioselectively alkylated and acylated offers an enormous number of possible α-, β-, γ-cyclodextrin derivatives (cf. Table 6 in Ref. [22]) which are not always readily accessible and may require tedious purification steps. Occasionally, cyclodextrin derivatives such as octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E) **17** are highly enantioselective for the gas-chromatographic separation of enantiomers of a wide array of classes of compounds [73].

The following more polar cyclodextrin derivatives coated on fused-silica capillary columns have been applied by Armstrong and co-workers [74–77]:

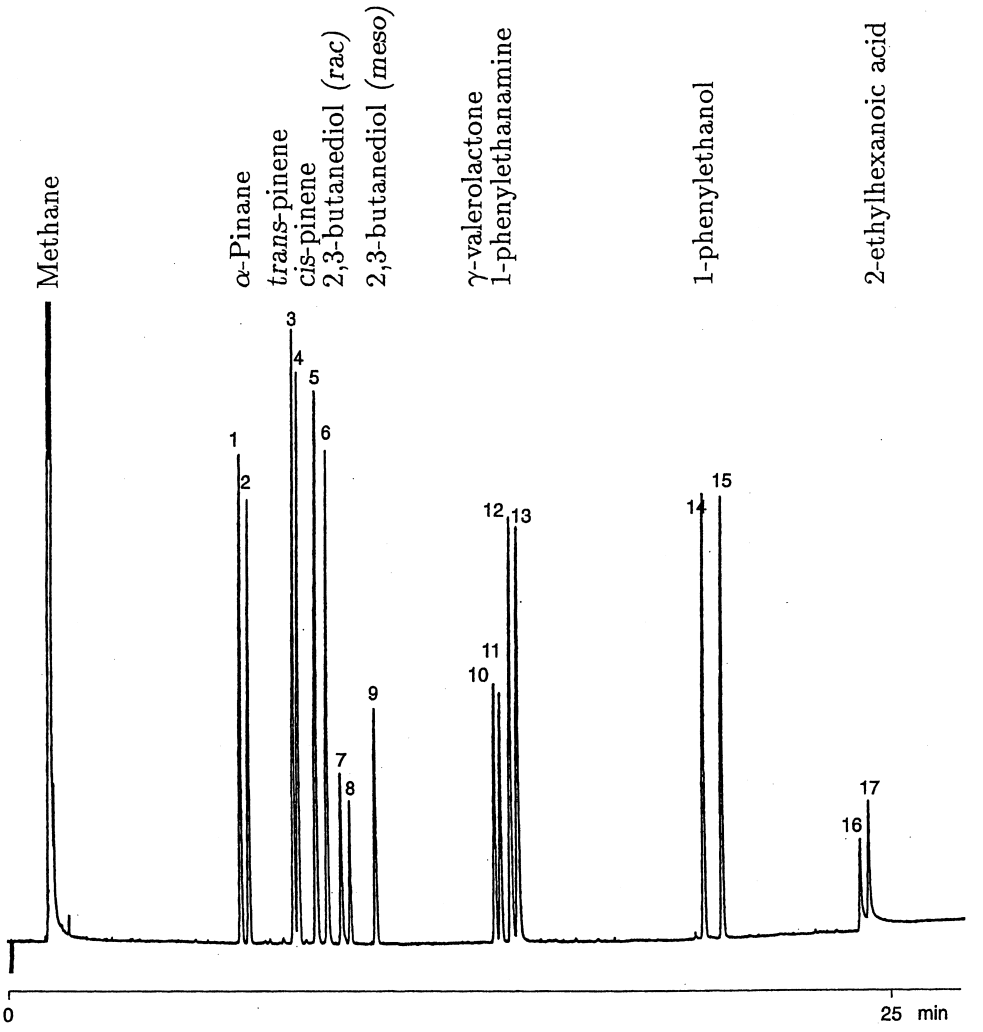

Fig. 4. Separation of the enantiomers of a test mixture [68] comprising of α-pinene (1, 2), *trans*-pinane (3, 4), *cis*-pinane (5, 6), 2,3-butanediol (rac) (7, 8), 2,3-butanediol (meso) (9), γ-valerolactone (10, 11), 1-phenylethylamine (12, 13), 1-phenylethanol (14, 15) and 2-ethylhexanoic acid (16, 17) by gas chromatography on permethylated β-cyclodextrin **11** dissolved in OV-1701 (10%, w/w, in OV 1701, 0.25 μm). Column: 25 m×0.25 mm I.D. fused-silica capillary. 70°C followed by 3°C/min, 0.65 bar hydrogen (reproduced with permission from Varian (Chrompack International), Middelburg, The Netherlands).

hexakis(per-*O*-(*S*)-2-hydroxypropyl)-per-*O*-methyl)-α-cyclodextrin (PMHP-α-CD) **18**

heptakis(per-*O*-(*S*)-2-hydroxypropyl)-per-*O*-methyl)-β-cyclodextrin (PMHP-β-CD) **19**

hexakis(2,6-di-*O*-*n*-pentyl)-α-cyclodextrin (dipentyl-α-CD) **20**

heptakis(2,6-di-*O*-*n*-pentyl)-β-cyclodextrin (dipentyl-β-CD) **21**

heptakis(3-*O*-trifluoracetyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin (DPTFA-β-CD) **22**

The introduction of bulky substituents, e.g., the *tert*.-butyldimethylsilyl (TBDMS) group [78] (a typical

protecting group for synthetic purposes) at the primary C6-hydroxy groups influences the conformation of the cyclodextrin and blocks the entrance to the cavity at the smaller rim which in turn has a strong impact on enantioselectivity. Thus, heptakis-(2,3-di - *O* - acetyl-6-*O*-*tert*.-butyldimethylsilyl)-β-cyclodextrin **23** and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*.-butyldimethylsilyl)-β-cyclodextrin **24** have been employed as useful complementary CSPs by Dietrich et al. [79,80] (cf. Fig. 5).

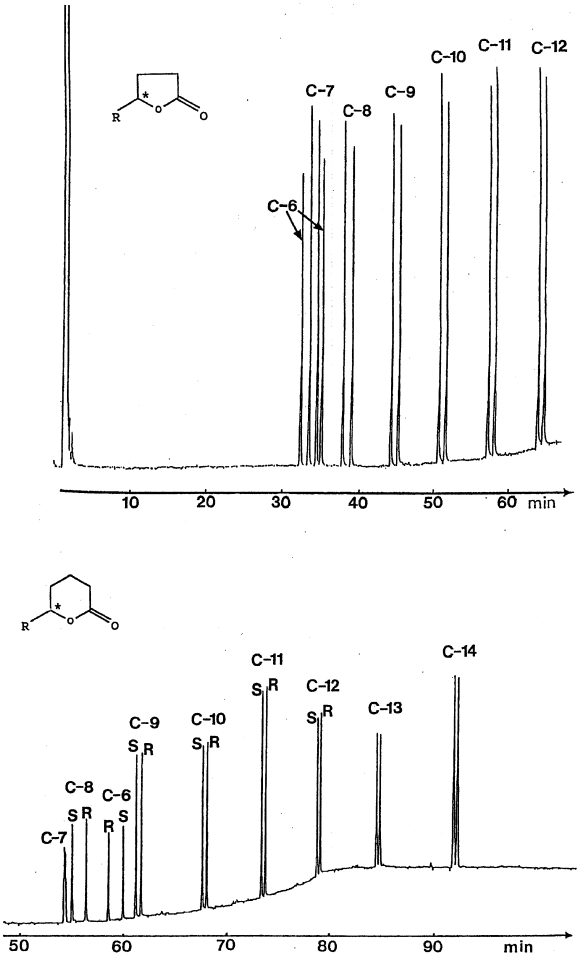

Fig. 5. Separation of the enantiomers of γ-lactones (top) and δ-lactones (bottom) by gas chromatography on 50% heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*.-butyldimethylsilyl)-β-cyclodextrin **23** in OV-1701(Vi) (0.25 μm). Column: 35 m×0.23 mm I.D. (top) and 50 m×0.23 mm I.D. (bottom) glass capillary, carrier gas hydrogen (165 kPa), temperature-programming (reproduced with permission from Ref. [79]).

A combination of the advances (i) and (ii), i.e., the dissolution of various cyclodextrin derivatives in polysiloxanes, e.g., OV-1701, represents the most useful approach to separation of enantiomers. In diluted systems the chiral separation factor α becomes concentration-dependent [81,82]. It has been inferred by theoretical considerations, and verified by experiments, that α does not linearly increase with the concentration of the cyclodextrin derivative in the polysiloxane [83]. The experimental data imply that the optimum is often reached at low concentrations and, consequently, no further improvement of enantioselectivity is gained above a cyclodextrin weight percentage of approximately 30% for permethylated β-cyclodextrin or 50% for derivatives with high molecular masses, i.e., γ-cyclodextrins containing *n*-pentyl groups. Thus, the use of undiluted modified cyclodextrins [20] is more and more discontinued.

As proposed by Schurig and Nowotny [66], an obvious extension of the diluted system consists in the fixation of the enantioselective cyclodextrin selector to a polysiloxane backbone by a permanent chemical linkage (analogous to Chirasil-Val **4**) an approach which was later realised [24,25,84]. The thermal immobilisation of the resulting Chirasil-Dex stationary phase **25** (cf. Scheme 3) on the fused-silica surface represented another refinement of this approach [85–87].

Dependent on the reaction conditions, the monooctamethylene spacer may be linked to the 2- or 6-position in one glucose unit of the cyclodextrin. Unambiguous regioselective substitution at the 6-position employing protecting group chemistry was advanced by Donnecke et al. [87]. The immobilised Chirasil-Dex stationary phases are resistant to column bleeding, compatible with solvent intake (and thus amenable to on-column injection techniques) and insensitive to temperature shock. The temperature range extends from −25°C to 250°C. Low operating temperatures are required, e.g., for the separation of enantiomers of chiral molecules consisting of only few atoms. Thus, bromochlorofluoromethane was separated into enantiomers by König on **12** at 20°C [20] (cf. Fig. 6, bottom) and by Grosenick et al. on Lipodex E **17** [73] which was chemically bonded to dimethylpolysiloxane (Chirasil-γ-Dex) **26** (Scheme 3) [88] at −22°C [89].

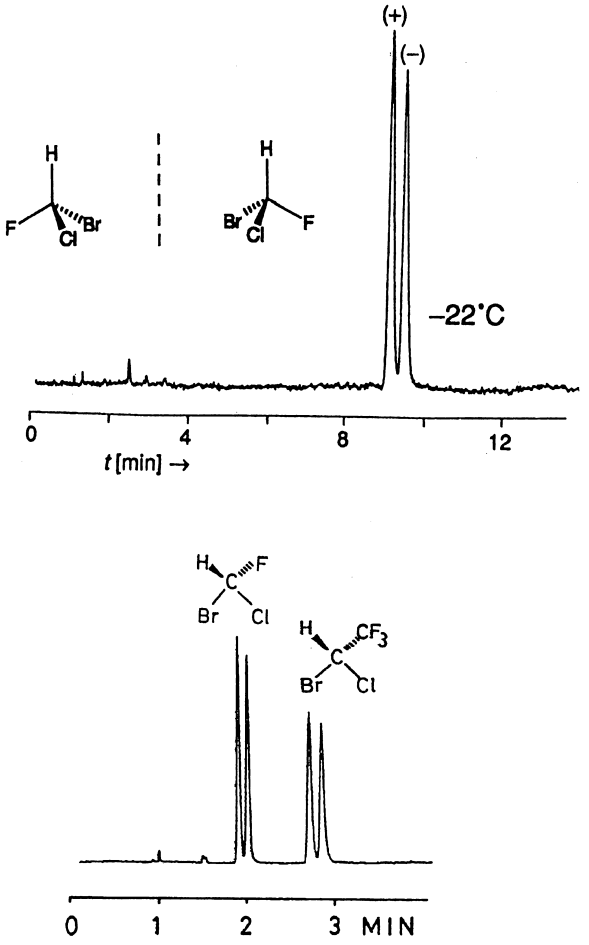

Fig. 6. Separation of the enantiomers of bromochlorofluoromethane at −22°C by gas chromatography on Chirasil-γ-Dex **26** obtained from Lipodex E **17** [73] (0.18 μm). Column: 10 m×0.25 mm I.D. fused-silica capillary, carrier gas hydrogen (50 cm/s) (reproduced with permission from Ref. [89]) (top) and of bromochlorofluoromethane and halothane at 20°C by gas chromatography on neat hexakis(2,3,6-tri-*O*-*n*-pentyl)-α-cyclodextrin (Lipodex A) **12**. Column: 25 m fused-silica capillary, carrier gas hydrogen (50 cm/s) (reproduced with permission from Ref. [20]) (bottom).

The polysiloxane matrix obviously kept the CSP in a liquid state at the cryogenic condition employed (cf. Fig. 6, top).

Interesting variants of Chirasil-Dex-type CSPs **25** have also been described [90–93]. Fused-silica columns coated with Chirasil-β-Dex **25** bear advantages such as:

- Use of a nonpolar polysiloxane matrix (in which cyclodextrin derivatives cannot be physically diluted) resulting in low elution temperatures for polar analytes
- High degree of inertness allowing analysis of polar compounds without prior derivatisation
- Higher cyclodextrin concentration resulting in increased separation factors α
- Long-term stability with absence of droplet formation leading to breakdown of efficiency
- Compatibility with all injection techniques
- Immobilisation by crosslinking and/or surface bonding

The immobilisation of Chirasil-β-Dex **25** is a prerequisite for separation of enantiomers of involatile enantiomers with solvating mobile phases in supercritical fluid chromatography (SFC), open-tubular liquid chromatography (o-LC) and open-tubular electrochromatography (o-CEC) [86,94,95]. In Fig. 6 a new example of the unified approach employing a single column for the separation of enantiomers of 1-(2-naphthyl)-ethanol by four different chromatographic methods is demonstrated [96] (cf. Fig. 7).

Gas chromatographic columns coated with modified cyclodextrins are applied for enantiomer analysis in many different fields of contemporary research such as the authenticity control of essential oils, flavours and fragrances [79,97,98] (and references cited therein), alcoholic beverages [99,100], clinical chemistry [101], terpenoids [102], enzymatic reactions [103], organochlorine pesticides [104,105] (cf. Fig. 8) and alkyl nitrates as atmospheric constituents [106] (cf. Fig. 9).

Reasonably volatile pharmaceutical compounds can also be investigated [18,20]. In Fig. 10 the separation of the enantiomers of derivatised amino acids on Chirasil-γ-Dex **26** (Scheme 3) is shown [13]. There are some marked enantioselectivity differences as compared to the use of Chirasil-Val **4** (cf. Fig. 1).

3.4. Mechanistic considerations on enantioselectivity involving modified cyclodextrins

The rationalisation of chiral recognition involving modified cyclodextrins is difficult since almost all classes of chiral compounds, ranging from apolar to highly polar (even including metal coordination compounds) are susceptible to separation of enantio-

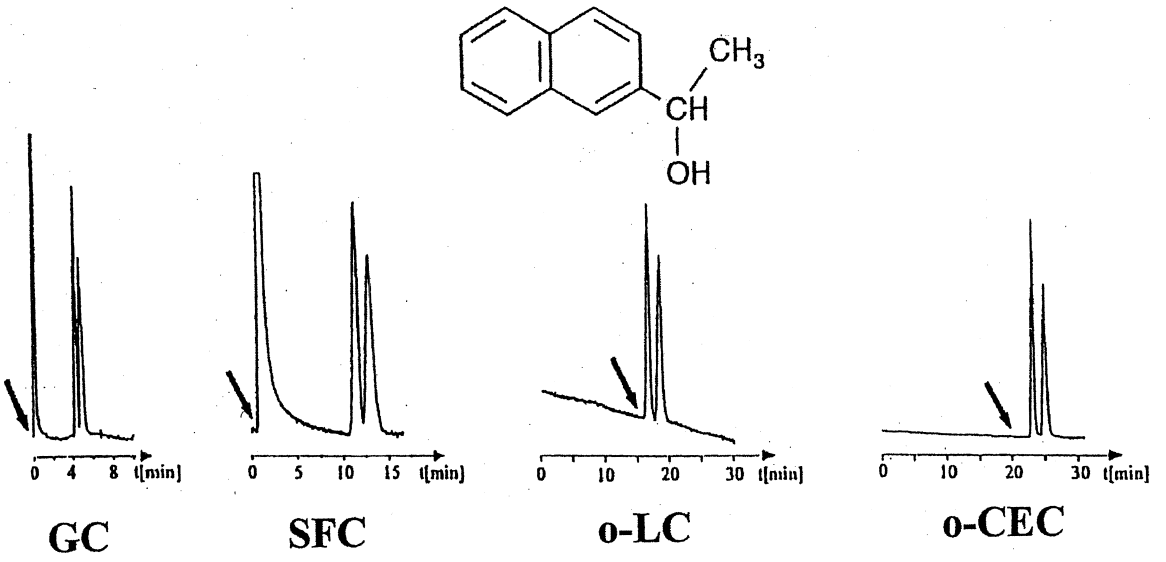

Fig. 7. Unified enantioselective chromatography. Separation of the enantiomers of 1-(2-naphthyl)-ethanol on Chirasil-β-Dex **25** (0.14 μm) by GC, SFC, o-LC and o-CEC. Column: 100 cm×0.05 mm I.D. fused-silica capillary. GC: $T=120$°C, $p=1.1$ bar (He); SFC: $p=68$ (CO_2), $T=55$°C; o-LC: $p=0.14$ bar, $T=35$°C 20 m*M* phosphate buffer, pH 7; o-CEC: $U=30$ kV, 10 m*M* borate/phosphate buffer, pH 7.5, $T=60$°C. The arrows in the chromatograms indicate the approximate breakthrough (dead) times t_M (reproduced with permission from Ref. [96]).

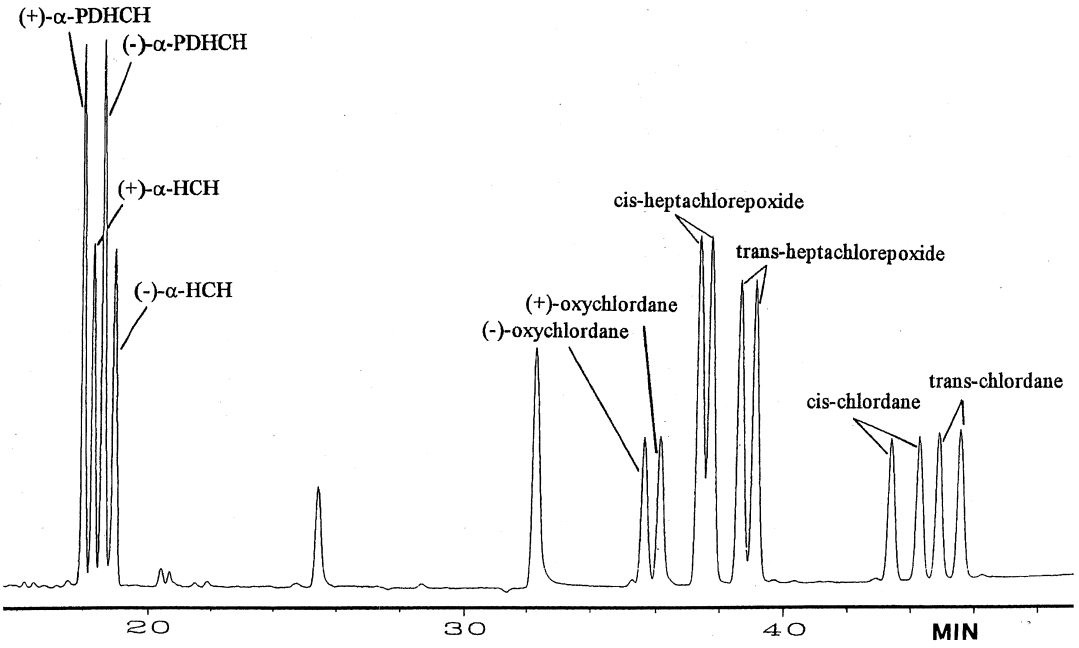

Fig. 8. Separation of the enantiomers of a multicomponent mixture of organochlorine pesticides (HCH=hexachlorocyclohexane, PDHCH=pentachlorocyclohexene) on 35% heptakis(2,3-di-*O*-methyl-6-*O*-*tert*.-butyldimethylsilyl)-β-cyclodextrin **24** in OV-1701 (0.15 μm). Column: 20 m×0.25 mm I.D. fused-silica capillary, carrier gas nitrogen, temperature-programming (reproduced with permission from Ref. [105]).

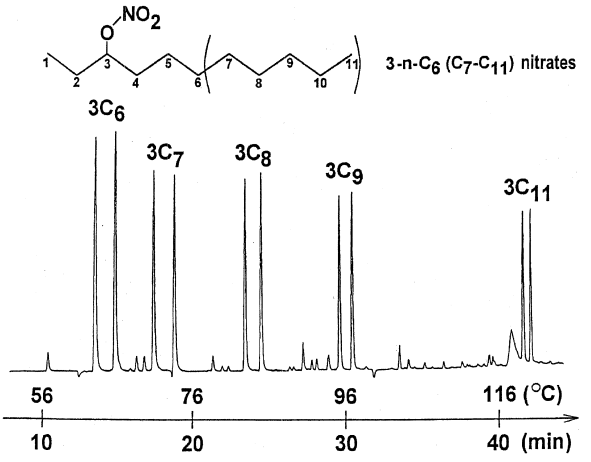

Fig. 9. Separation of the enantiomers of homologous 3-*n*-alkyl nitrates on neat heptakis(3-*O*-acetyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin (Lipodex D) **15**. Column: 25 m×0.25 mm I.D. fused-silica capillary, carrier gas hydrogen (55 cm/s at 70 kPa), temperature-programming (reproduced with permission from Ref. [106]).

mers on a certain cyclodextrin-derived CSP, often with no logical dependence on molecular shape, size and functionalities of the selectand and the selector (α, β, γ). Obviously multimodal recognition processes, which may involve inter alia inclusion, hydrogen-bonding, dispersion forces, dipole–dipole interactions, electrostatic interactions and hydrophobic interactions, are important [6,18,107,108]. Since separation of enantiomers has also been observed with per-*n*-pentylated amylose [109], molecular inclusion into the cavity may not be a prerequisiste for chiral recognition with modified cyclodextrins. Due to the high resolving power of capillary gas chromatography, Gibbs free energy differences as low as 0.02 kcal/mol corresponding to a separation factor of only $\alpha = 1.02$ at ambient temperature already lead to a quantitative separation of enantiomers [14]. Consequently, it does not come as a surprise that at such minute energy differences of the diastereomeric selector–selectand association equilibria, changes of the elution order in homologous series of compounds are frequently observed [110]. Consequently, predictions of absolute configuration vs. elution order are often arbitrary. Since the error of molecular calculations of enantioselectivity may be in the range of up to 1 kcal/mol, corresponding to a separation factor $\alpha \gg 2$, in the first modelling study of a gas chromatographic separation of enantiomers only inclusion was proved [18] (cf. page 953). Subsequent accounts [111–113] must be treated with some caution as far as low separations factors α are involved and experimentally observed elution orders are predicted. Köhler and co-workers, however, modelled a system of high enantioselectivity, i.e., the enantiomers of methyl 2-chloro-propanoate on **16,** and the authors could corroborate the data by nuclear magnetic resonance (NMR) measurements [114,115].

Important contributions to the molecular modelling of cyclodextrin-mediated separation of enantiomers were made by Lipkowitz et al. [116–118]. These authors stressed the dichotomy between the location of the preferred binding site of a selectand within the cavity and the location of the optimum chiral discrimination domain which are a priori not necessarily identical. It was also proposed that short-range dispersion forces are important as intermolecular forces.

It should be noted that a strong molecular association is not always a prerequisite to efficient chiral discrimination [18]. Often a weak selectand–selector interaction (e.g., for chiral saturated hydrocarbons) can lead to appreciable chiral recognition. It may even be predicted that one enantiomer is included in the cyclodextrin cavity while the other enantiomer is excluded for steric reasons thereby producing a very high separation factor α. The largest chiral discrimination of modified cyclodextrins is exhibited toward halogenated hydrocarbons, i.e., 2-haloalkanes [107], alkyl 2-haloalkanoates [77,114] and various chlorinated/fluorinated ethers (inhalation anesthetics) [81,119] (cf. Fig. 11). A recent case involves the unprecedented high $\alpha = 8$ in enantioselective gas chromatography of a breakdown product of the inhalation anaesthetic desflurane, i.e., CH_3OCF_2-$CH(CF_3)OCH_2F$ on Chirasil-γ-Dex **26** (Scheme 3) [120].

4. Thermodynamics of separation of enantiomers by gas chromatography

Scrutiny of the mechanism of chiral recognition should always be based on reliable experimental

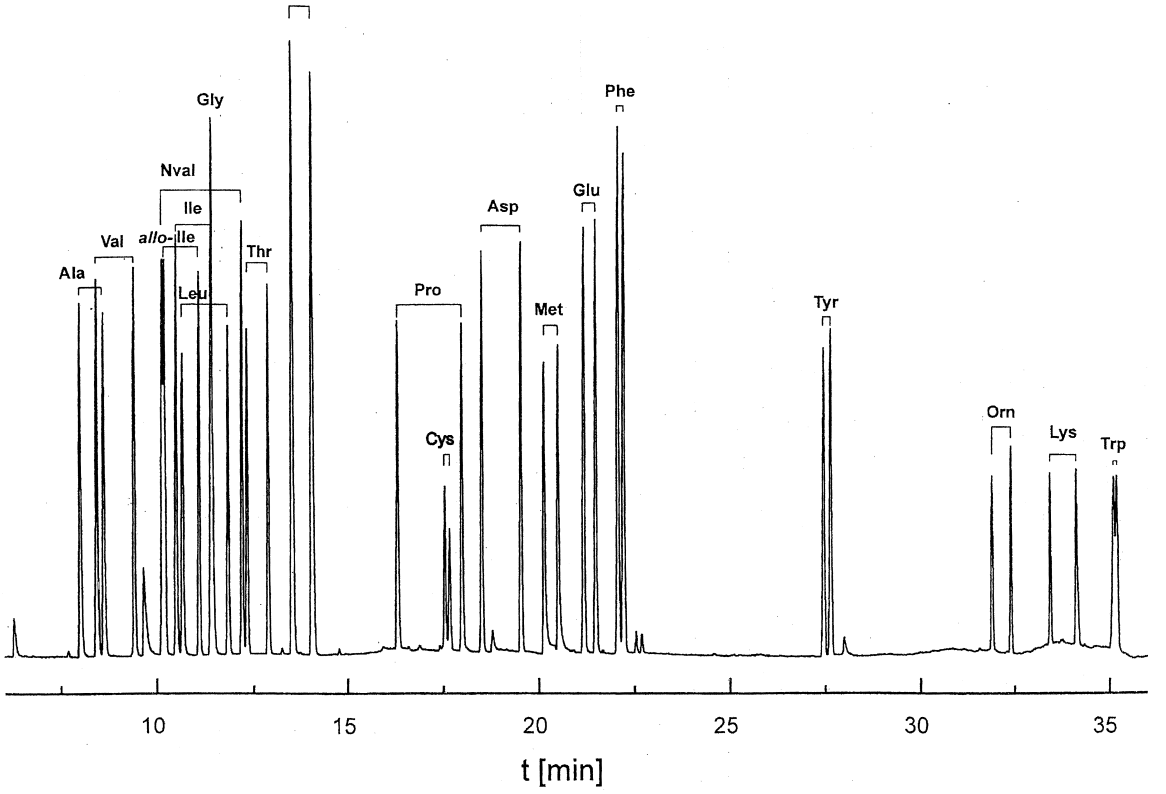

Fig. 10. Separation of the enantiomers of amino acids as *N*-trifiuoroacetyl ethyl esters on immobilised Chirasil-γ-Dex **26** obtained from Lipodex E **17** [73] (0.2 μm). Column: 25 m×0.25 mm I.D. fused-silica capillary. Temperature program: 65°C, 2 min isothermal, 3.5°C/min to 180°C. Carrier gas hydrogen (60 kPa). Arginine is not eluted. Histidine was not subjected to further derivatisation at the cyclic nitrogen, hence, it is also not eluted. All L-amino acids are eluted after the D-amino acids except for Pro and Thr with a reversed order (reproduced with permission from Ref. [13]).

thermodynamic parameters. Such data may easily be acquired from the measurement of gas chromatographic retention data [14,81–83,121–123].

Separation of enantiomers by gas chromatography on CSPs is based on fast kinetics and is governed by thermodynamics [42]. It results from the difference in the Gibbs free energy $-\Delta_{R,S}(\Delta G)$ of the diastereomeric association equilibria between the enantiomers (selectand) and the CSP (selector). The chemical association equilibria in the stationary phase are described by the thermodynamic association constants K_R and K_S, with R arbitrarily referring to the second eluted enantiomer and S to the first eluted enantiomer. For separation of enantiomers, the Gibbs–Helmholtz equation (Eq. (1)) applies:

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

For a 1:1 molecular association, the quantities $\Delta_{R,S}(\Delta S)$ and $\Delta_{R,S}(\Delta H)$ display an opposite effect on $\Delta_{R,S}(\Delta G)$. At the isoenantioselective temperature T_{iso}:

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

peak coalescence occurs [$\Delta_{R,S}(\Delta G)=0$, $K_R=K_S$, no separation of enantiomers]. Above T_{iso} the sign of enantioselectivity changes, leading to peak inversion (peak reversal). Below the coalescence temperature,

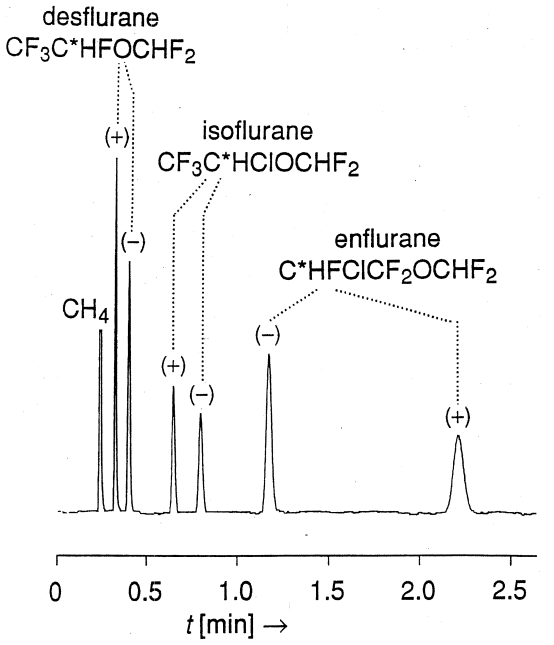

Fig. 11. Separation of the enantiomers of the inhalation anesthetics desflurane, isoflurane and enflurane by gas chromatography on immobilised Chirasil-γ-Dex **26** obtained from Lipodex E **17** [73] (0.18 μm) at 28°C. Column: 10 m×0.25 mm I.D. fused-silica capillary (reproduced with permission from Ref. [119]).

the sign of enantioselectivity $\Delta_{R,S}(\Delta G)$ is governed by $\Delta_{R,S}(\Delta H)$ and above it by $\Delta_{R,S}(\Delta S)$. In most cases, even at high temperatures, enantioselectivity is dominated by enthalpy-control and separation factors α increase with decreasing temperature. Therefore, it is recommended to use the lowest possible temperature for separation of enantiomers by gas chromatography. In order to lower the elution temperature, short columns are recommended whereby the loss of efficiency is outweighed by a gain in selectivity (cf. Section 9). In few rare cases, a temperature-dependent reversal of enantioselectivity in gas chromatographic separation of enantiomers on a single CSP arising from enthalpy/entropy compensation or from multimodal chiral recognition mechanisms has been observed [45,46,52,57,59,124,125].

For undiluted CSPs, the quantity $-\Delta_{R,S}(\Delta G)$ can directly be obtained from the separation factor α_{undil} according to Eq. (3) [106,107]:

$$-\Delta_{R,S}(\Delta G) = RT \ln \alpha_{\text{undil}} \tag{3}$$

whereas for diluted systems $-\Delta_{R,S}(\Delta G)$ is obtained according to Eq. (4) (note that α_{dil} becomes concentration-dependent [83]):

$$-\Delta_{R,S}(\Delta G) = RT \ln \frac{K_R}{K_S} = RT \ln \frac{R'_R}{R'_S} \tag{4}$$

with:

$$R' = \frac{t'_{\text{R}} - t'^{\,0}_{\text{R}}}{t'^{\,0}_{\text{R}}} = K\,a \tag{5}$$

where $t^{0\prime}_{\text{R}}$ = adjusted retention time of the enantiomer (R or S) measured with a reference column without CSP and t'_{R} adjusted retention time of the enantiomer (R or S) measured with the reactor column containing the CSP in a solvent (polysiloxane), K = thermodynamic association constant, a_{A} activity (or concentration at high dilution) of the CSP in the solvent.

5. Assignment of absolute configurations by enantioselective gas chromatography?

The determination of absolute configurations of chiral compounds is an important task in enantiomer analysis. Absolute configurations of minute amounts of chiral samples may be determined *directly*, and free of chiroptical evidence, by gas chromatography *via* coinjection of a reference compound of known stereochemistry. In complexation gas chromatography, absolute configurations have been predicted *indirectly* by empiric rules which correlate the absolute configuration and the order of elution for enantiomers belonging to homologous series of compounds such as oxiranes [126]. Although consistent relationships between the order of elution and absolute configuration of related compounds have been observed in complexation gas chromatography and in hydrogen-bonding systems, e.g., for amino acids (usually D before L on an L-selector) [14,15], remarkable inconsistencies are also known [16]. As a rule, such comparisons, if any, should be restricted to measurements at the same temperature since peak reversals may occur at different temperatures as the result of enthalpy vs. entropy compensation (cf. Section 4) or multimodal chiral recognition mechanisms (cf. Section 3.4). Thus, the indirect assignment

of absolute configurations by gas chromatography may be ambiguous.

6. The method of enantiomer labelling

The amount of enantiomers in complex matrices can be determined by enantioselective gas chromatography employing the method of ''enantiomer labelling'' [127,128]. In the absence of diastereomeric effects between enantiomers in nonideal solutions (''EE-effect'' [129]), the enantiomeric ratio er (ee or ec; for definitions cf. Ref. [5]) is not influenced by sample manipulations (achiral derivatisation, dilution, injection, detection, chemical and physical losses). With the method of ''enantiomer labelling'' [128] a known quantity of an enantiomerically pure standard is added to the mixture (or an aliquot of it) and the amount of the enantiomer originally present is calculated from the change of er after the addition of the standard. The method of enantiomer labelling presupposes the precise knowledge of the enantiomeric ratios, er, of the sample and the standard.

The following relations apply [21]:

(i) For enantiomer labelling with a pure enantiomer: A (mg) $=(\mathrm{er}\cdot C)/(\mathrm{er}'-\mathrm{er})$; B (mg) $=A$ (mg)/er.

(ii) For enantiomer labelling with the racemate: A (mg) $=\{(1-\mathrm{er}')/[(\mathrm{er}'/\mathrm{er})-1]\}\cdot C/2$; B (mg) $=[(1-\mathrm{er}')/(\mathrm{er}'-\mathrm{er})]\cdot C/2$.

with: er, measured before addition of the label; er′, measured after addition of the label; A = amount of major enantiomer; B = amount of minor enantiomer; C = amount of label (all in mg).

The quantitative determination of the inhalation anaesthetic isoflurane in blood samples during and after surgery using enantiomer labelling has been performed by enantioselective headspace GC–MS [130]. A comparative study of enantiomer and isotopic labelling in enantiomer analysis has been carried out with gas–liquid chromatography–MS in the SIM mode [131].

7. Enantiomerisation studies by dynamic gas chromatography

When enantiomers invert their configuration (or conformation) during the time scale of gas chromatographic separation, transient elution profiles are obtained which are characterised by plateau formation between the terminal peaks of the enantiomers. This phenomenon was predicted [132] and subsequently observed for the enantiomers of the spiroketal 1,6-dioxaspiro[4.4]nonane [16] and the invertomers of 1-chloro-2,2-dimethylaziridine by complexation gas chromatography [16,60]. The term ''enantiomerisation'' [16] was introduced to unambiguously describe the process of a reversible monomolecular interconversion of one enantiomer into the other by inversion of configuration (or conformation) [60]. The common term racemisation, i.e., the macroscopic interconversion of one enantiomer into the racemate [133], was considered inappropriate in this context since in fact a racemic mixture was investigated. Furthermore, enantiomerisation in chromatography was linked with the term ''dynamic'' [16,60] to indicate a process of interconversion as a special case of reaction gas chromatography [134].

It was stated previously that plateau-formation in chromatographic separation of enantiomers represents a diagnostic tool [60] for the recognition of the lability of stereogenic elements (chiral centres, chiral axes, chiral planes) requiring only minute amounts of the racemic mixture instead of isolated enantiomers. The methodology gained recent interest in connection with the legislation of chiral drugs. Thus, the US Food and Drug Administration (FDA) requires that

> *the stability protocol for enantiomeric drug substances and drug products should include a method or methods capable of assessing the stereochemical integrity of the drug substance and drug product*

[135].

The barrier of enantiomerisation ($\Delta G^{\ddagger}$) can be determined by dynamic gas chromatography via peak form analysis of interconversion profiles and by the comparison of experimental and simulated chromatograms [60]. Very recently a fast computer program (ChromWin99) has been developed permitting the determination of rate constants of enantiomerisation by peak-form analysis of chromatographic interconversion profiles on a personal computer [136] thus replacing the former program Simul which required excessive calculation times [137].

The application of the principle of microscopic reversibility [60] requires that the rates of inter-

conversion of the two enantiomers are different in the environment of the chiral selector. Yet no deracemisation is observed because the second eluted enantiomer is formed more rapidly but is also depleted to a greater extent due to its longer residence time in the column [137]. It is predicted that a deracemisation process in favour of the second eluted enantiomer can be accomplished by increasing the flow-rate (enhanced-flow-technique) after the first eluted enantiomer left the column.

The determination of the enantiomerisation barrier of homofuran by computer simulation of interconversion profiles at various temperatures obtained by complexation gas chromatography yielded kinetic data in very good agreement with that obtained by chiroptical measurements [138]. More recent examples featuring enantiomerisation and the determination of enantiomerisation barriers are concerned with atropisomers [139,140], chiral allenes [141] (cf. Fig. 12) and planar-chiral paracyclophanes [142,143].

When the chiral selectand inverts its configuration (or conformation) very rapidly during the chromatographic time scale only a single peak is observed for both enantiomers. An example is provided by the conformationally labile enantiomers of *cis*-1,2-di-

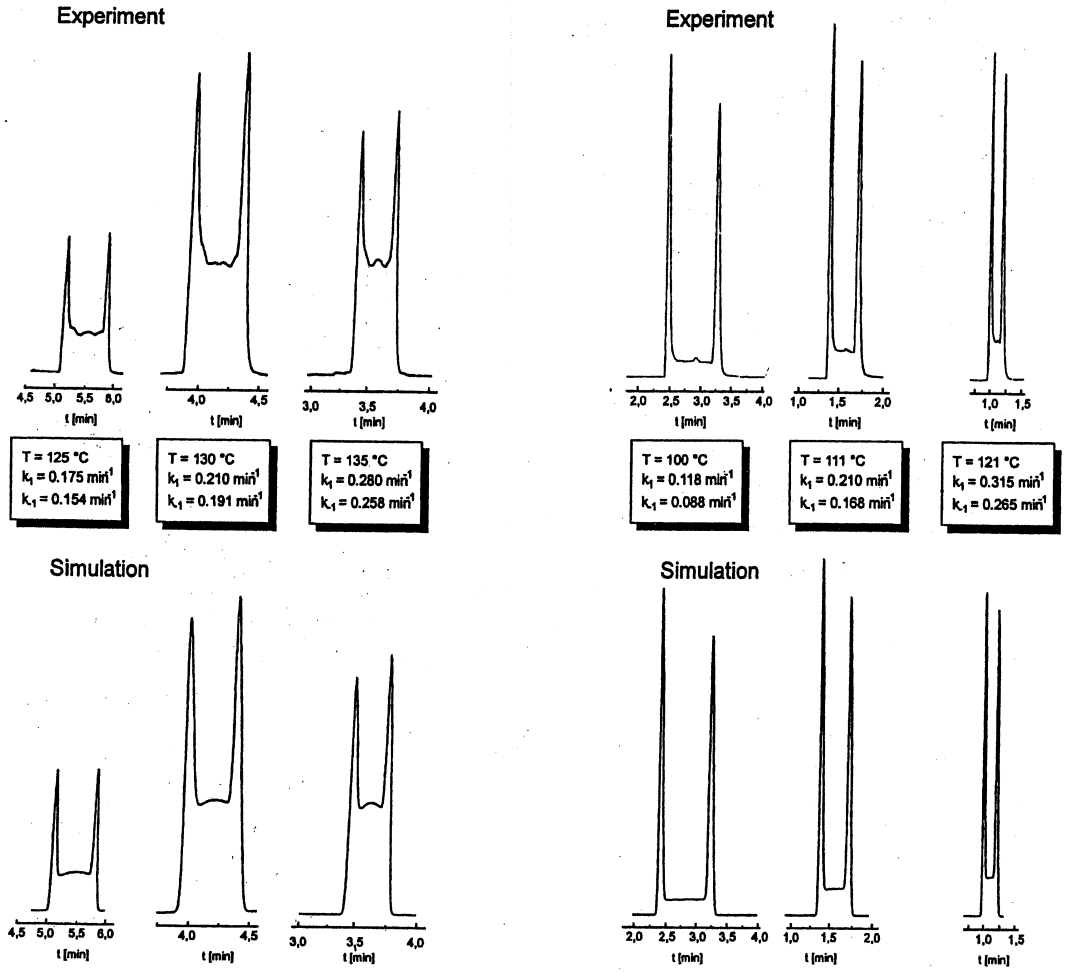

Fig. 12. Enantiomerisation of the axially chiral allene dimethyl-2,3-pentadienedioate. Experimental (top) and simulated (bottom) chromatograms. Left: separation of enantiomers by gas chromatography on Chirasil-Nickel **10** (0.25 μm). Column: 19 m×0.25 mm I.D. fused-silica capillary, carrier gas helium (1 bar). Right: separation of enantiomers by gas chromatography on Chirasil-β-Dex **25** (0.2 μm). Column: 10 m×0.25 mm I.D. fused-silica capillary, carrier gas helium (1 bar) (reproduced with permission from Ref. [141]).

methylcyclohexane which interconvert from one chiral chair conformation (image) to the other chiral chair conformation (mirror image) while the configuration at the two chiral centres remains unchanged. The appearance of only one peak by gas chromatography on permethylated β-cyclodextrin was therefore interpreted as an extremely rapid enantiomerisation leading to complete peak coalescence [58].

Enantiomerisation studies by dynamic gas chromatography (DCC) has subsequently been extended to dynamic liquid chromatography (DLC) [144–147] and to supercritical fluid chromatography (DSFC) [148].

8. Enantiomerisation studies by stopped-flow gas chromatography

Enantiomerisation studies by stopped-flow techniques have only recently been reported by Weseloh et al. in capillary electrophoresis [149], by Lorenz et al. in liquid chromatography [150] and by Schurig et al. in gas chromatography [151]. The enantiomerisation of the one-column separated enantiomers proceeds at elevated temperatures in a small segment of the column at stopped-flow conditions. While the preceding section of the column is used for the quantitative separation of the enantiomers, the subsequent section of the column is used for the determination of the enantiomeric ratio er of the two enantiomerised fractions [151]. Instead of two peaks in a conventional set-up, four peaks are obtained under stopped-flow conditions. When the enantiomerisation is performed in the middle of the column, only three peaks are expected as the newly formed fractions overlap at the centre of the chromatogram. In the one-column approach, unfortunately, enantiomerisation proceeds in the presence of the CSP. The chiral environment of the CSP is likely to interfere enantioselectively in the enantiomerisation process at ambient temperatures [148]. Therefore, a stopped-flow multidimensional gas chromatographic technique (sf-MDGC) has been developed which consists of three separate columns operated in series [152,153] (cf. Fig. 13). Thus, the enantiomers are quantitatively separated in the first column on the CSP. The first or the second eluted enantiomer is transferred into the empty second column (devoid of the CSP) and the flow is stopped. Enantiomerisation leading to racemisation proceeds in this reactor column during the time *t* at the temperature *T*. Afterwards the enantiomerised fraction is cryo-focussed and the er is determined in the third column on the same CSP. In principle, the same experiment can be performed with only two columns, i.e., a separating column and a reactor column. In this case, the

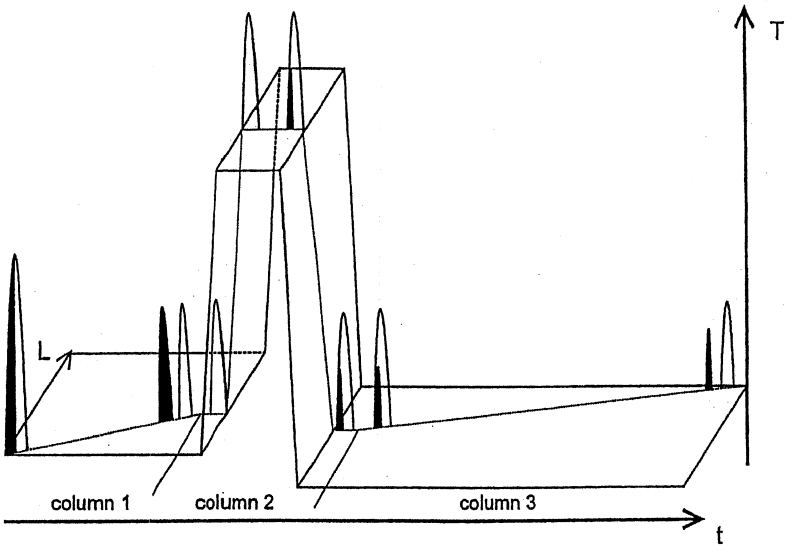

Fig. 13. Schematic representation of stopped-flow multidimensional gas chromatography (reproduced with permission from Ref. [153]).

flow must be reversed after it was stopped during enantiomerisation. Also a cyclic arrangement involving two columns is feasible.

Enantiomerisation barriers as high as 185 kJ/mol have been determined for atropisomeric polychlorinated biphenyls (PCBs) by sf-MDGC [153]. The enantiomerisation barriers of Tröger's base [154] and of 1-chloro-2,2-dimethylaziridine [155] have also been determined by this experimentally simple method. It should be noted that enantiomerisation proceeds in the inert environment of the carrier gas (e.g., helium) and only minute amounts of the racemic compound are required by this novel technique.

9. Miniaturisation in enantioselective gas chromatography

For separation of enantiomers by gas chromatography 10–25 m×0.25 mm I.D. glass or fused-silica capillaries coated with a CSP are customarily employed. Unless enantiomers are analysed in complex matrices, or a multitude of enantiomers is investigated simultaneously (e.g., all proteinogenic amino acids, cf. Fig. 1), the whole separation window offered by long columns is not required for a simple binary system comprising of only one enantiomeric pair as it is usually encountered in practice. With short columns the elution temperature can be reduced resulting in a gain of selectivity (α) which compensates for the loss of efficiency (N). In most cases the speed of analysis is improved with short columns and the sharp peaks thus obtained improve detectability.

The first use of short columns in enantioselective gas chromatography involving modified cyclodextrins were reported by Lindström [156]. In the unified approach of enantioselective chromatography involving one single column for the separation of enantiomers by GC, SFC, LC and CEC (cf. Fig. 7), dimensions of 100 cm×0.05 mm I.D. represented the best compromise for the four different techniques, each operating under suboptimal conditions [95]. As depicted in Fig. 14, the fastest separation of enantiomers reported by gas chromatography, namely that of enflurane on immobilised Chirasil-γ-Dex **26** (Scheme 3), requires only few seconds [119].

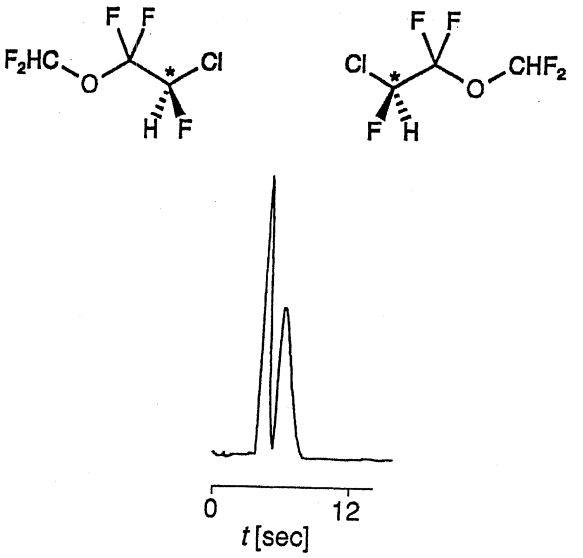

Fig. 14. Fast separation of the enantiomers of enflurane by gas chromatography on immobilized Chirasil-γ-Dex **26** obtained from Lipodex E **17** [73] (0.25 μm) at 26°C. Column: 50 cm×0.05 mm I.D., carrier gas hydrogen (0.4 bar) (reproduced with permission from Ref. [119]).

Another very fast separation of enantiomers by complexation gas chromatography has been described for 2-methyl-tetrahydrofuran on Chirasil-Nickel(II) **10** [24].

The influence of column length and column diameter on the resolution factor R_s is demonstrated in Fig. 15 for the separation of enantiomers of Tröger's base on Chirasil-β-Dex **25** [157]. The elution temperature can indeed be reduced with short columns. A faster analysis time is achieved with the short column at the unchanged inside diameter. In order to keep the phase ratio β constant, a thin film has to be employed with the 50 μm I.D. column. Consequently, the sample capacity of the column is low.

10. Preparative enantioselective gas chromatography

Unfortunately, preparative enantioselective gas chromatography is inherently restricted to volatile racemic compounds. However, contrary to liquid chromatography, the removal of the mobile phase

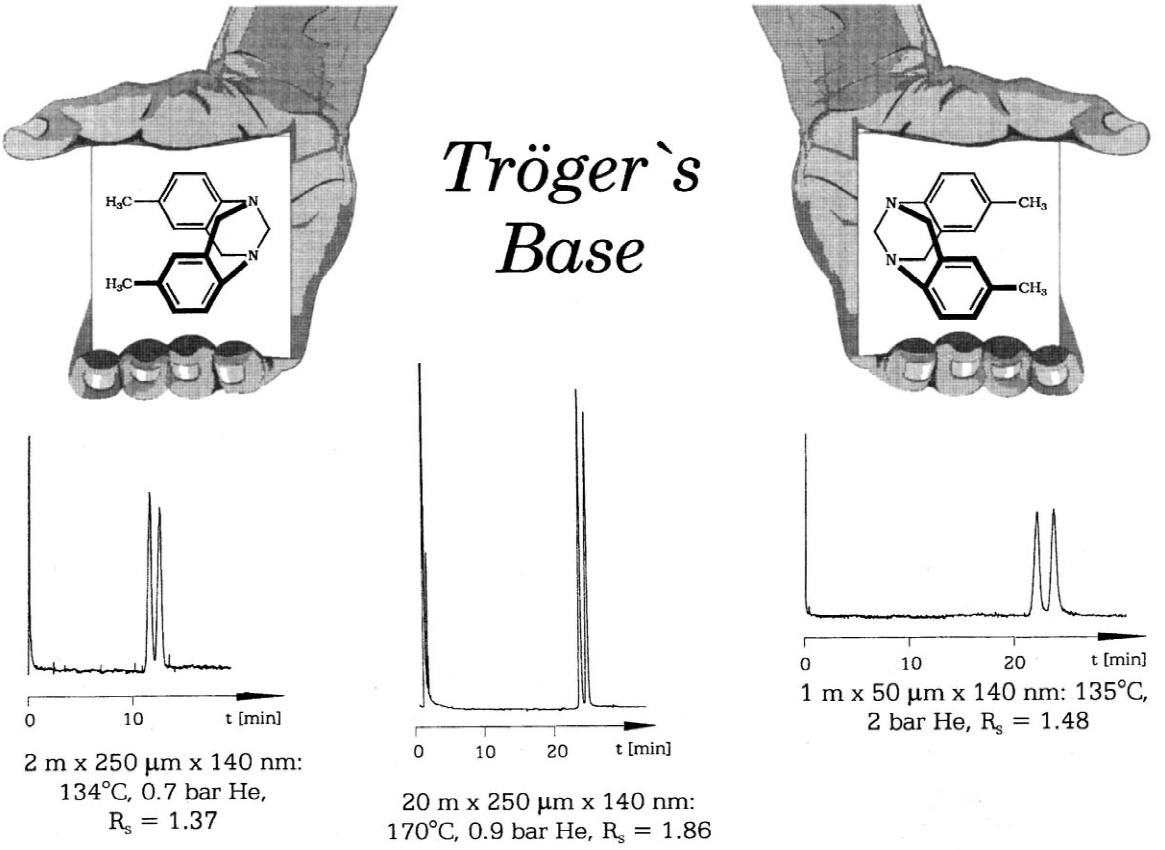

Fig. 15. Separation of the enantiomers of Tröger's base by gas chromatography on immobilised Chirasil-β-Dex **4** under various experimental conditions (cf. insertions in the figure) [157].

does not present any problems in gas chromatography involving volatiles. A separation factor of $\alpha > 1.3$ is considered as a prerequisite for efficient preparative gas chromatographic separation of enantiomers. For semi-preparative purposes, however, separation factors as low as $\alpha = 1.1$ are sufficient according to Hardt and König [158].

Interestingly, in the development of enantioselective gas chromatography, packed columns containing supported CSPs were initially employed, i.e., for a hydrogen-bonding-type CSP [3], a coordination-type CSP [159] and an inclusion-type CSP [61,62]. In complexation gas chromatography, semi-preparative enantiomer separations at the mg-scale have been reported for spiroketals (among them pheromones) [55,160]. The preparative invertomer separation of 1-chloro-2,2-dimethylaziridine permitted the determination of chiroptical data, the absolute configuration and the inversion barrier [161]. Very large separation factors α were observed for the saturated hydrocarbons *cis*- and *trans*-pinane and camphane on a mixture of α-cyclodextrin and formamide impregnated on celite [61,62]. The preparative separation of enantiomers of camphene was subsequently realised on a packed column [162].

The large separation factors α observed for the inhalation anesthetics enflurane, isoflurane and desflurane on a mixture of Lipodex E **17** [73] and the polysiloxane SE-54 (cf. Fig. 11) was exploited for preparative separation of enantiomers [163] followed by biomedical trials. The continuous separation of enantiomers of enflurane (F_2HC-O-CF_2-C*HFCl)

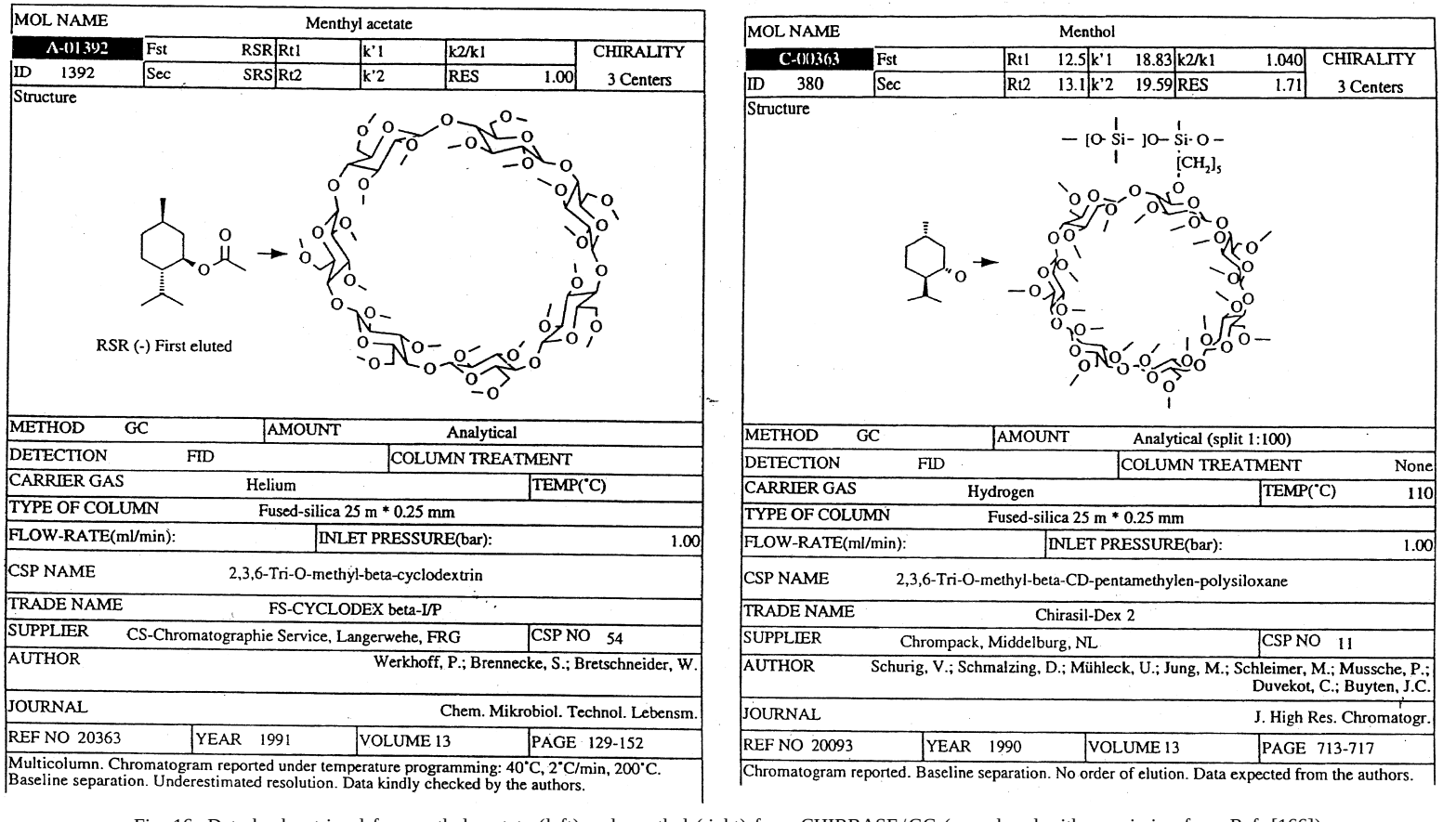

Fig. 16. Data bank retrieval for menthyl acetate (left) and menthol (right) from CHIRBASE/GC (reproduced with permission from Ref. [166]).

with the first enantioselective gas chromatographic simulated moving bed (SMB) unit was described recently [164].

11. Data retrieval for enantioselective gas chromatography

The wealth of information on enantioselective gas chromatography has been documented in the Chirbase/GC data bank [4,165,166] (cf. Fig. 16). This data bank has also been exploited for predictive searches of intrinsic molecular properties that influence enantiomer discrimination for chiral compounds containing three-membered rings [167] and four-membered rings [168].

12. Miscellaneous

Topics such as precision and accuracy of enantioselective gas chromatography, practical hints and recommendations have been treated comprehensively in former accounts [7,15,20,21,104].

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