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Salient Features of Enantioselective Gas Chromatography: The Enantiomeric Differentiation of Chiral Inhalation Anesthetics as a Representative Methodological Case in Point

Volker Schurig

Abstract The enantiomeric differentiation of the volatile chiral inhalation anesthetics enflurane, isoflurane, and desflurane by analytical and preparative gas chromatography on various modified cyclodextrins is described. Very large enantioseparation factors α are obtained on the chiral selector octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E). The gas-chromatographically observed enantioselectivities are corroborated by NMR-spectroscopy using Lipodex E as chiral solvating agent and by various sensor devices using Lipodex E as sensitive chiral coating layer. The assignment of the absolute configuration of desflurane is clarified. Methods are described for the determination of the enantiomeric distribution of chiral inhalation anesthetics during narcosis in clinical trials. The quantitation of enantiomers in a sample by the method of enantiomeric labeling is outlined. Reliable thermodynamic parameters of enantioselectivity are determined by using the retention-increment R' approach for the enantiomeric differentiation of various chiral halocarbon selectands on diluted cyclodextrin selectors.

Keywords Absolute configuration · Chiral inhalation anesthetics · Enantiomer labeling · Enantioselective gas chromatography · Enantioselective sensors · Modified cyclodextrins · Retention-increment

Contents

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1 Introduction

There are a number of contemporary reviews available on the gas-chromatographic separation of enantiomers on chiral stationary phases (CSPs) [1–5]. In the present account, the various aspects of the technique will be illustrated via the gas-chromatographic analytical and preparative enantioseparation of the chiral volatile inhalation anesthetics *enflurane*, *isoflurane*, and *desflurane* on modified cyclodextrins.

The use of inhalation anesthetics allows patients to undergo medical treatment without distress and pain. They have changed the operating room from a chamber of horrors, where patients felt and noticed everything, to a place in which medical care is provided in a tranquil atmosphere [6]. Inhaled narcotics enter the brain and induce profound sleep, passive state, muscle relaxation, and analgesia. Anesthetics should have a rapid onset and a rapid recovery, returning the patient from a deep hypnotic sleep to an awakened state, i.e., to resume "street fitness" as rapidly as possible. At the same time the anesthetics should produce few or no side effects and exit the body essentially unchanged [6]. Diethyl ether was described by Valerius Cordus in the sixteenth century and its anesthetic properties were adopted in the same century by Paracelsus. In 1842, Crawford Long in Jefferson used diethyl ether as a surgical anesthetic [6, 7], while the dentist William T.G. Morton introduced it at Massachusetts General Hospital at Boston in 1846 [8] (Fig. 1).

The "fluorine revolution" [9] enabled the introduction of new anesthetics into medicinal practice such as enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether = 2-chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane), isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether = 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane), and desflurane (difluoromethyl-1,2,2,2-tetrafluoroethyl ether = 2-(difluoromethoxy)-1,1,1,2-tetrafluoroethane) (Fig. 2) [6, 7, 10]. Isoflurane is a structural isomer of enflurane and in desflurane the chlorine of isoflurane is

Fig. 1 Panel from a monument in Boston's Public Garden commemorating Morton's demonstration of diethyl ether's anesthetic use at Massachusetts General Hospital in 1846 (private photograph)

substituted by fluorine. Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) will not be treated here. The new achiral anesthetic sevoflurane (fluoromethyl-2,2,2-trifluoro-1-(trifluoromethyl)ethyl ether) = 1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane) [7] and its chiral decomposition product "compound B" (2-(fluoromethoxy)-3-methoxy-1,1,1,3,3-pentafluoropropane) will be addressed later on in this account.

As compared to diethyl ether, reduction in toxicity and flammability, resistance to peroxidation, reduction or absence of metabolic conversion, increase in volatility, and potency guided the design of modern haloethers for anesthesia. Yet the unintended introduction of a stereogenic center into the molecules and its impact on enantioselective biological action has totally been ignored from the outset. Although chiral inhalational anesthetics have been produced and administered as racemic mixtures up to now, in vivo and in vitro studies have suggested differences in their pharmacological properties notably for isoflurane [11, 12]. However, these differences appeared not as striking as to warrant actions by legislative authorities for the use of single enantiomers. Yet the study of enantioselective effects in

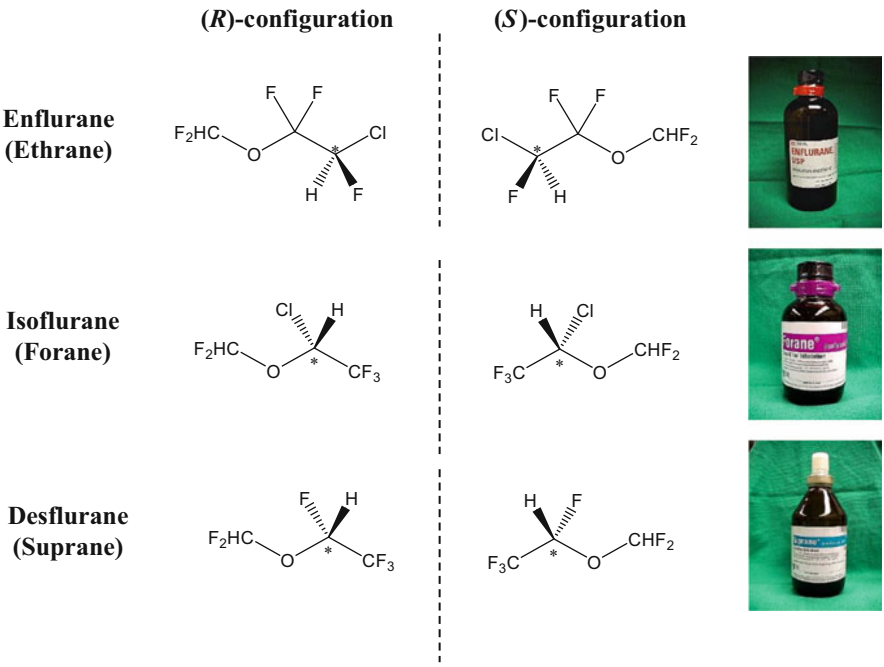

Fig. 2 Molecular structures of modern chiral inhalation anesthetics

pharmacokinetics and pharmacodynamics may help to unravel the still unknown pharmacological action of inhalation anesthetics. For this purpose, apart from enantioselective synthetic approaches [9], the development of analytical and preparative gas-chromatographic methodologies for the enantiomeric differentiation of enflurane, isoflurane, and desflurane became essential [12].

2 Analytical Gas-Chromatographic Enantioseparation of Enflurane, Isoflurane, and Desflurane

Owing to the lack of suitable functionalities, chiral haloethers are not amenable to gas-chromatographic enantioseparation via hydrogen-bonding CSPs [13] or by metal-complex containing CSPs [14]. However, with the advent of modified cyclodextrin CSPs, inclusion-type enantioselective GC became feasible and it soon emerged as an important tool for the enantioseparation of almost each class of chiral compounds [2, 15, 16]. Moreover, most chiral selectands are not required to be derivatized for effective enantiomeric differentiation on cyclodextrin-derived selectors. For gas-chromatographic enantioseparation using alkylated and/or acylated cyclodextrin (CD) derivatives, two different approaches have been developed. (1) In order to combine the enantioselectivity of modified cyclodextrins with the high efficiency encountered with silicone stationary phases, permethylated β-cyclodextrin was diluted

in semipolar polysiloxanes (e.g., OV-1701) and then coated on glass [17] or fused silica capillary columns [18]. Later on, permethylated β-cyclodextrin was chemically linked to an apolar polydimethylsiloxane backbone to improve column performance by yielding the CSP Chirasil-Dex [19]. The polymeric CSP can be immobilized via thermal surface bonding on the inner capillary wall and a single capillary column coated with Chirasil-Dex can be used in open-tubular enantioselective gas chromatography (*o*-GC), supercritical fluid chromatography (*o*-SFC), liquid chromatography (*o*-LC), and capillary electrochromatography (*o*-CEC) in a unified multichromatographic approach [20]. (2) Pentylated/acylated cyclodextrins are viscous liquids at room temperature and they can be used as undiluted CSPs coated on glass and fused silica columns [16, 21, 22]. In order to improve column performance, the pentylated/acylated cyclodextrins were later also diluted in polysiloxanes, e.g., octakis (3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) in OV-1701 [23]. It has been demonstrated that the enantioseparation factor α is concentration-dependent when modified cyclodextrins are diluted in polysiloxanes and it is not increased after a threshold value of dilution [24]. Thus the diluted selector approach [17] nowadays represents the method of choice of enantioseparation by GC using modified CDs.

At the outset, isoflurane has been enantioseparated by Meinwald et al. on a 25-m fused silica capillary column coated with hexakis(2,3,6-tri-*O*-pentyl)-α-cyclodextrin (column A) and on a 25 m Pyrex glass capillary column coated with octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (column B) at 30°C [25] using the *undiluted selector approach* of König [16]. Whereas isoflurane has been enantioseparated on both columns, halothane could be enantioseparated only on column A and enflurane only on column B [25]. Shitangkoon et al. enantioseparated enflurane, isoflurane, and desflurane on 30 m × 0.25 mm i.d. fused silica capillary columns coated with 0.25 μm thick films of undiluted 2,6-di-*O*-pentyl-3-*O*-trifluoroacetylated α-, β-, and γ-cyclodextrins [26], a CSP comprising of synthetic mixtures of isomers and homologues [22]. Other commercially available pentylated and hydroxypropylated cyclodextrins (Cyclodex) were also used as CSPs [26]. In a subsequent study emerging from an industrial laboratory engaged in the production of fluorocarbon anesthetics, various CSPs were tested for racemic enflurane, isoflurane, and desflurane and related synthetic analogues [27]. Octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-cyclodextrin and octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) were compared to probe the influence of substitution at the 3-position of the CDs, whereas hexakis(2,3,6-tri-*O*-pentyl)-α-cyclodextrin and heptakis(2,3,6-tri-*O*-pentyl)-β-cyclodextrin were tested to assess the role of the cavity size [27]. In few cases exceptionally high enantioseparation factors α were observed which could be correlated with the magnitude of chemical shift differences between ^{1}H- and ^{19}F-nuclei of the enantiomers in the presence of the modified cyclodextrins used as chiral solvating agents (CSA) in NMR spectroscopy [27]. It was therefore suggested that the simple and inexpensive NMR experiment could be used as predictive tool for proper CSP selection in enantioselective GC of fluoroethers employing CDs [27].

By the *diluted selector approach* of Schurig and Nowotny [17], octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) [28] diluted to 40 wt% in

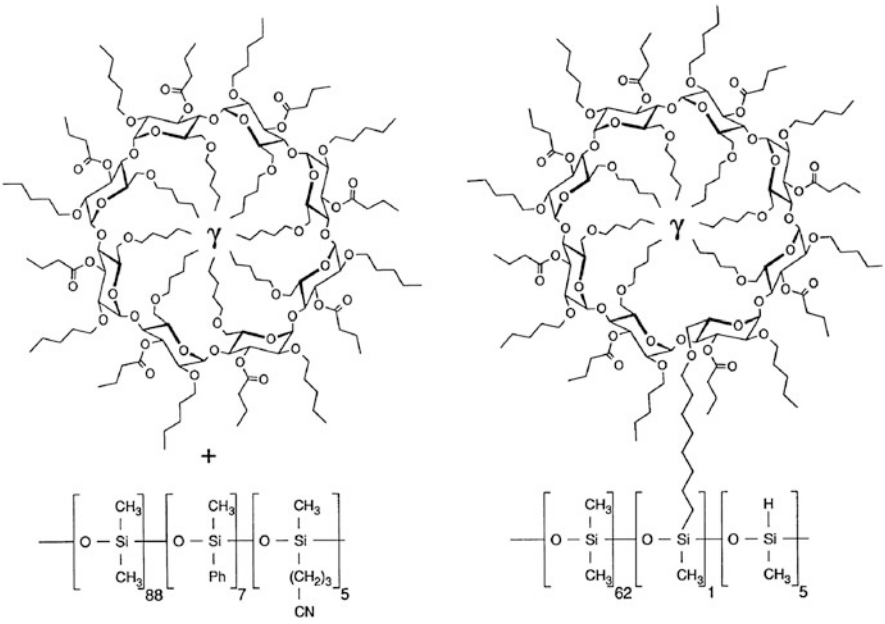

Fig. 3 *Left*: Octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) [28] diluted in OV-1701 [31]. *Right*: Octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) chemically linked via an octamethylene spacer to polydimethylsiloxane to yield Chirasil-γ-Dex [31, 32]

polysiloxane OV-1701 (5% cyanopropyl–7% phenyl-dimethylpolysiloxane) (Fig. 3, left) enantioseparated enflurane with $\alpha = 2.16$ and isoflurane with $\alpha = 1.36$ at 24°C (Fig. 4, left) [29]. Due to the large enantioseparation factors α observed, very fast enantioseparations within 30 s became feasible at elevated temperatures and higher carrier gas velocities (Fig. 4, right) [29]. For the enantiomeric analysis of enflurane by its enantioselective clathrate inclusion in tri-*o*-thymotide, a 25 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.25 µm Chirasil-β-Dex [19] has been used at −1 to 7°C [30]. The simultaneous analytical enantioseparation of enflurane, isoflurane and desflurane in 2.5 min has been achieved on a 25 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.5 µm octakis (3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) in polysiloxane SE-54 and on a 10 m × 0.25 mm (i.d.) fused silica capillary column coated with immobilized Chirasil-γ-Dex (Fig. 5) [31]. In Chirasil-γ-Dex the selector octakis (3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) [28] is chemically linked via a monooctamethylene spacer to polydimethylsiloxane at the 6-*O*- and/or 2-*O*-position of CD (Fig. 3, right) [32].

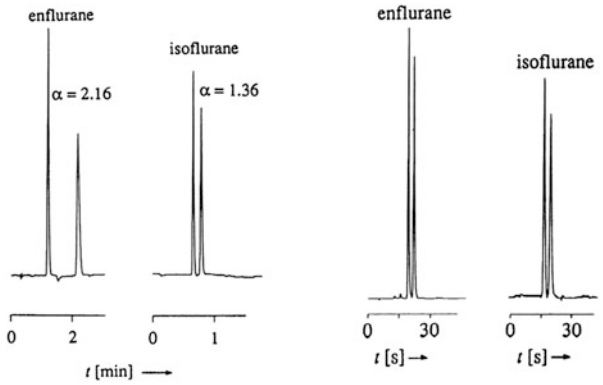

Fig. 4 *Left*: Analytical gas-chromatographic enantioseparation of enflurane and isoflurane. 6 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.25 μm octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) in OV-1701 (40 wt%) at 24°C and 45 cm/s dihydrogen [29]. *Right*: Rapid enantioseparation of enflurane at 60°C, 54 cm/s dihydrogen and isoflurane at 26°C, 100 cm/s dihydrogen [29]

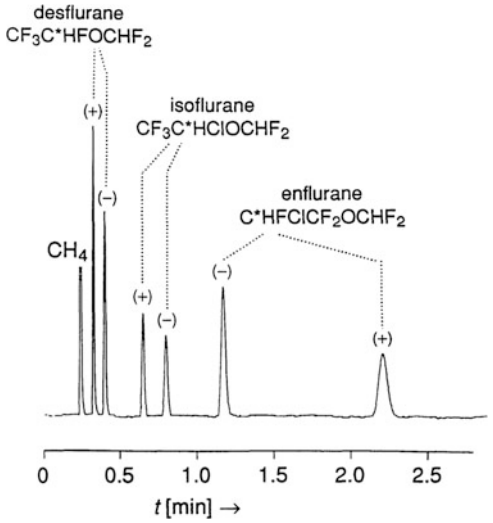

Fig. 5 Fast simultaneous gas-chromatographic enantioseparation of enflurane, isoflurane and desflurane. 10 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.18 μm immobilized Chirasil-γ-Dex at 28°C and 40 cm/s dihydrogen [31]

3 Preparative Gas-Chromatographic Enantioseparation of Enflurane, Isoflurane, and Desflurane

The state-of-the-art of enantioselective preparative GC has been reviewed [33]. This method is restricted to thermally stable and volatile compounds. Due to the absence of liquid mobile phases as compared to LC, the recovery of the isolated enantiomers from the gaseous mobile phase is straightforward when aerosol and mist formation is prevented by using specially designed collection vessels [33]. The absence of an isolation problem of separated compound from the solvent is especially beneficial for volatile racemates like those of chiral inhalation anesthetics. However, preparative enantioselective GC does not match the overwhelming success of preparative enantioselective LC [34, 35]. The preparative GC method has only been successful for racemates exhibiting enantioseparation factors $\alpha > 1.5$ [33]. Preparative enantioselective chromatography relies on a compromise between three variables: (1) peak resolution (governed by enantioselectivity, efficiency and retention), (2) speed of enantioseparation, and (3) chiral column sample capacity. Any of two desired goals may only be achieved at the expense of the third. If a large sample throughput is required in a short time, resolution must be high. However, if resolution is insufficient, either the column load is limited or the time of enantioseparation is long [33].

Interestingly, in the early development of enantioselective GC, packed columns containing supported CSPs were employed for a hydrogen-bonding-type CSP [36], a metal-complexation-type CSP [37], and an inclusion-type CSP [38]. In complexation GC, semi-preparative enantioseparations at the milligram-scale have been reported for spiroketals (among them pheromones) [14, 39]. The preparative invertomer separation of 1-chloro-2,2-dimethylaziridine permitted the determination of chiroptical data, the absolute configuration, and the inversion barrier at the stereogenic nitrogen atom [40]. Large enantioseparation factors α were observed for the saturated hydrocarbons *cis*- and *trans*-pinane on a mixture of α-cyclodextrin and formamide impregnated on celite [38]. The preparative enantioseparation of camphene was subsequently realized on a packed column [41].

With the advent of alkylated/acylated CDs as versatile CSPs for analytical enantioseparations by GC (Sect. 2), their potential for semi-preparative enantioseparations of flavours, fragrances, and terpenoids was recognized. Micro-preparative enantioseparations by GC on modified cyclodextrins have been achieved on thick-film wide-bore fused silica capillary columns [42]. The enantiomers of 2 mg of racemic methyl jasmonate were enantioseparated within 80 min on a 1.8 m × 4 mm (i.d.) stainless steel column packed with 5 wt% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin in polysiloxane OV-1701 (1:1, w/w) coated onto Chromosorb W-HP (100–120 mesh) at 120°C and 0.4 bar helium [43]. The analytical and semi-preparative enantioseparation of all-anti-*trans*-perhydrotriphenylene on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin has also been described [44], demonstrating that even a rather nonvolatile racemate exhibiting a low enantioseparation factor α was amenable to semi-preparative enantioseparation by GC.

As human inhalation anesthetics enflurane, isoflurane, and desflurane exhibited unprecedented high enantioseparation factors α on octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) (Fig. 5), their preparative enantioseparation represented an attractive target [33]. The availability of sufficient amounts of single enantiomers of the chiral haloethers was the prerequisite for medical trials and for the determination of chiroptical data and their absolute configurations. The absence of suitable functionalities precluded resolution of the racemates via diastereomers. The liquid phase chromatographic enantioseparation on CSPs is hampered by the difficulty in separating the volatile compounds from the liquid mobile phases. Enantioselective GC was therefore the method of choice [33]. Two approaches were advanced. (1) Racemic isoflurane was semi-preparatively enantioseparated on a 2 m × 10 mm (i.d.) stainless steel column containing 23.4 wt% of undiluted octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-cyclodextrin coated on Chromosorb W (AW, 80–100 mesh) using helium as carrier gas at 40°C [45, 46]. Employing a sampling interface between the column end and the fraction collector, the enantiomeric excess (ee) was detected online by an analytical enantioselective column. This set-up allowed the calculation of ee, recovery rate, and production rate. Racemic enflurane was enantioseparated on a 1 m × 10 mm (i.d.) stainless steel column containing 25.0 wt% of octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-cyclodextrin using dihydrogen as carrier gas at 40°C [46]. Up to 24 mg of the first eluted enantiomers with ee ~ 100% could be obtained when 75 mg of the racemate was injected whereas only 6 mg of the second eluted enantiomer with ee ~ 100% was obtained upon injecting 20 mg of the racemate in the optimized system. Higher production rates could only be obtained by sacrificing the enantiomeric excess (ee) [46]. (2) Whereas the above described endeavor utilized an undiluted CD selector, the dilution of modified CDs in semipolar polysiloxanes, previously applied successfully in analytical enantioselective GC [17], also proved useful for packed columns in a conventional GC apparatus (Fig. 6). Thus 30 μL (47 mg) racemic enflurane was enantioseparated in 45 min at 26°C into the first eluted (*R*)-enantiomer and at 50°C into the second eluted (*S*)-enantiomer on a 4 m × 7 mm (i.d.) glass column containing 95 g Lipodex E diluted in SE-54 (dimethyl-phenyl(5%)-polysiloxane) (10 wt%) and coated onto Chromosorb P (AW, DMCS, 80–100 mesh) (20 wt%) (Fig. 7, left) [29, 47]. A chemical purity of at least 99.9% and an enantiomeric excess (ee) of at least 99.8% were achieved for both enflurane enantiomers. The eluates from the column were split 1:1,500 (Fig. 6) between a flame-ionization detector and a cold-trap cooled with dry ice/acetone or liquid dinitrogen. Although the separation of the enantiomers from the carrier gas helium is straightforward, some mist formation during collection reduced the amount of recovered enantiomers (~12 mg each). An overloading study is depicted in Fig. 7, right [29].

Repetitive injections allowed the collection of 250 mg each of enflurane enantiomers of ee = 99.8% daily. By using the same procedure, only 6 mg of racemic isoflurane could be baseline enantioseparated isothermally at 26°C in 28 min [29]. However, with isothermal runs, repetitive injections in close succession and at short intervals were possible (Fig. 8) [29].

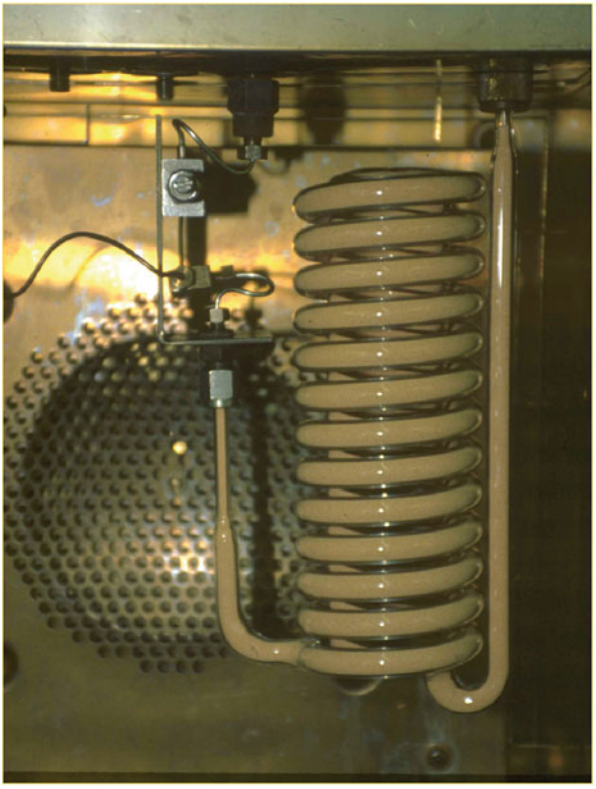

Fig. 6 Instrumental set-up of enantioselective preparative GC in a conventional apparatus (Carlo-Erba, HRGC 5300). The home-made packed 4 m × 7 mm i.d. glass column consisted of a modified condenser of a discarded rotary evaporator [33]

The above-described set-up was insufficient for large scale preparative enantioseparations of isoflurane and desflurane. Therefore an up-scaled column design was employed [48]. A packed 1 m × 24 mm (i.d.) stainless steel column filled with Chromosorb P (AW-DMCS, 80–100 mesh) which was coated with 20.3 wt% of the mixture of 10.6 wt% octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) in polysiloxane SE-54, was installed in an automated Hupe & Busch preparative GC (built in the 1960s). It required the synthesis of 500 g CSP consisting of 10.75 g unpurified Lipodex E and 90.5 g SE-54 coated onto 400 g Chromosorb P. Unpurified Lipodex E contains under- and over-pentylated species and, interestingly, the crude selector showed a higher enantioseparation factor α for isoflurane (from 1.3 to 1.6 at 30°C) but reduced values for desflurane and enflurane. Here 300 mg of the single enantiomers of isoflurane were obtained daily with 130 automated repetitive injections isothermally at room temperature (Fig. 9) [48, 49]. The very high enantiomeric excess of both isoflurane enantiomers (ee = 99.8%) was determined by analytical enantioselective GC (Fig. 10). Desflurane could only be enantioseparated under overlapping conditions and recycling or discharging the middle fractions [48].

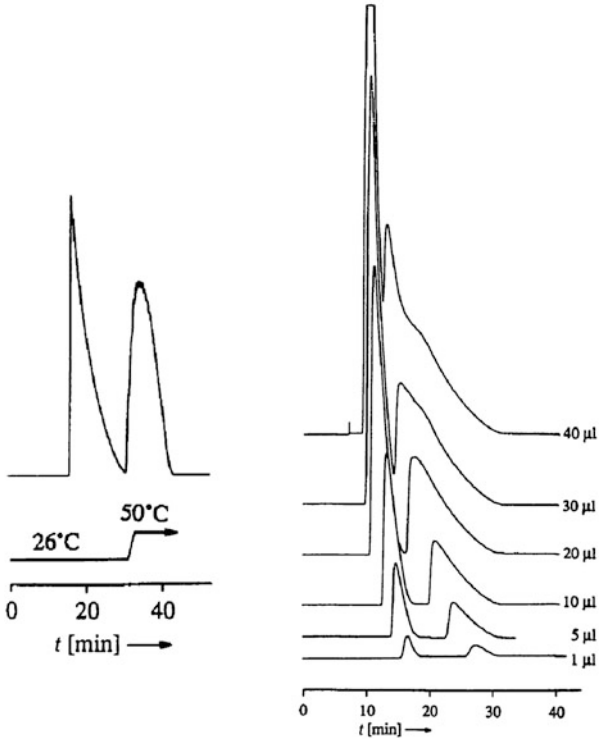

Fig. 7 *Left*: Temperature-programmed semi-preparative gas-chromatographic enantioseparation of enflurane. 4 m × 7 mm (i.d.) glass column filled with 95 g Chromosorb P-AW-DMCS coated with 20.3 wt% Lipodex E in SE-54 (10 wt%). Injected amount: 30 µL, 8.4 cm/s helium. *Right*: Overloading experiment (1–40 µL of racemic enflurane) at 40°C and 8.4 cm/s helium [29]

Then 500 mg of the first eluted enantiomer (ee = 91%) and 450 mg of the second eluted enantiomer (ee only 68%) were obtained daily. This amount was drastically reduced when higher ee values were required.

With the availability of pure enantiomers of the inhalation anesthetics with ee ~ 99.8% (corresponding to 0.1% of enantiomeric impurity; for enantiomers of isoflurane, cf. Fig. 9, right), various topics could be investigated: (1) in olfaction the enantiomers of isoflurane possessed a different odor [27], (2) the absolute configurations of (+)-isoflurane and (+)-desflurane were determined by cryoscopic X-ray crystallography (Sect. 5), (3) biological trials showed only small differences in the MAC values of isoflurane enantiomers in rodents [12, 50], (4) the use of single isoflurane enantiomers as novel chiral additive to the mobile phase in GC did not exhibit any enantiomeric differentiation for a host of tested racemates [51], (5) single enantiomers of enflurane, isoflurane, and desflurane were used as analytes for enantioselective sensor devices (Sect. 4), (6) single enflurane enantiomers were employed for NMR studies with CDs used as CSAs (Sect. 10), and (7) single isoflurane enantiomers were required for the enantiomer labelling method (Sect. 7).

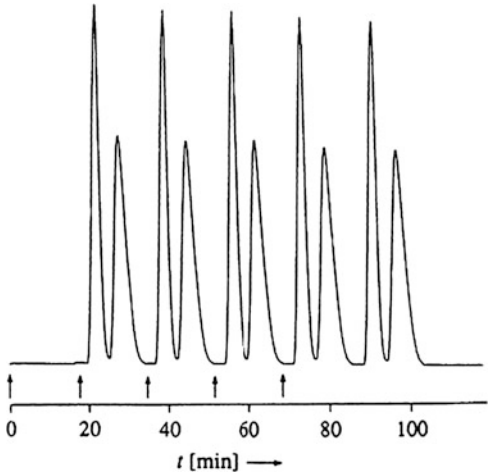

Fig. 8 Semi-preparative gas-chromatographic enantioseparation of racemic isoflurane with repetitive injections of 4 µL during one run. 4 m × 7 mm (i.d.) glass column filled with 95 g Chromosorb P-AW-DMCS (80–100 mesh) coated with 20.3 wt% Lipodex E in SE-54 (10.0 wt%) at 26°C and 8.4 cm/s helium [29]

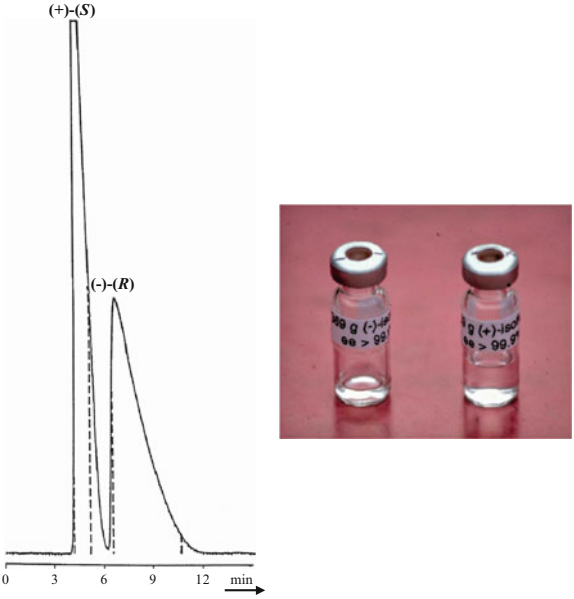

Fig. 9 *Left*: Preparative gas-chromatographic enantioseparation of isoflurane. 1 m × 24 mm (i.d.) steel column filled with 450 g Chromosorb P-AW-DMCS (80–100 mesh) coated with 20.3 wt% of unpurified Lipodex E in SE-54 (10.6 wt%) at 27°C and 1 bar dinitrogen [48]. *Right*: isolated single enantiomers of isoflurane with ee = 99.9%

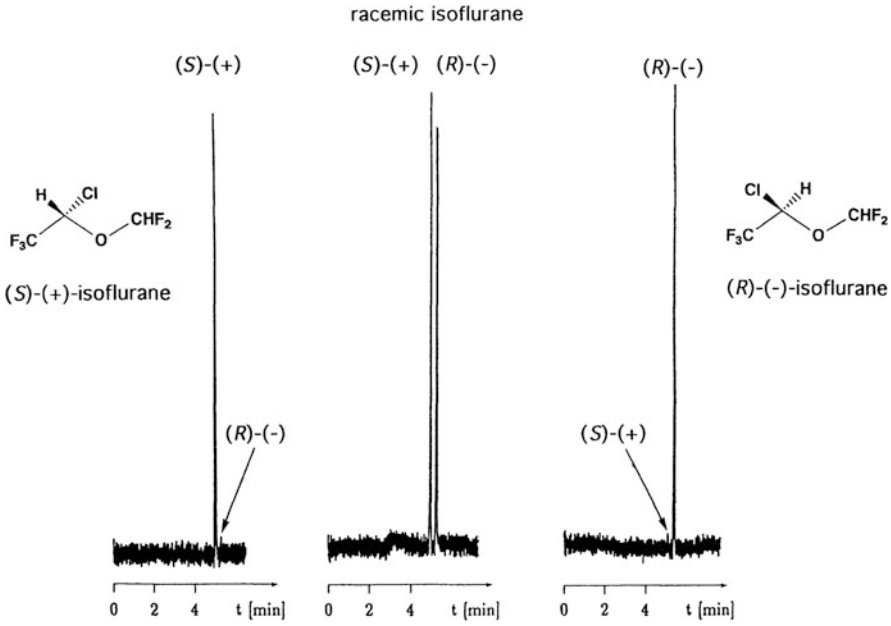

Fig. 10 Proof of the very high ee of isoflurane enantiomers. 25 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.5 μm octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) in SE-54 (10 wt%) at 26°C and 1.1 bar helium (courtesy, Dr. M. Juza)

In an attempt to scale-up the discontinuous preparative GC approach by a continuous enantioseparation of inhalation anesthetics, the simulated moving bed (SMB) technology has been employed in enantioselective GC, adapting the previous findings obtained in the batch-wise processes described above. Preliminary studies included enflurane [52, 53] and isoflurane [54], which were enantioseparated on unpurified octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) diluted in polysiloxane SE-54 and coated on Chromosorb P (AW, DMCS, 80–100 mesh). An optimized version of the enantioselective SMB unit later on consisted of eight 80 cm × 15 mm (i.d.) stainless steel columns assembled in a home-made SMB-GC unit (Fig. 11) operated at 35°C. Each column with an adsorption bed volume of 140 mL contained 20% unpurified Lipodex E diluted in SE-54 and coated on Chromosorb A (NAW, 20–30 mesh) (17 wt%). Under carefully optimized conditions the SMB-GC pilot unit furnished a total of 20 g of each single enantiomers of enflurane with an averaged ee of 96.6% with dinitrogen as carrier gas [55]. This set-up represented the first gas-chromatographic SMB-GC pilot plant for preparative enantioseparations.

In enantioselective GC-SMB, the required continuous counter-current flow of the fluid and of the solid adsorbent ("moving bed chromatography") is simulated by periodically switching the different inlets and outlets in the eight-column-array (Fig. 11). The preparative enantioseparation of enflurane by SMB and pressure swing adsorption (PSA) was theoretically investigated by dynamic simulations [56]

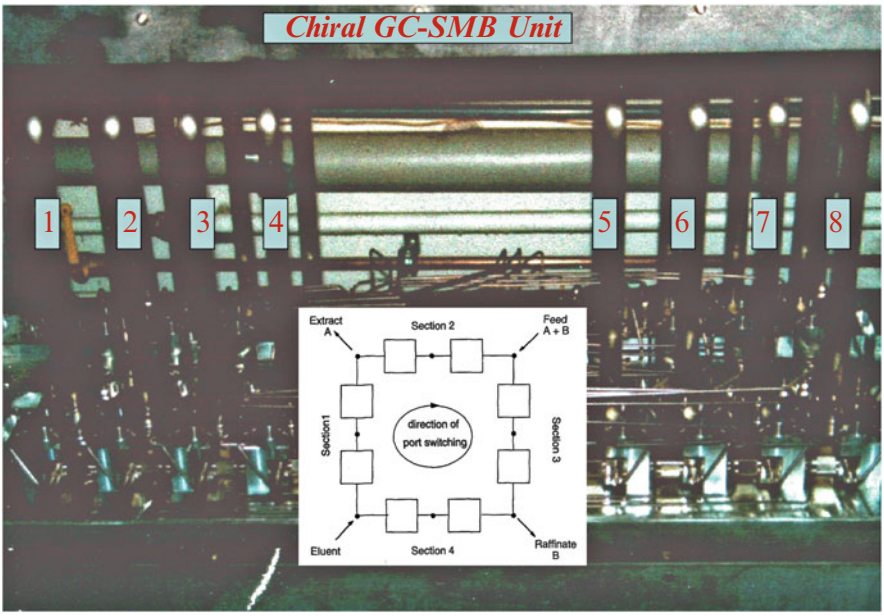

Fig. 11 An enantioselective GC-SMB-plant. (Courtesy Prof. M. Morbidelli, ETH, Zürich) containing eight columns. *Inserted*: schematic principle of SMB [55]

based on model parameters reported in previous experimental investigations [55]. A key difference between the enantioselective liquid and gas phase SMB is the strong influence of the pressure drop on the flow rate in the latter approach and the limited solubility of the feed in the gas phase which in turn results in limited productivities [57].

4 Enantioselective Sensor Devices for Inhalation Anesthetics

The gas-chromatographically obtained single enantiomers of enflurane, isoflurane, and desflurane were used for enantioselective trials using a chiral quartz microbalance sensor system [58, 59]. The quartz crystal was coated with octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) diluted in polysiloxane SE-54. A schematic representation of the thickness-shear mode resonator (TSMR) employed is shown in Fig. 12, top.

Upon exposure of the TSMR to single enantiomers of enflurane, the sorption of analyte molecules by the cyclodextrin coating leads to a change in the oscillating mass which in turn causes a shift of the operating frequency. As depicted in Fig. 12, middle, representative experiments consisted of the alternating exposure of the coated TSMR to sample vapour (in ppm) and dry synthetic air, respectively. In addition to the chiral sensor, an achiral reference device coated with pure SE-54 (devoid of enantiomeric

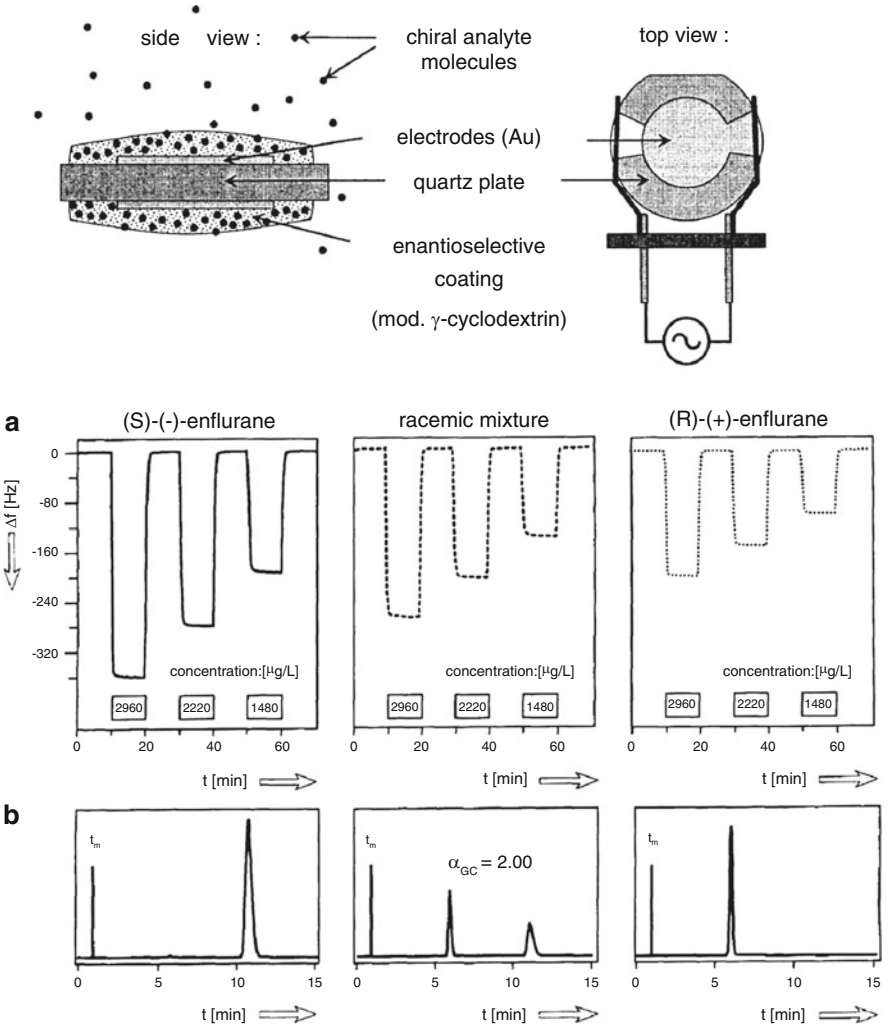

Fig. 12 *Top*: Principle of the enantioselective TMSR sensor. *Bottom*: Comparison of enantioselective TMSR sensor device (**a**) and enantioselective GC (**b**) for single enantiomers of enflurane and its racemic mixture exposed to 50 wt% Lipodex E in SE-54. The sensor responses upon alternate exposure to enantiomer-loaded to pure synthetic air are given in Hz, the analyte concentration in µg/L. The GC retention times obtained with a 25 m × 0.25 mm (i.d.) capillary column are displayed in min [58, 59]

differentiation) was used for the purpose of artefact adjustment including fluctuating gas phase concentrations and analyte contaminations. The sensor responses upon the exposure to the single enflurane enantiomers showed an unprecedented frequency difference and the observed enantioseparation factor α agreed well with that of the gas-chromatographic experiment. Similar results were obtained for the enantiomers of isoflurane and desflurane [60] (cf. also Fig. 15 in [61]). The sensor experiment and the

gas-chromatographic set-up displayed the same sign and magnitude of enantioselectivity (Fig. 13). As the recognition step in the case of a gas sensor involves just one theoretical plate while GC enantioseparation arises from some thousands of subsequent absorption-desorption-steps in single theoretical plates, the suitability of TSMR for enantiomer discrimination was far from being obvious at the outset of the experiments [58–60].

5 Determination of Absolute Configurations in Enantioselective GC

The assignment of absolute configurations [62] of minute amounts of chiral compounds is an important task in trace enantiomeric analysis. *By direct evidence*, absolute configurations can be determined by enantioselective GC (free of chiroptical evidence) via the simultaneous injection of a reference compound with an established stereochemistry. *By indirect evidence*, it is tempting to correlate the absolute configuration of the enantiomers of structurally related compounds (congeners, homologues) with their order of elution from a given CSP in enantioselective GC. Indeed, on L-valine diamide selectors, the derivatized L-enantiomers of all proteinogenic α-amino acids were found to elute as the second peak [4]. By coincidence, the same elution order was also observed for Chirasil-γ-Dex (Lipodex E, comprised of D-glucose moieties, anchored to polydimethylsiloxane [32], Fig. 3, right), except for proline and threonine where the L-enantiomers eluted first [63]. In enantioselective complexation GC, a rather consistent correlation between absolute configuration and elution order of 2-alkyl- and 2,3-dialkyl-substituted oxiranes on metal(II)-bis(perfluoroacyl-(1*R*)-camphorates), was found, leading to the formulation of a quadrant rule [14]. However, striking exceptions to the rule were also noted for structurally related racemates. Thus, *trans*-(2*S*,3*S*)-dimethyloxirane (a "hard" donor selectand) and *trans*-(2*R*,3*R*)-dimethylthiirane (a "soft" donor selectand) were both eluted as the second peak on nickel(II) bis(3-heptafluorobutanoyl-(1*R*)-camphorate) despite their opposite configurations, whereas (*R*)-methyloxirane and (*S*)-methylthiirane showed the expected opposite elution order on the same selector [64]. (*S*)-2-*Methyl*-tetrahydrofuran was eluted as the first peak while (*S*)-2-*ethyl*-tetrahydrofuran was eluted as the second peak from nickel(II) bis(3-heptafluorobutanoyl-(1*R*)-camphorate) [65]. These examples vividly showed that predictions of absolute configuration from retention behavior for homologues or structurally related compounds may be ambiguous. Moreover, the temperature-dependent reversal of the elution order may obscure assignments of absolute configurations by enantioselective chromatography as the result of enthalpy–entropy compensation (Sect. 8) within an extended temperature-range of operation. Thus, isopropyloxirane as compared to other alkyloxiranes exhibited the remarkably low $T_{iso} = 64°C$ on nickel(II) bis(3-heptafluorobutanoyl-10-ethylidene-(1*S*)-camphorate) [66]. Thus peak inversion occurs above T_{iso}, and a non-consistent elution order would result between isopropyloxirane and other

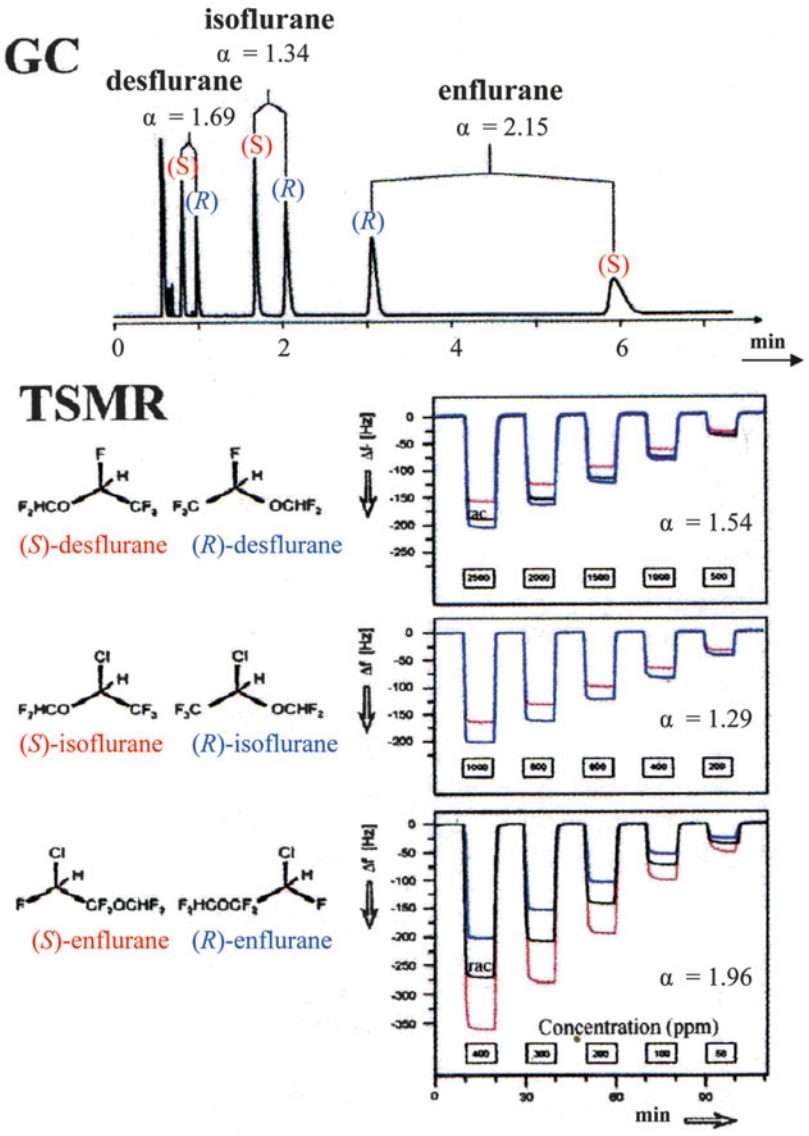

Fig. 13 *Top*: Analytical gas-chromatographic enantioseparation of desflurane, isoflurane and enflurane (structures see *bottom*, *left*). 25 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.5 μm octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) in SE-54 (10 wt%,) at 26°C and 1.1 bar helium [48]. *Bottom*, *right*: Sensor responses of a quartz thickness-shear mode resonator (TSMR) coated with Lipodex E in SE-54 (50 wt%) in Hz for μg/L amounts of single enantiomers and its racemic mixture of desflurane, isoflurane and enflurane, depicted as an overlay of strongly and weakly interacting enantiomers and, in between, for the racemate (the latter except for isoflurane) [61]

homologues alkyloxiranes with identical absolute configuration at elevated temperatures in the GC experiment on simple thermodynamic grounds.

Some confusion existed previously in the assignment of the absolute configurations of isoflurane vs desflurane. The two molecules differ only by chlorine vs fluorine at the stereogenic carbon atom (Fig. 2). The enantiomers of isoflurane and desflurane were isolated by preparative enantioselective GC on the γ-cyclodextrin CSP Lipodex E (Sect. 3) and the dextrorotatory enantiomers (+)-isoflurane and (+)-desflurane were eluted before the levorotatory enantiomers (–)-isoflurane and (–)-desflurane [31] (optical rotations refer to the sodium-*D*-line at 20°C). Thermodynamic considerations using the retention-increment R' approach (Sect. 8) supported the reasonable assumption that the enantiomers of isoflurane and desflurane with the same configuration possess the same sign of optical rotation and the same elution order on Lipodex E [31].

The assignment of absolute configuration to dextro- and levorotatory isoflurane and desflurane was carried out by vibrational circular dichroism (VCD) spectra [67]. Thus the recorded spectrum of (+)-isoflurane in CCl_4 was compared with the theoretical spectra calculated by ab initio methods for different conformers of (*R*)- and (*S*)-isoflurane [68]. The theoretical VCD spectrum obtained as the sum of those for the two lowest energy conformers with (*S*)-configuration was found to match well with the experimental VCD spectrum for (+)-isoflurane. Therefore it was concluded that (+)-isoflurane had the (*S*)-configuration and hence (–)-isoflurane had the (*R*)-configuration [68]. However, in order to approximate better the experimental and theoretical VCD spectra, different ratios of the two conformers were applied. Moreover, the influence of the solvent on the VCD spectra was not considered. An analogous study with desflurane implied that (+)-desflurane had the (*R*)-configuration and hence (–)-desflurane had the (*S*)-configuration [69]. The surprising reversal of the sign of optical rotation vs absolute configuration (i.e., (+)-(*S*)- isoflurane vs (–)-(*S*)-desflurane) was also in contradiction to other consequences resulting from the assignment by VCD. The preparative conversion of (–)-isoflurane to (+)-desflurane (likely an S_{N2} process) would proceed with retention of configuration whereas the decarboxylation of a desflurane precursor would proceed with inversion of configuration; cf. references in [31, 70] (Fig. 14, left). Interestingly, single crystals obtained under cryogenic conditions of (+)-isoflurane and (+)-desflurane, which were previously isolated by preparative enantioselective GC (Sect. 3) [31], allowed the assignment of absolute configuration by anomalous X-ray crystal structural analysis at −180°C. Dextrorotatory isoflurane and desflurane were both found to possess (*S*)-configuration, thus confirming the VCD assignment (+)-(*S*)-isoflurane, but revising the VCD assignment of (+)-(*R*)-desflurane to (+)-(*S*)-desflurane [70]. Although the revised X-ray assignment of (+)-(*S*)-desflurane was still not unambiguous due to the small spatial differences between hydrogen and fluorine atoms and an unfavorable Flack parameter [70], it was in agreement with the general reaction stereochemistry of S_{N2} reactions proceeding with Walden inversion of configuration and decarboxylations at a stereogenic center proceeding with retention of configuration [71] (Fig. 14, right). The preliminary assignment of absolute configuration by VCD to (+)-(*R*)-desflurane has later been revised to (–)-(*R*)-desflurane and had been explained as arising from a wrongly labeled specimen [72, 73]. An extensive theoretical VCD

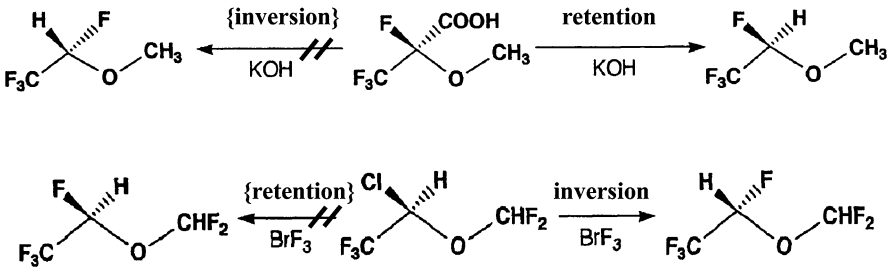

Fig. 14 Revised stereochemical course of chemical reactions relevant to the preparation of enantiomeric inhalation anesthetics. *Top*: Decarboxylation. *Bottom*: S_{N2}-substitution

study of isoflurane and desflurane has subsequently been advanced by Biedermann et al. [74].

Based on the previous VCD configurative assignment of (+)-(*S*)-desflurane, the decarboxylation of a desflurane precursor was described to proceed with inversion of configuration (Fig. 14, left, top) [75, 76] (despite some reservation; cf. footnote in [28] in [76]) but has later been corrected as proceeding with retention of configuration [77] (Fig. 14, right, top). Thus the gas-chromatographic elution order of isoflurane and desflurane enantiomers on Lipodex E is consistent with their absolute configurations [31, 70]. Furthermore, the magnitude of NMR shifts of protons of isoflurane and desflurane enantiomers in the presence of Lipodex E is compatible with their absolute configurations [70] and the frequency shifts of isoflurane and desflurane enantiomers on a quartz sensor coated with Lipodex E are also in line with their absolute configurations (Sect. 4) [58]. The enantiomers of isoflurane and desflurane are identified and differentiated by their chiroptical properties, i.e., the sign of optical rotation and circular dichroism. General methods for the assignment of absolute configuration of dextro- and levorotatory enantiomers rely on VCD and anomalous X-ray methodologies. The present elaboration shows that different approaches finally contribute to the clarification of a difficult research topic and that enantioselective chromatography plays an important role in it [62].

In the US patent 5,114,714 of May 19, 1992 it is claimed that (*R*)-isoflurane and (*R*)-desflurane (essentially free of the (*S*)-enantiomers), while inducing and maintaining anesthesia, are insufficient to cause adverse effects associated with the (*R*,*S*)-racemates [78]. In a follow-up US patent 5,114,715 of May 19, 1992 it is claimed that (*S*)-isoflurane and (*S*)-desflurane (essentially free of the (*R*)-enantiomers), while inducing and maintaining anesthesia, are insufficient to cause adverse effects associated with the (*R*,*S*)-racemates [79]. The two patents are identical in the wording except for the interchanged stereochemical descriptors (*R*) and (*S*) (Fig. 15). The "enantiomeric" patents are entirely devoid of physical characterizations and pharmacological data of the compounds claimed. No experimental details on the enantioselective preparation of the (*R*)- vs (*S*)-enantiomers are given. The identification of the enantiomers by their absolute configuration is also missing. Although no supporting details were disclosed, the two patents imply that either the (*R*)- or the (*S*)-enantiomers of isoflurane and desflurane do not possess adverse effects as

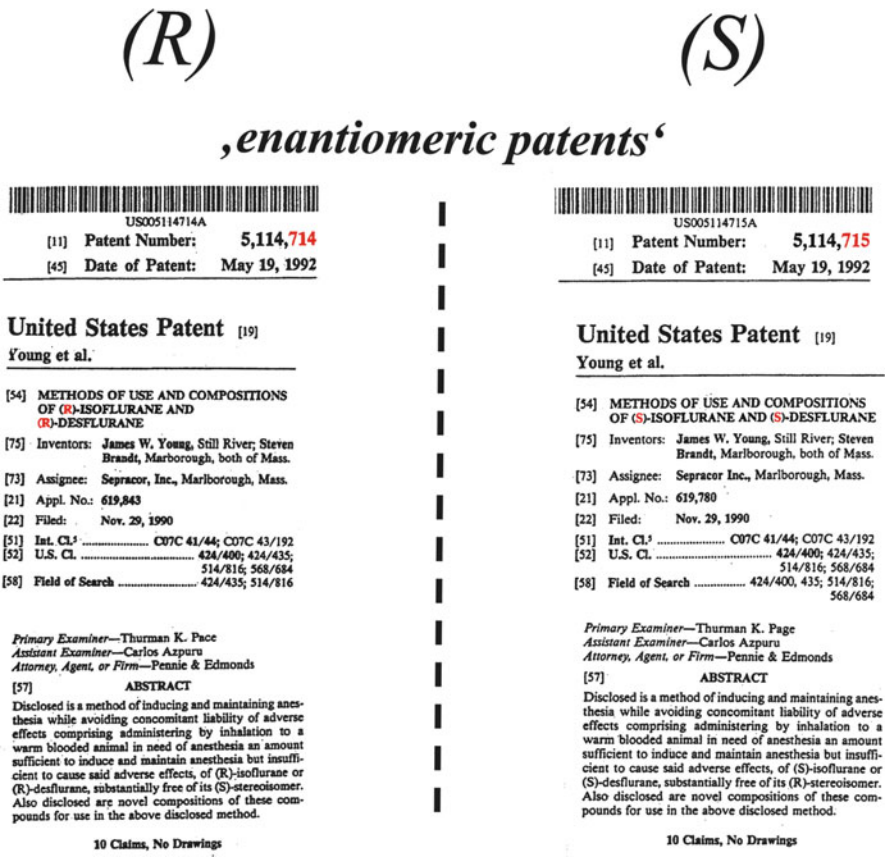

Fig. 15 "Enantiomeric" US patents 5,114,714 [78] and 5,114,715 [79] of May 19, 1992, claiming that (*R*)-isoflurane and (*R*)-desflurane (first patent) and (*S*)-isoflurane and (*S*)-desflurane (second patent) while inducing and maintaining anesthesia do not cause adverse effects associated with the (*R*,*S*)-racemates of isoflurane and desflurane

compared to the racemic composition in which (*R*)- and (*S*)-enantiomers are present. Unfortunately, the (*R*)- and (*S*)-enantiomers were not identified by their sign of optical rotation and the incertitude in the assignment of absolute configuration of desflurane could only be resolved later on (see above).

6 In Vivo Studies of the Enantiomeric Distribution of Administered Isoflurane and Desflurane in Humans After Anesthesia

Due to the availability of enantiomeric differentiation of isoflurane by chiral GC (Sect. 2), a small preponderance of the (+)-(*S*)-enantiomer over the (–)-(*R*)-enantiomer could readily be demonstrated for the first time in blood samples collected

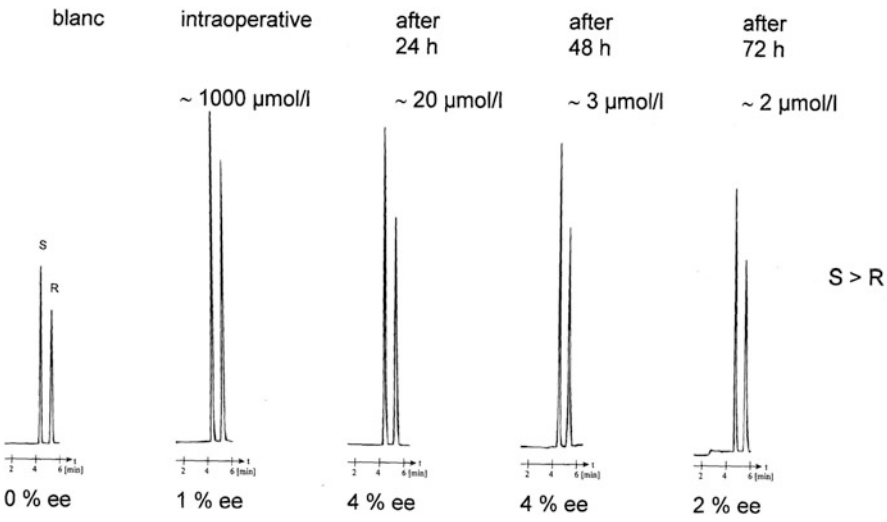

Fig. 16 Deviation from the 1:1 ratio of racemic isoflurane ((*S*) > (*R*)) during anesthesia in a single patient. 30 m × 0.25 mm (i.d.) fused-silica capillary column coated with 0.28 µm octakis (3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E) in PS 255 (30 wt%) at 28–29°C and 1.62 bar dihydrogen [80]

during anesthesia with racemic isoflurane in a routine surgery via a self-experiment (V.S. in 1997, Fig. 16) [80]. This finding was subsequently confirmed in a small group of patients undergoing eye surgery (Fig. 17) [81]. The highest enantiomeric bias of 51.8% (+)-(*S*)-isoflurane (ee = 3.6%) was observed after 1 day (1,440 min). This result initiated a comprehensive clinical study to detect differences in the pharmacokinetics of isoflurane enantiomers in humans involving a group of 41 volunteers (of whom 25 were females) who underwent anesthesia maintained with racemic isoflurane [82, 83].

Isoflurane enantiomers were analyzed in blood samples drawn before induction, at onset of anesthesia, after tracheal extubation, and daily for up to eight postoperative days by venous puncture. The following procedure was employed [82]. Blood samples were treated with the anticoagulant EDTA to prevent coagulation and 1 mL of the blood plasma was placed into 2-mL GC headspace vials and stored at −18°C. Prior to analysis, the sample vials were equilibrated for 10 min at 50°C at a preheating station before injection. A multipurpose sampler (Gerstel® MPS) was used for the headspace GC–MS analysis and it was combined with a cold injection system (Gerstel® CIS 3) for cold-trapping, enrichment, and cryo-focusing of the analyte (Gerstel, Mülheim an der Ruhr, Germany). The enantiomeric ratio of isoflurane was determined on a 30 m × 0.25 mm fused silica capillary column coated with 0.28 µm (film thickness) octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E) [28, 31] which was diluted in poly(vinylmethylsiloxane) PS 255 (30 wt%). The enantiomers were detected in the selected ion monitoring mode (GC–MS-SIM) with the ions 117 and 149 *m*/*z*. Thus interference of co-eluted volatiles (e.g., acetone, 2-propanol and, occasionally, ethanol!) was excluded (Fig. 18) [82]. Upon anesthesia, approximately

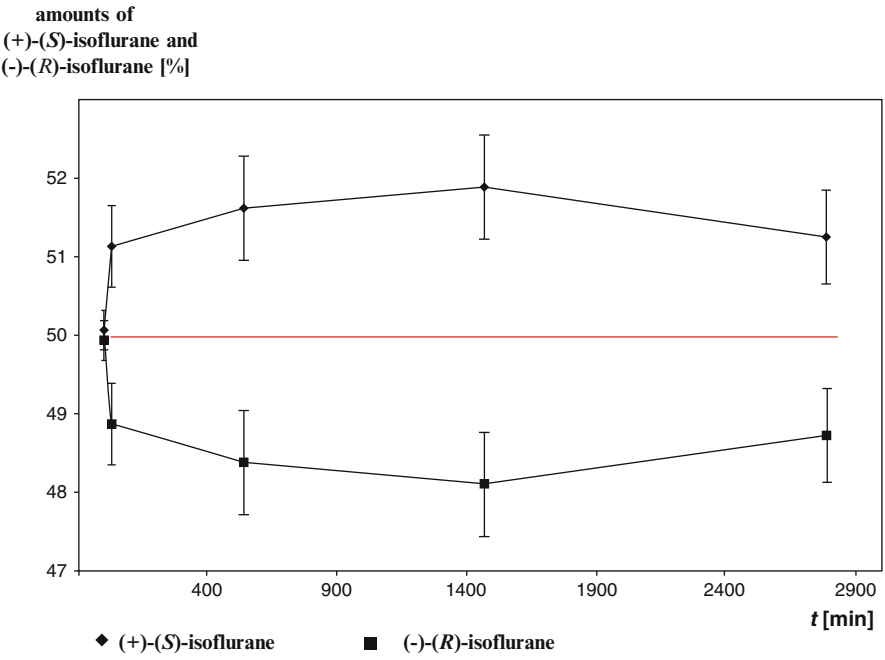

Fig. 17 Enrichment of (+)-(*S*)-isoflurane (first eluted enantiomer) in blood samples taken during and after eye surgery under narcosis with racemic isoflurane (mean of 36 measurements) [81]

1.2 vol.% of racemic isoflurane were added to the gas mixture of N_2O and O_2 (70:30, v/v) yielding blood concentrations of approximately 0.5 µmol/mL or ~0.1 g/L, which was well amenable to FID-detection of the anesthetic.

An enrichment of (+)-(*S*)-isoflurane was found in all blood samples drawn after anesthesia [82, 83] in agreement with the former studies (Figs. 16 and 17) [80, 81]. The highest level between 52% and 54% as opposed to 50% for the racemic form was reached on day 2 for most of the volunteers. Blood samples drawn after several days of anesthesia showed isoflurane concentrations below 1 nmol/mL blood (Sect. 7). It was therefore necessary to increase sensitivity by optimizing the headspace GC/MS method. The mass selective detector was used in the SIM mode (Fig. 18) with two monitored ions 117 and 149 *m*/*z* (Fig. 19) [82].

In a follow-up study, patients were categorized according to body mass index, duration of anesthesia, and pre-existing lung disease. None of these factors had any significant influence on the shift toward accumulation of (+)-(*S*)-isoflurane, although in obese patients the preponderance of the (+)-(*S*)-enantiomer exceeded that in non-obese patients [83]. Quite unexpectedly, and due to the extraordinary sensitivity of the GC set-up, isoflurane enantiomers were already present in blood samples which were drawn pre-operatively before the actual onset of anesthesia as the result of the omnipresence of traces of isoflurane in the operating room and/or contamination of medical instrumentation with minute amounts of isoflurane [80, 81].

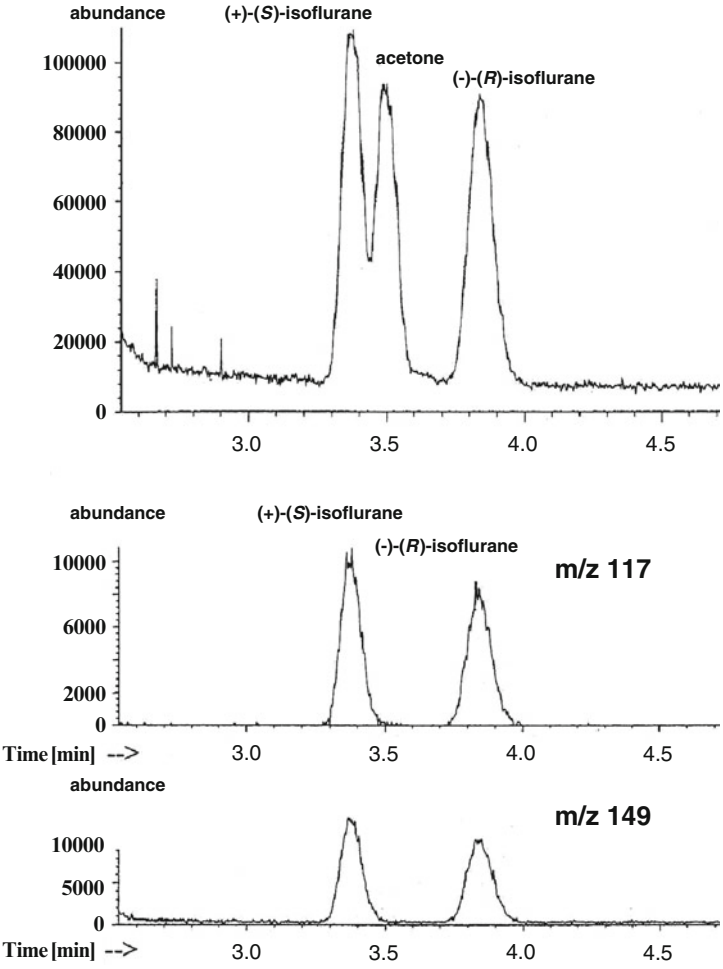

Fig. 18 *Top*: Total ion chromatogram (scan 40–200 m/z). *Bottom*: ion chromatogram by headspace GC/MS of a blood sample obtained at onset of isoflurane anesthesia [82]

The data obtained rely on the conditions that the distribution of the enantiomers in the headspace is not biased by the presence of enantiomeric constituents in the liquid (e.g., chiral proteins) and that the ionization of isoflurane enantiomers is not affected by the presence of co-eluted contaminants (e.g., acetone). Consequently, after each of 21–30 sample measurements (corresponding to samples of three patients), quality control experiments were performed with blank blood samples spiked by variable amounts of racemic isoflurane which gave the expected unbiased 1:1 ratio of the enantiomers within experimental error. The controls were analyzed in series with the patient specimens to exclude any deleterious effect (e.g., memory effects) of the analytical method [82]. In addition, the integration parameters (slope-test) were optimized [80, 82] which constitutes an essential requirement

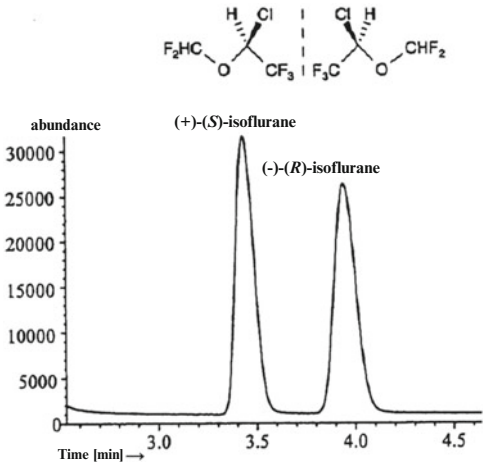

Fig. 19 Isoflurane analysis of a blood sample by GC/MS/SIM (117 and 149 *m/z*). 30 m × 0.25 mm (i.d.) fused-silica capillary column, retention gap 1.5 m, coated with 0.28 μm octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E) [28, 31] in PS 255 (30 wt%) at 33°C and 60 kPa helium [82]

when true 1:1 ratios of racemic mixtures, and small deviations therefrom, are quantified with high precision [84]. The mean enantiomeric enrichment (52.1 ± 0.1%) of (+)-(*S*)-isoflurane observed in vivo in the clinical study on day 2 as compared with the racemic isoflurane controls (50.0 ± 0.1%) was considered to be statistically significant [82]. The observed enrichment of (+)-(*S*)-isoflurane is important in view of the fact that isoflurane is subject to little (maximum 0.2%) or no biotransformation [85]. The distribution of volatile chlorofluoroethers into different tissues depends on uptake and arterial concentration, perfusion of the tissue, and the partition coefficient into the different compartments (blood plasma, interstitial fluid, fat tissue, intra- and transcellular fluid). The two enantiomers of the vaporized racemate may have different pharmacokinetic properties during uptake and redistribution. Enantioselective partitioning between gas and liquid may already occur in the lung, somewhat reminiscent of the principle of enantioselective gas chromatography! It has been concluded previously that anesthetics act on membranes rather than on lipids whereby isoflurane was shown to activate potassium channels (which silence the cell via hyperpolarization) whereby the (+)-(*S*)-enantiomer was about twice as effective as the (–)-(*R*)-enantiomer [86, 87].

The same methodology has been used to determine the enantiomeric ratio of isoflurane in urine samples collected from patients undergoing anesthesia. Comparable to blood samples, in urine samples isoflurane enantiomers could be determined up to 9 days after surgery [88, 89]. Yet in contrast to blood, in urine (–)-(*R*)-isoflurane was enriched to a low degree (up to 51.3%) [88].

In analogy to the isoflurane study, the time-dependent distribution of desflurane enantiomers in the blood and urine of five patients undergoing general anesthesia

with racemic desflurane was scrutinized [90]. EDTA-treated blood samples were drawn immediately after tracheal extubation. In the following 3 days, 1-mL blood and urine samples were collected and transferred in 2-mL GC-headspace vials. In all blood samples, an enrichment of (+)-(*S*)-desflurane (up to 53.6%) was found, whereas in urine (–)-(*R*)-desflurane (up to 52.9%) was abundant. For controls in which blood and urine samples were spiked with racemic desflurane, the expected 1:1 ratio within experimental error was found. The enantiomeric enrichment in the samples of the patients was appreciably higher than the standard deviation of the control measurements.

As isoflurane possesses a negligible metabolic rate (<0.2%) in humans [85], it is mostly eliminated via respiration in the course of anesthesia. Isoflurane could be detected in breath even 23 days after surgery [88]. The percentage of isoflurane enantiomers in human breath, although the anesthetic is administered as a racemate, varied significantly with the time of exhalation [91, 92]. Breath samples were collected using a 1-L-tedlar bag and were then transferred onto a freshly conditioned thermodesorption tube filled with Tenax®. Analysis was performed by direct thermodesorption-GC–MS (Gerstel® TDS 2) combined with the cold injection system (Gerstel® CIS 3). Any remaining water on the adsorbent was removed by a stream of helium at a TDS temperature of 20°C. Thermodesorption was achieved by raising the temperature to 220°C at a rate of 60°C/min and then keeping it there for 10 min. The temperature of the CIS was held at −150°C to cryo-focus the components efficiently and then heated to 250°C at a rate of 12°C/s. Isoflurane enantiomers in breath were quantified up to 19 days after surgery [91, 92]. During the early postoperative phase, (+)-(*S*)-isoflurane was slightly enriched (50.5% at day 3) whereas up to 5 days after surgery a noticeable excess of (–)-(*R*)-isoflurane (51.8% at day 19) was observed [91].

In view of the existing, but low, bias of isoflurane enantiomers during human anesthesia, and because the minimum alveolar concentrations (MACs necessary to prevent movement in response to a painful stimulus in rats) differ only minimally for isoflurane enantiomers [50], the use of single enantiomers of isoflurane, as insinuated in two consecutive patents (Sect. 5) [78, 79], is clearly unwarranted. Besides, enantioselective syntheses or preparative chromatographic enantioseparations of excessive amounts of chiral inhalation anesthetics would appear to be unrealistic and cumbersome. The findings also show that enantiomers do not always display striking differences in a biological chiral environment.

7 Quantitation of Enantiomers in a Sample by the Method of Enantiomer Labeling

The enantiomer of opposite configuration represents an ideal internal standard for the quantitation of the target enantiomer in a mixture. This has first been established in chiral α-amino acid analysis. Thus the amount of an L-amino acid in a sample has been extrapolated from the change of the enantiomeric ratio after addition of a *known*

amount of the oppositely configurated D-amino acid (or the DL-racemate) employed as an internal label. This method was first conceived by Bonner [93, 94] via referring to the use of an *enantiomer marker*. The approach, which was subsequently termed *enantiomer labeling* [95–97], is intriguing because enantiomers possess identical (non-chiroptical) properties in an achiral environment and therefore the enantiomeric composition is not influenced by sample manipulation (isolation, derivatization, fractionation, storage) or by chromatographic manipulations (dilution, partitioning, splitting, injection, detection). Not even thermal or catalytic decomposition, losses, or incomplete isolation will obscure the analytical result. Thus the added enantiomer serves as an overall internal standard through the whole analytical procedure. The method relies on the absence of self-recognition between enantiomers, undergoing molecular association (e.g., dimerization), in concentrated nonracemic mixtures (the *EE*-effect) [98]. However, such nonlinear effects in enantioselective chromatography [99] constitute a rather rare phenomenon and can probably be totally ignored in diluted systems.

The amount of a particular enantiomer (X_a) present in the sample is calculated from the ratio of the peak areas of the (*R*)-enantiomer (A_R) and the enantiomeric (*S*)-label (A_S) multiplied by the amount of the (*S*)-label (m_S) added as the internal standard [95]:

$$X_a = m_S \cdot \left(\frac{A_R}{A_S}\right). \tag{1}$$

Substance specific calibration factors *f* need not be considered by the enantiomer labeling method [95]. The method of enantiomer labeling can also be used for chiral compounds in samples and standards possessing only incomplete enantiomeric purities even including racemic compositions. The method only requires the precise knowledge of the enantiomeric ratios of the sample and the standard which are readily accessible by the same enantioselective GC method. The amount of the chiral component in a sample after addition of the chiral standard can be obtained as follows [95]:

$$X_i = m_S \cdot \left[\frac{(A_R - A_S \cdot C_S)(1 + C_R)}{(A_S - A_R \cdot C_R)(1 + C_S)}\right], \tag{2}$$

where A_R is the peak area of the (*R*)-enantiomer after addition of the standard, A_S is the peak area of the (*S*)-enantiomer after addition of the standard, C_R is the enantiomeric ratio (*S*)/(*R*) of the sample, C_S is the enantiomeric ratio (*R*)/(*S*) of the standard, m_S is the amount of enantiomeric standard (*S*) added, and X_i is the amount of the chiral component *i* (as sum of its enantiomers) present in the sample.

The amount of a racemate ((*S*)+(*R*)) present in a complex matrix can also be quantitatively determined via the enantiomer labeling method when a known amount of a single enantiomer ((*S*) or (*R*)) is added to the sample and the change of the peak areas is determined by enantioselective GC.

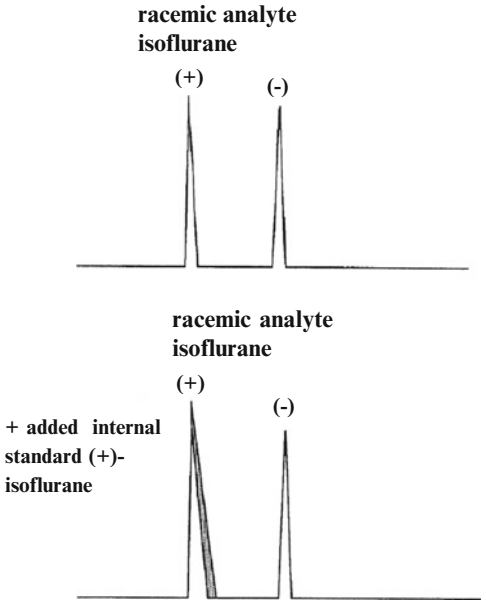

Fig. 20 Schematic chromatograms for enantiomeric labeling. *Top*: first analysis: enantioseparation of isoflurane in a sample. First and second peak: isoflurane enantiomers. *Bottom*: second analysis: enantioseparation of isoflurane in a sample after addition of the enantiomerically pure internal standard (+)-(*S*)-isoflurane. First peak: isoflurane enantiomer spiked with a known amount of the pure (+)-(*S*)-enantiomer (in practice the shaded peak will merge with the unshaded peak thereby enhancing the overall peak height). Second peak: non-labelled isoflurane enantiomer [81]

The quantitative determination of the inhalation anesthetic isoflurane in blood samples during and after surgery has been performed by enantioselective headspace GC–MS (Sect. 6), employing enantiopure (+)-(*S*)-isoflurane obtained by preparative GC (Sect. 3, Fig. 9, right) [48] as an internal standard [80, 81]. The method of enantiomer labeling requires two measurements on the enantioselective GC set-up (Fig. 20).

For quantitation, (3) has been used [81]:

$$P_{\text{isoflurane}} = \frac{W_{\text{standard}}}{W_{\text{sample}}} \cdot \frac{1}{\frac{A_{i,2}A_{k,1}}{A_{i,1}A_{k,2}} - 1}, \tag{3}$$

where $P_{\text{isoflurane}}$ is the percentage amount of each single enantiomer contained in the blood sample prior to the addition of the enantiopure standard (+)-(*S*)-isoflurane, W_{standard} is the weight of the internal standard of (+)-(*S*)-isoflurane solution added to the blood sample and being eluted as the first peak, W_{sample} is the weight of the blood sample in the headspace vial, $A_{i,1}$ and $A_{i,2}$ are the peak areas obtained for the first eluted enantiomer in the first measurement (before addition of the standard solution) and the second measurement (after addition of the standard solution), and

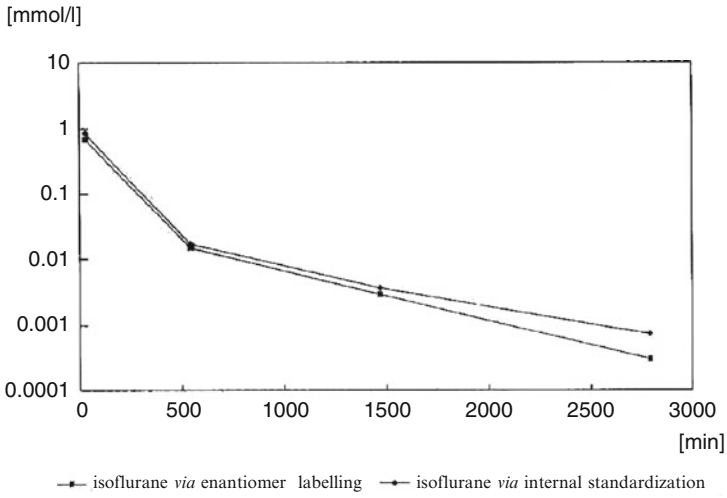

Fig. 21 Elimination of isoflurane in blood samples determined by enantiomer labeling and internal standardization [81]

$A_{k,1}$ and $A_{k,2}$ are the peak areas of the second eluted enantiomer in the first and second measurement.

The equation compensates for all errors caused by different injection volumes in the first and second measurement. The following procedure was used. Approximately 4 mL of blood specimens of patients undergoing eye surgery were collected before, during, and after surgery every 12 h for 3 days and stored after addition of citrate buffer at −20°C in vials sealed with silicon septa. A stock solution of a concentration of 0.80 μm/g of (+)-(*S*)-isoflurane in Ringer's solution was employed. Thus 1 g standard solution contained 0.15 mg (+)-isoflurane standard (molecular weight = 184.5 g/mol). For headspace GC analysis, 1 mL blood samples were transferred into 12-mL vials, sealed and equilibrated at 27°C for 30 min. The first analysis was performed prior to, and the second analysis after, addition of the stock solution containing (+)-(*S*)-isoflurane. Between 20 and 100 μL of the headspace were injected. Cryo-focusing was carried out to avoid band broadening (Sect. 6). The isoflurane enantiomers were then determined by enantioselective GC–MS (SIM) (Sect. 6). The elimination profile of isoflurane is depicted in Fig. 21. Isoflurane is washed out in blood samples in the range of 1,000–1 nmol/mL within 2 days after narcosis. In an independent study involving five patients undergoing general surgery under isoflurane anesthesia and employing the enantiomer labeling method by using (2), the isoflurane concentration decreased rapidly from ~500 nmol/mL blood sample (mean) directly after onset of anesthesia to ~0.5 nmol/mL (mean) 8 days after anesthesia (Table 1) [82].

The high volatility and the low gas–liquid partition coefficient (concentration in the liquid vs concentration in the solvent [100]) of isoflurane may cause analytical difficulties during the determination of its concentration in liquids. Apart from the problematic preparation of the standards at the concentrations in the therapeutic

Table 1 Isoflurane concentration (both enantiomers) in blood samples of five patients [82]

	Days after anesthesia								
Patient[a]	0	1	2	3	4	5	6	7	8
1	247.8	7.5	3.5	1.6	0.7	0.6	n.q.[b]	n.q.[b]	0.2
2	609.9	31.8	10.6	5.9	3.9	3.6	1.8	1.6	0.9
3	498.9	20.0	8.2	3.1	2.0	1.5	0.2	0.7	0.4
4	254.4	22.8	11.8	6.2	4.6	4.9	2.4	2.2	0.7
5	303.4	24.5	23.5	13.6	10.2	7.9	2.5	2.6	0.8

[a]Values are in nmol/mL blood
[b]Not quantified

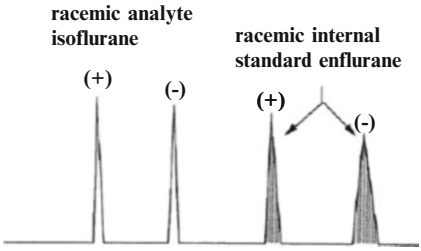

Fig. 22 Schematic chromatograms for internal standardization. First and second peak: isoflurane enantiomers; third and forth peak: known amount of internal standard racemic enflurane [81]

range (~1 mmol/L), any step in the storage and transfer of the standard which exposes the liquid to the gas phase will introduce an error via partitioning of enflurane from the liquid to the gas phase. Therefore the enantiomer labeling method is the method of choice as the internal standard possesses identical properties in complex mixtures.

The enantiomer labeling method was also compared with the common internal standard method (Fig. 22) [81]. As internal standard the racemic anesthetic enflurane (Fig. 2) has been selected. Isomeric enflurane possesses the same molecular weight as isoflurane. The method of internal standardization (Fig. 22) requires only a single measurement on the enantioselective GC set-up in contrast to the enantiomer labeling method (Fig. 20).

For quantitation, (4) has been used [81]:

$$P_{\text{isoflurane}} = \frac{W_{\text{standard}}}{W_{\text{sample}}} \cdot f_{\text{rr}} \cdot \frac{A_{\text{isoflurane}}}{A_{\text{standard}}}, \tag{4}$$

where $P_{\text{isoflurane}}$ is the percentage amount of each single enantiomer of isoflurane contained in the blood sample, W_{standard} is the weight of the internal standard of racemic enflurane solution added to the blood sample being eluted as two peaks, f_{rr} is the dimensionless relative response factor determined separately, and $A_{\text{isoflurane}}$ and $A_{\text{enflurane}}$ are the peak areas obtained for the two compounds.

Equation (4) can be used for the quantitation of either enantiomer of isoflurane by either enantiomer of enflurane. The total amount of isoflurane in the blood sample corresponds to the sum of both enantiomers. Since racemic enflurane is resolved into two peaks, it represents a twin standard molecule, i.e., double-checking of the analytical result is feasible in the case where the second eluted

peak of enflurane is not unduly broadened (Fig. 22). Figure 21 shows a small discrepancy when the two methods for the quantitation of isoflurane in blood samples were employed [81].

8 The Retention-Increment R' Approach and Thermodynamic Parameters of Enantiomeric Differentiation by GC

The gas-chromatographic enantiomeric differentiation is governed by thermodynamics and requires a *fast* and *reversible* 1:1 association equilibrium of the injected selectand enantiomers D and L and the enantiopure selector A present in the CSP. Different stabilities of the transient diastereomeric association complexes AD and AL are the prerequisite of gas-chromatographic enantiomeric differentiation. The true thermodynamic enantioseparation factor α^{assoc} is defined as the ratio of the association constants $K_{\text{D}}^{\text{assoc}}$ and $K_{\text{L}}^{\text{assoc}}$ of the energetically distinct diastereomeric complexes AD and AL according to $\text{A} + \text{D} \rightleftharpoons \text{AD}\,(K_{\text{D}}^{\text{assoc}})$ and $\text{A} + \text{L} \rightleftharpoons \text{AL}\,(K_{\text{L}}^{\text{assoc}})$, whereby D is arbitrarily eluted after L from the GC column [101]:

$$\alpha^{\text{true}} = \frac{K_{\text{D}}^{\text{assoc}}}{K_{\text{L}}^{\text{assoc}}}. \tag{5}$$

Thus, α^{true} quantifies the *true enantioselectivity* imparted by the enantiopure and undiluted selector A on the selectand enantiomers D and L. As shown for the gas-chromatographic enantioseparation of enflurane, isoflurane, and desflurane, (5) applies to the undiluted CD selector [26]. However, in contemporary enantioselective GC, chiral selectors (amino acid derivatives, metal complexes, or modified cyclodextrins) are either diluted in, or chemically bonded to, polysiloxanes used as achiral solvent matrix S. In the case of a diluted selector A present in S, the apparent enantioseparation factor α^{app} is defined as the ratio of the retention factors k_{D} and k_{L} of the selectand enantiomers D and L observed on the total CSP (A in S) [101]:

$$\alpha^{\text{app}} = \frac{k_{\text{D}}}{k_{\text{L}}}, \tag{6}$$

α^{app} being customarily used as a practical measure of enantioselectivity. However, when the selector A is diluted in an achiral solvent S, the retention factors k_{D} and k_{L} of the enantiomers D and L are determined (1) by non-enantioselective contributions to retention which arise from the achiral solvent S and which are identical for the enantiomers and (2) by the different enantioselective contribution to retention due the distinct diastereomeric molecular association of the enantiomers D and L with the chiral selector A. Due to the presence of an achiral matrix in the total CSP, i.e., the solvent S containing the chiral selector A, α^{app} is always lower than α^{true}.

In order to obtain data on the true thermodynamics of enantiomeric recognition by (5), the achiral contributions to retention must be separated from the chiral contribution to retention via the retention-increment R' approach [102]. The method requires the determination of retention data of the enantiomers D and L (1) on a reference column containing only the pure solvent S and (2) on a column containing the selector A diluted in, or chemically bonded to, the solvent S.

In the reference column, the enantiomers D and L are distributed between the mobile gas phase and the stationary phase containing the solvent S and the general equation of chromatography applies (k = retention factor, K_c = distribution constant, β = mobile phase over stationary phase volume ratio, t_R = total retention time, t'_R = adjusted retention time, t_M = unretained-peak holdup time with $t'_R = t_R - t_M$; the circle above the parameters refers to the reference column):

$$k^\circ = \frac{t'^\circ_R}{t^\circ_M} = \left(\frac{t^\circ_R}{t^\circ_M}\right) - 1 = \frac{K^\circ_c}{\beta^\circ}, \tag{7}$$

k° being identical for the enantiomers D and L on the achiral solvent S. If a chiral selector A, which interacts with the enantiomers, is diluted in the solvent S, enantioselectivity is introduced into the separation process according to the different diastereomeric association constants K_D^{assoc} and K_L^{assoc}. For each enantiomer, a distinct retention-increment R' is observed. It can be calculated from the retention factor k of the enantiomer measured on the enantioselective column containing A in S:

$$k = \frac{t'_R}{t_M} = \left(\frac{t_R}{t_M}\right) - 1 = \frac{K_c}{\beta}, \tag{8}$$

and the retention factor k° of the same enantiomer measured on the reference column containing only the solvent S (7). With $K_c = K^\circ_c + K^\circ_c\, K^{assoc} - a_A$ and $\beta^\circ = \beta$, the following equation for the retention-increment R' has been derived (K^{assoc} = association constant and a_A = activity of A in S) [66]:

$$R' = \left(\frac{k}{k^\circ}\right) - 1 = K^{assoc} \cdot a_A. \tag{9}$$

The retention-increment R' quantifies the increase of retention due to the association of each single enantiomer D and L with A in S. Equation (9) requires that the holdup times, t°_M and t_M, and the phase ratios, β° and β, of the reference column (S) and the enantioselective column (A in S) are identical. Column dimensions as well as the amount of solvent S in both columns must also be the same. In order to become independent of all column parameters, relative retentions r ($r = k/k_{ref} = t'_R/t'_{Rref}$) and r° ($r^\circ = k^\circ/k^\circ_{ref} = t'^\circ_R/t'^\circ_{Rref}$), correlated to an *inert reference standard*, have been used in practice [66, 102, 103]. Equation (9) can thus be rewritten as

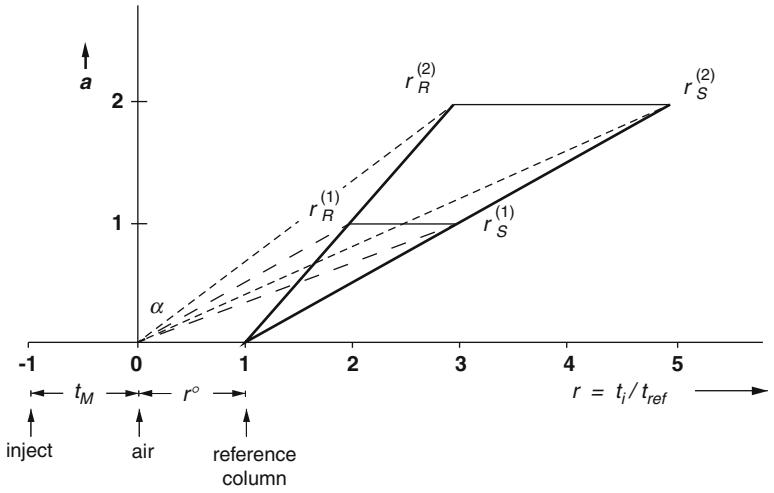

Fig. 23 Schematic representation of the distinction between (1) the non-enantioselective contribution to the relative retention of r°, and (2) the enantioselective contribution to the retention of the enantiomers (*R*) and (*S*), $r_i - r^\circ$, leading to a constancy of the retention-increment ratio R'_S/R'_R as required by (11). t_M and r° were arbitrarily set at unity, and the ratio R'_S/R'_R was arbitrarily set 2:1 [104]

$$R' = \left(\frac{r}{r^\circ}\right) - 1 = K^{\text{assoc}} \cdot a_A. \tag{10}$$

Thus, for the enantiomers D and L, irrespective of a_A, the ratio $K_D^{\text{assoc}}/K_L^{\text{assoc}}$ is directly related to the ratio of the retention-increments R'_D/R'_L which is readily accessible from the relative retentions r_D, r_L, and r°, and the true enantioselectivity $-\Delta_{D,L}\Delta G^{\text{true}}$ in diluted selector systems is then obtained as follows:

$$\begin{aligned} -\Delta_{D,L}\Delta G^{\text{true}} &= -\Delta_{D,L}\Delta H^{\text{true}} + T\Delta_{D,L}\Delta S^{\text{true}} = \text{RT ln}\, a^{\text{true}} \\ &= \text{RT ln}\left(\frac{K_D^{\text{assoc}}}{K_L^{\text{assoc}}}\right) = \text{RT ln}\left(\frac{R'_D}{R'_L}\right) = \text{RT ln}\left(\frac{r_D - r^\circ}{r_L - r^\circ}\right). \end{aligned} \tag{11}$$

Equation (11) links the true enantioselectivity $-\Delta_{D,L}\Delta G^{\text{true}}$ (and the enthalpic and entropic parameters $\Delta_{D,L}\Delta H^{\text{true}}$ and $\Delta_{D,L}\Delta S^{\text{true}}$ via the Gibbs–Helmholtz equation) with the individual retention-increments R' of the enantiomers D and L. From a thermodynamic point of view the distinction between α^{app} and α^{true} is mandatory. A schematic representation of the inherent differences between r° and r underscoring the differences between RT ln α^{true} and RT ln α^{app} is depicted in Fig. 23 [102, 104].

It follows from (11) that the expression $-\Delta_{D,L}\Delta G$ {=} RT ln α^{app}, linking enantioselectivity with the apparent enantioseparation factor being frequently used in the literature, is inappropriate when diluted selectors A are employed in enantioselective GC.

For α^{app} the new (12) is obtained by substituting k_D and k_L in (6) via the expression of (9):

$$a^{app} = \frac{K_D^{assoc} a_A + 1}{K_L{}^{assoc} a_A + 1} = \frac{R_D' + 1}{R_L' + 1} \tag{12}$$

Thus α^{app} approaches α^{true} of (5) only when the association constants K^{assoc} and/or the activity a_A of A in S are high, resulting in $R' >> 1$ [24], or when non-enantioselective contributions to retention are negligible, i.e., $r^\circ \sim 0$ (Fig. 23). Moreover α^{true}, and hence the true enantioselectivity $-\Delta_{D,L}\Delta G^{true} = RT \ln \alpha^{true}$, is independent of the activity a_A (or concentration c_A at high dilution) of A in S, whereas α^{app} is concentration-dependent due a mixed retention mechanism [24, 101]. However, a concentration-dependence is not compatible with a true thermodynamic quantity. The retention-increment R' approach has also been applied for binary selector systems, i.e., diluted mixed CSPs containing two different chiral selectors [101].

The differentiation of apparent and true enantioselectivity in enantioseparations was at first employed in enantioselective complexation GC employing metal chelate CSPs [103] and was later extended to enantioselective inclusion GC utilizing modified cyclodextrin selectors [24, 104, 105]. The distinction between non-enantioselective and enantioselective interactions has also been adopted in enantioselective LC [106–108].

It should be recognized that the enantioselectivity, as expressed by $-\Delta_{D,L}\Delta G^{true}$, is only determined by the *ratio* of the association constants of the selectand enantiomer and the chiral selector, $RT \ln(K_D^{assoc}/K_L^{assoc})$, regardless of whether the average associations of the selectand enantiomers D and L and the selector A, $-\Delta G = RT \ln K^{assoc}$, are weak, intermediate, or strong. Thus, high enantioselectivities are often observed at quite low retention-increments R' (due to weak molecular association) while only low enantioselectivities may arise despite high retention-increments R' (due to strong molecular association). Consequently, the value of a virtual enantiorecognition factor $\chi = -\Delta_{D,L}\Delta G/-\Delta G$ is unpredictable and varies at random [109]. The enantioselectivity is usually pronounced when the selectand/selector interaction is impaired by steric congestion.

The Gibbs–Helmholtz parameters $-\Delta_{D,L}\Delta H$ and $\Delta_{D,L}\Delta S$ of the thermodynamic enantioselectivity are readily accessible by linear van't Hoff plots when measurements are performed at different temperatures T according to

$$R \ln\left(\frac{R_D'}{R_L'}\right) = \frac{-\Delta_{D,L}\Delta G}{T} = \frac{-\Delta_{D,L}\Delta H}{T} + \Delta_{D,L}\Delta S. \tag{13}$$

According to the Gibbs–Helmholtz equation (11), the true enantioselectivity $-\Delta_{D,L}\Delta G$ is governed by an enthalpy term $-\Delta_{D,L}\Delta H$ and an entropy term $\Delta_{D,L}\Delta S$, where the latter term is linked with the temperature T. For a 1:1 association equilibrium both quantities oppose each other in determining $-\Delta_{D,L}\Delta G$. Thus *enthalpy/entropy compensation* arises due to the fact that the more tightly bonded

complex ($-\Delta H_D > -\Delta H_L$) is more ordered ($\Delta S_D < \Delta S_L$). Since the entropy term increases with temperature T, an *isoenantioselective temperature* will be reached at the compensation temperature $T_{iso} = \Delta_{D,L}\Delta H/\Delta_{D,L}\Delta S$ at which $\Delta_{D,L}\Delta G$ is rendered zero (i.e., absence of enantioselectivity), since $K_D^{assoc} = K_L^{assoc}$. Peak coalescence (no enantioseparation) takes place at T_{iso} [110]. Above T_{iso} enantioseparation commences with inversed elution order. Below T_{iso} enantioseparation is governed by the predominant enthalpic contribution to enantiorecognition, whereas above T_{iso} it is governed by the predominant entropic contribution ($\Delta S_D < \Delta S_L$) to enantiorecognition with the stronger bonded enantiomer D ($-\Delta H_D > -\Delta H_L$) bizzarrely eluted as the first peak. Whereas the sign of the enantioselectivity changes at T_{iso}, the association constants K_D^{assoc} and K_L^{assoc} between the selectand enantiomers D and L and selector A steadily decrease with increasing temperature T. The existence of an isoenantioselective temperature T_{iso} in enantioselective GC has been observed independently in hydrogen-bonding equilibria [111, 112] and in metal complexation equilibria [113, 114] whereby the intriguing peak reversal for the enantiomers below and above T_{iso} was clearly evident in the gas-chromatograms. A report of a peak reversal for the enantiomers of methyl lactate on a modified cyclodextrin selector (Lipodex E) [115] could not be ascertained [112] while a temperature-induced reversal of the elution order has been observed for the enantiomers of *N*-trifluoroacetyl-α-amino acid ethyl esters on the selector Chirasil-Dex [112]. Thus the derivatives of valine and leucine showed a peak reversal on Chirasil-Dex below and above $T_{iso} = 70°$ and only a single peak was observed at the coalescence temperature. For the isoleucine derivative the isoenantioselective temperature was as low as $T_{iso} = 30°$ [112]. A different enthalpy/entropy compensation approach based on the plot of ln R' vs ΔH for the second eluted enantiomer has also been described [105].

Enthalpy/entropy compensation must be considered for molecular modeling studies whereby the importance of entropy changes should be taken into account. Most gas-chromatographic enantioseparations on modified cyclodextrins are governed by the enthalpy term of the Gibbs–Helmholtz equation. Consequently, enantioselectivity increases by reducing the elution temperature. This will be illustrated in the GC enantioseparation of halocarbons on CDs in the following sections. As nonvolatile racemates usually require a high elution temperature, it is advisable to use short columns (1–10 m × 0.25 mm i.d.). The loss of efficiency arising from the smaller theoretical plate number N of a short column is often overcompensated by the gain of enantioselectivity below T_{iso}, due to an increased enantioseparation factor α^{true} at the lower elution temperature.

9 Determination of Thermodynamic Parameters of the Enantioselectivity of Enflurane, Isoflurane, and Desflurane on Diluted Lipodex E

The pronounced enantioselectivity between the haloethers enflurane, isoflurane, and desflurane on Lipodex E diluted in SE 54 allows quantitative enantioseparations within 2.5 min (Fig. 5) [31]. The apparent enantioseparation factor α^{app} strongly increases in the order: isoflurane < desflurane < enflurane and the observed $\alpha^{app} > 2.0$ for enflurane can still be increased to $\alpha^{app} = 2.7$ at 0°C. In order to get thermodynamic data on the true enantioselectivity by the retention-increment R' approach (Sect. 8), three columns were used. The reference column (50 m × 0.25 mm i.d.) was coated statically with pure polysiloxane (polydimethylsiloxane SE 54 containing 5% phenyl, and 1% vinyl, CP polarity index 8) while the enantioselective columns (25 m × 0.25 mm i.d.) were coated statically with ~5% and ~10 wt% Lipodex E in polysiloxane SE 54. The stationary phase film thicknesses were 0.5 μm. The haloethers, methane (as holdup time marker) and the reference standards (*n*-pentane, *n*-hexane, *n*-heptane, or diethylether) were mixed in headspace vials and were split-injected (1:100). Highly precise data for the true enantioselectivity $-\Delta_{DL}\Delta G^{true}$ were obtained [104] irrespective of the choice of the reference standards (C5–C7, diethylether) and the concentration of the selector in the polysiloxane (5% vs 10%), thus underlining the validity of (11) and the need to separate rigorously achiral from chiral contributions to retention in (10) via the concept of the retention-increment R'. The true enantioselectivity $-\Delta_{DL}\Delta G^{true}{}_{303}$ at 30°C is 2.0 (0.02) kJ/mol for enflurane, 0.9 (0.03) kJ/mol for isoflurane and 1.55 (0.03) kJ/mol for desflurane obtained on the column with 10% selector concentration and as mean value for all reference standards used. Temperature-dependent data for the true enantioselectivity $-\Delta_{DL}\Delta G^{true}$ obtained at five temperatures between 0 and 60°C and measured at both concentrations of the selector (5 wt% and 10 wt%) and four reference standards furnished the following mean thermodynamic parameters via the strictly linear van't Hoff plots according to (13) [104]:

$$\text{enflurane}:$$
$$-\Delta_{DL}\Delta H^{true} = 7.2(0.2)\ \text{kJ/mol}$$
$$\Delta_{DL}\Delta S^{true} = -17.2(0.6)\ \text{J/mol K}$$
$$T_{iso} = 420(40)\text{K},$$

$$\text{isoflurane}:$$
$$-\Delta_{DL}\Delta H^{true} = 4.1(0.2)\ \text{kJ/mol}$$
$$\Delta_{DL}\Delta S^{true} = -10.6(0.5)\ \text{J/mol K}$$
$$T_{iso} = 390(60)\text{K},$$

$$\text{desflurane}:$$
$$-\Delta_{DL}\Delta H^{true} = 9.4(0.6)\ \text{kJ/mol}$$
$$\Delta_{DL}\Delta S^{true} = -25.7(2.1)\text{J/mol K}$$
$$T_{iso} = 365(40)\ \text{K}.$$

The highest enantioselectivity $-\Delta_{DL}\Delta G$ was found for enflurane whereas the largest quantities for $-\Delta_{DL}\Delta H$ and $\Delta_{DL}\Delta S$ were observed for desflurane. When comparing isoflurane and desflurane, substitution of chlorine by fluorine leads to more than a doubling of the thermodynamic quantities. The large enthalpy term for desflurane implies that temperatures as low as possible should be employed for enantioseparation and that $-\Delta_{DL}\Delta G$ of desflurane will exceed that of enflurane below −15°C [104]. The ratio between $-\Delta_{DL}\Delta H$ and $\Delta_{DL}\Delta S$ is not constant, furnishing different values of T_{iso} for enflurane, isoflurane, and desflurane. The high volatility of the haloethers precluded the experimental verification of the existence of T_{iso} above 100°C.

For $2 \times 3 \times 4 \times 6 = 144$ measurements, involving the two enantiomers of three haloethers related to four references standards at six temperatures, all ratios of the retention-increments R' for the two columns 1 and 2, containing different concentrations of the selector A (~5% vs ~10%), were identical, i.e., ${R'_L}^{(2)}/{R'_L}^{(1)} = {R'_D}^{(2)}/{R'_D}^{(1)} = {a_A}^{(2)}/{a_A}^{(1)} = 1.64 \pm 0.04$. According to (9) the constant factor directly reflected the true activity ratio ${a_A}^{(2)}/{a_A}^{(1)}$ of the two columns 1 and 2, their absolute values being unknown. By the known activity ratio and the measured sets of relative retentions $r^{(1)}$ and $r^{(2)}$, the r° values of all three haloethers expected for the reference column were graphically extrapolated (or calculated via (14), Sect. 11) and a reasonably good agreement (0.5–18% standard deviation) indicated that r° values can even be assessed without resorting to a reference column [104]. The results also confirmed the validity of the retention-increment R' approach to distinguish enantioselective and non-enantioselective contributions to retention in chiral GC (Fig. 23) [104].

The enantioselectivity $-\Delta_{DL}\Delta G^{true}$ for enflurane and Lipodex E has been corroborated by ^{1}H-NMR spectroscopy employing the modified cyclodextrin selector as CSA in d_{12}-cyclohexane for enantiomeric differentiation [31, 116]. It was observed that the proton resonance absorptions of (*S*)-enflurane were shifted more downfield than those of (*R*)-enflurane in the presence of Lipodex E. Likewise (*S*)-enflurane is eluted well after (*R*)-enflurane on Lipodex E in the GC experiment. A ratio of the association constants $K_S^{assoc}/K_R^{assoc} = 2.25$ was found as mean value for the α- and β-proton of enflurane in d_{12}-cyclohexane in the presence of Lipodex E yielding $-\Delta_{SR}\Delta G_{298} = 2.00$ kJ/mol [116]. This value compares well with the GC data of $-\Delta_{SR}\Delta G_{298} = 2.08$ kJ/mol in SE-54 at 25°C [104] whereby the absolute association constants K^{assoc} of the GC and NMR experiments differed by a factor of 5 due to different experimental conditions of the two methods. Notwithstanding, the result confirms the synergism between enantioselective NMR spectroscopy and enantioselective GC.

For an undiluted selector, thermodynamic parameters can be directly obtained from the equation RT ln $\alpha = -\Delta_{D,L}\Delta G$ (Sect. 8). Temperature-dependent measurements in the range 35–50°C gave the following averaged enantioselectivity data for the haloethers enantioseparated by GC on octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-cyclodextrin [26]:

$$\text{enflurane}: -\Delta_{DL}\Delta H = 4.51\ \text{kJ/mol},\ \Delta_{DL}\Delta S = -10.9\ \text{J/mol K}$$
$$\text{isoflurane}: -\Delta_{DL}\Delta H = 6.09\ \text{kJ/mol},\ \Delta_{DL}\Delta S = -15.7\ \text{J/mol K}$$
$$\text{desflurane}: -\Delta_{DL}\Delta H = 3.79\ \text{kJ/mol},\ \Delta_{DL}\Delta S = -9.4\ \text{J/mol K}.$$

The isoenantioselective temperatures T_{iso} were estimated at around 400 K (~125°C) but could not be verified experimentally. All thermodynamic data represent only average values owing to a range of interactions between the anesthetics and the cyclodextrin selectors as the CSP octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-cyclodextrin employed was comprised of a mixture of isomers and homologues [26].

10 ^{1}H-NMR NOE Difference Spectroscopy of Enflurane and Lipodex E

Intermolecular ^{1}H-NMR-nuclear Overhauser effect (NOE) investigations were performed to obtain insights into the molecular complexes formed by the stronger interacting (*R*)-enantiomer of enflurane and octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) [116]. A negative NOE intensity change for the H-α- and H-β-proton signals of (*R*)-enflurane was detected upon irradiation of the inner protons H-3 and H-5 of Lipodex E while no NOE intensity changes were observed upon irradiation of the corresponding outer protons H-1, H-2, H-4, H-6A, H-6B, or either of the diastereotopic butanoyl protons of Lipodex E (Fig. 24). Analogous results were obtained for (*S*)-enflurane and Lipodex E. Thus it was concluded that both enflurane enantiomers are clearly located inside the cyclodextrin cavity. Internal and external contributions to enantiomeric differentiation involving cyclodextrins have been discussed previously [5] and the total absence of molecular inclusion is manifested by the efficient enantioseparation capability of modified linear dextrins ("acyclodextrins") [117]. The established inclusion properties of Lipodex E for haloethers have been ascribed to internal dipole–dipole interactions and hydrogen-bonding involving acidic H-α- and H-β-protons of enflurane [116]. Precise molecular modeling studies have been elusive thus far.

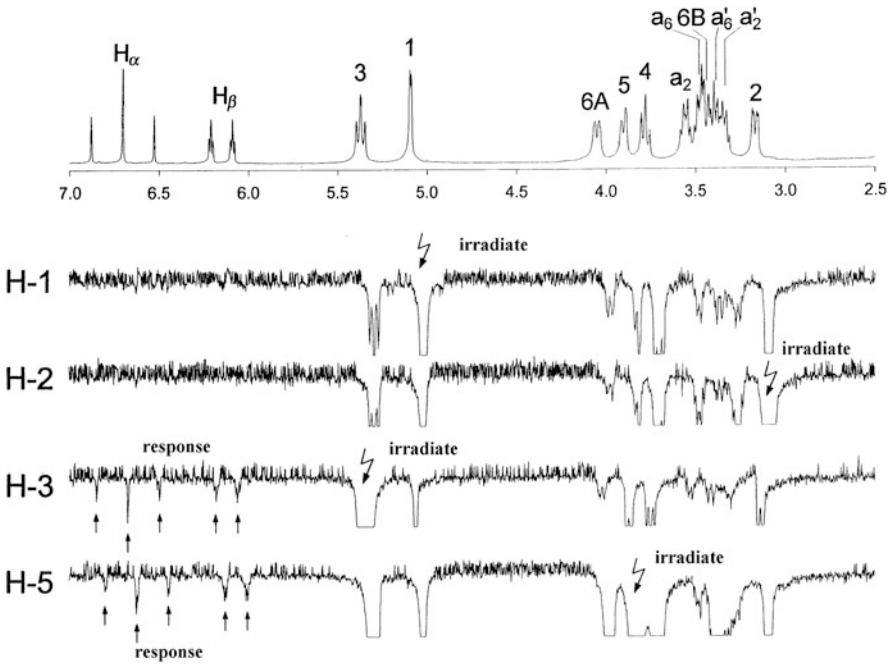

Fig. 24 400 MHz ^{1}H-NMR NOE difference spectra of 0.078 M (*R*)-enflurane in the presence of 0.013 M of the CSA Lipodex E in d_{12}-cyclohexane [116]. *Top*: Spectrum of enflurane at 6–7 ppm and spectrum of Lipodex E at 3.0–5.5 ppm. *Bottom*: Irradiation of external cyclodextrin H-1 and H-2 (no response) and of internal cyclodextrin protons H-3 and H-5 (with response)

11 An Extraordinary Enantiomeric Differentiation Between "Compound B" and Lipodex E

Enantiomeric differentiations by GC employing modified cyclodextrins are usually characterized by low enantioselectivities [2, 16]. Yet, due to the high resolving power of capillary GC, very low enantioseparation factors of $1.01 < \alpha < 1.10$ are sufficient for quantitative analytical enantioseparations in a short time. However, for an understanding of chromatographic enantioselectivity [118, 119], small values of α are detrimental to reliable mechanistic studies of enantiorecognition [105, 120], e.g., by molecular modeling calculations involving cyclodextrins [119, 121–123]. Low enantioselectivities are also totally inappropriate for predictions of the elution order of enantiomers on modified cyclodextrins (Sect. 5). The correlation of the elution order and configuration of the separated enantiomers may further be obscured by the possibility of peak reversals due to enthalpy-entropy compensation (Sect. 8), thus requiring extended temperature-dependent studies. High values of $\alpha > 1.5$ are only rarely encountered for selectand enantiomers in enantioselective GC employing modified cyclodextrin selectors. Yet unexpected large enantioseparation factors α has been observed with enflurane,

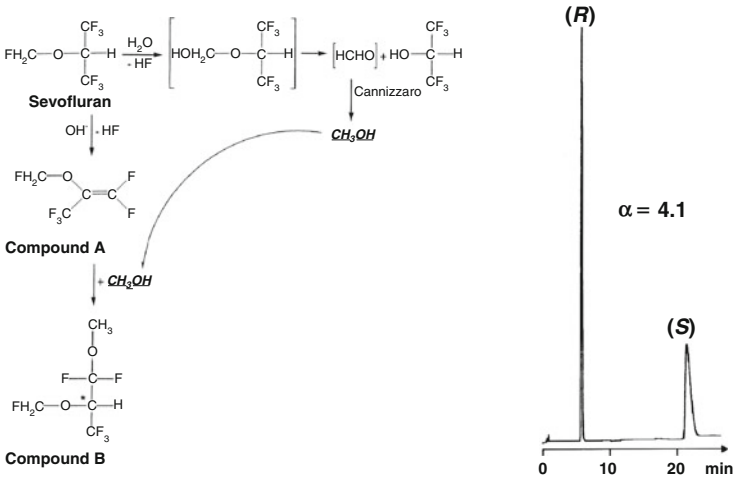

Fig. 25 *Left*: Decomposition products of sevoflurane in an alkaline environment [129]. *Right*: Analytical gas-chromatographic enantioseparation of "compound B." 10 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.25 μm heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS 86 (~15% diphenyl, ~85% dimethyl-PS, 20 wt%) at 30°C and 1.25 bar dihydrogen [129]

isoflurane, and desflurane (Sect. 2). This observation is in line with a general trend of favourable enantioselectivities occurring with halogenated chiral compounds and modified cyclodextrins. Even the smallest five-atomic halocarbons bromochlorofluoromethane and chlorofluoroiodomethane can be resolved by enantioselective inclusion GC (Sect. 12).

Methyl 2-chloropropanoate has been enantioseparated on undiluted heptakis (3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin (Lipodex D) [124] with $\alpha = 2.02$ corresponding to $-\Delta_{S,R}\Delta G = 2.1$ kJ/mol at 60°C, whereby the (*S*)-enantiomer is eluted after the (*R*)-enantiomer, and NMR studies and molecular dynamics calculations were carried out for this efficient enantioselective system [125, 126]. Methyl 2-chloropropanoate was also enantioseparated on octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) with $\alpha = 2.27$ corresponding to $-\Delta_{S,R}\Delta G = 2.34$ kJ/mol, $-\Delta_{S,R}\Delta H = 13.8$ kJ/mol, and $-\Delta_{S,R}\Delta S = 33.5$ J/mol K at 70°C [127]. The enantioseparation of methyl 2-chloropropanoate on undiluted heptakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-β-cyclodextrin at 50°C gave the following thermodynamic data: $-\Delta_{S,R}\Delta G = 3.01$ kJ/mol, $-\Delta_{S,R}\Delta H = 13.4$ kJ/mol, $-\Delta_{S,R}\Delta S = 31.4$ J/mol K, and $T_{iso} = 150$°C [128]. All thermodynamic data refer to true values since the selectors were used in the undiluted form (Sect. 8).

The enantioseparation of "compound B" (2-(fluoromethoxy)-3-methoxy-1,1,1,3,3-pentafluoropropane) (Fig. 25, left) on modified CDs exhibited the highest enantioseparation factor α ever observed in enantioselective GC [129]. This unexpected result implies that one enantiomer undergoes a strong molecular association, whereas the other enantiomer does not. The very high GC enantioseparation factors α for "compound B" on modified CDs have been found by mere chance as the target

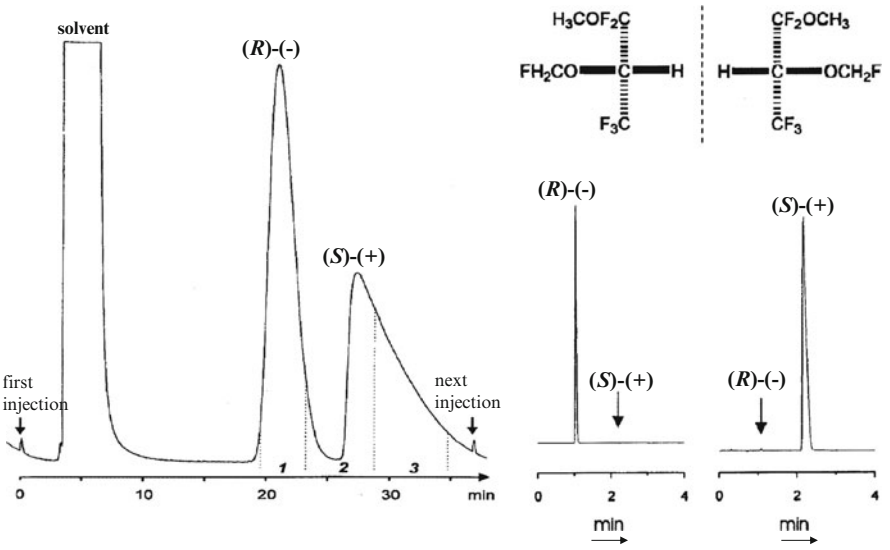

Fig. 26 *Left*: Preparative gas-chromatographic enantioseparation of "compound B" dissolved in diethylether. 1 m × 18 mm (i.d.) stainless steel column packed with Chromosorb P-AW-DMCS (80–100 mesh) coated with 19.1 wt% of heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS 86 (15.2 wt%) at 70°C and 1.4 bar dinitrogen. The *dotted lines* indicate the three fractions collected [129]. *Right*: Gas-chromatographic determination of the high enantiomeric excess (ee) of the isolated single enantiomers (conditions as in Fig. 25, *right*) [129]

compound represents just a minor chiral decomposition product of the achiral new generation anesthetic sevoflurane (1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane) (Fig. 25, left) [130]. Since volatile anesthetics are recycled during anesthesia in re-breathing units, the exhaled carbon dioxide is trapped with soda lime. In the alkaline environment, methanol is formed by a Cannizzaro reaction from formaldehyde which is the result of hydrolysis of sevoflurane. Methanol is then added to the double bond of "compound A" which is generated by hydroformic acid abstraction from sevoflurane by soda lime (Fig. 25, left). The perfluorodiether "compound B" represents a chiral molecule. It has been analytically enantioseparated on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin [131] diluted in polysiloxane PS 86 (~15% diphenyl, ~85% dimethyl-PS, 20 wt%), exhibiting the large enantioseparation factor $\alpha = 4.1$ at 30°C (Fig. 25, right) [129]. The high enantioselectivity allowed the isolation of the enantiomers of "compound B" by preparative GC (Sect. 3) and the subsequent determination of their specific rotation by polarimetry and their absolute configurations both by anomalous X-ray diffraction [129] and by VCD [132]. Thus in ten repetitive runs under the conditions detailed in Fig. 26 (left), a total of 275 mg of the first eluted enantiomer were collected as first fraction with ee $> 99.9\%$ and 73 mg of the second eluted enantiomer as third fraction with ee $> 99.7\%$, whereby the middle fraction was not discarded but re-injected [129]. The high enantiomeric excess (ee) of both enantiomers has been determined analytically (Fig. 26, right). By X-ray evidence, the levorotatory enantiomer had the (–)-(*R*)-configuration while the dextrorotatory

enantiomer had the (+)-(*S*)-configuration. The elution order of the enantiomers of "compound B" on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin is (*S*) after (*R*) [129]. In order to gain information on the origin of enantiomeric differentiation, ^{1}H- and ^{19}F-NMR-spectroscopic studies have been performed whereby heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin has been employed as a CSA for inducing chemical shift anisochrony of the externally enantiotopic nuclei of racemic "compound B" in d_{12}-cyclohexane as solvent [133]. Experiments with single enantiomers of "compound B" obtained by preparative GC revealed larger chemical shifts of the protons of the (*S*)-enantiomer as compared to the (*R*)-enantiomer by ^{1}H-NMR in the presence of the cyclodextrin selector in agreement with the GC experiment where (*S*) is eluted later than (*R*) [129]. A ratio of the association constants $K_S^{\text{assoc}}/K_R^{\text{assoc}} = 1.5$ was found as mean value for the α- and β-proton of enflurane in d_{12}-cyclohexane in the presence of the cyclodextrin selector yielding $-\Delta_{SR}\Delta G_{298} = 1.00$ kJ/mol. This value is lower than expected from the GC experiment exhibiting $\alpha = 4.1$ at 30°C corresponding to $-\Delta_{SR}\Delta G_{303} = 3.5$ kJ/mol [129]. Also by ^{19}F-NMR spectroscopy a large anisochrony was observed whereby the fluorine resonances of the (*S*)-enantiomer were shifted more downfield than those of the (*R*)-enantiomer [133]. In order to elucidate the association mechanisms of the complexes formed in solution, intermolecular rotating-frame Overhauser effect spectroscopy (1D- and 2D-ROESY) was performed with equimolar solutions of racemic "compound B" and heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in d_{12}-cyclohexane [133]. The results implied that the CH_3O-group of "compound B" is included at the wider opening of the cyclodextrin cavity whereas the CH_2F- and CF_3-groups interact with the external acetyl and silyl groups of the cyclodextrin used as CSA [133]. Thus the important role of the functional groups on the cyclodextrin rim and the occurrence of partial molecular inclusion in the enantiomeric differentiation mechanism were established.

The largest enantioseparation factor of $\alpha = 10.6$ at 26°C ever observed in enantioselective GC has been detected for "compound B" on octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) [28] diluted in polysiloxane PS 255 (Fig. 27, left) [134].

The described finding was the result of mere serendipity rather than rational design as the structural requirements for efficient enantiomeric differentiation involving haloethers and modified cyclodextrins are currently unknown. This is also borne out by the unexpected observation that the enantioseparation factor drops to $\alpha = 2.1$ on heptakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-β-cyclodextrin and to $\alpha = 1.0$ (no enantioselectivity at all!) on hexakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-α-cyclodextrin [134]. This unusual dependence of enantioselectivity on the cavity size of CDs in enantioselective inclusion GC warrants an explanation and it may also be of interest to test the δ-CD congener of Lipodex E for the enantiomeric differentiation of "compound B" by GC and NMR in the future. One tentative explanation for the exclusive versatility of Lipodex E based on γ-cyclodextrin for the enantioseparation of many racemates in general [28], and of "compound B" in particular [134], may be associated with self-inclusion of *n*-pentyl groups into the cavity of the

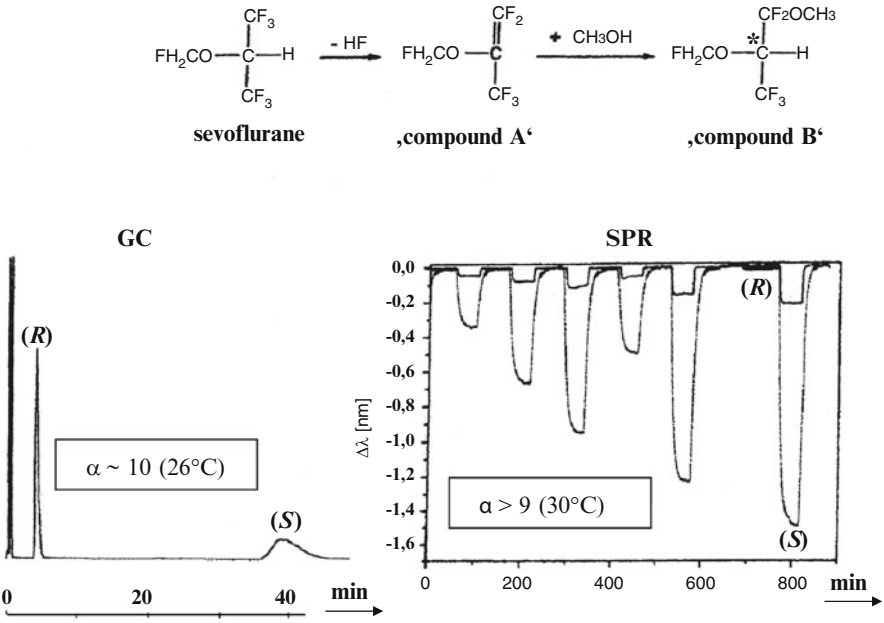

Fig. 27 *Left*: Analytical gas-chromatographic enantioseparation of "compound B." 5 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.28 μm octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E) in PS 225 (30 wt%), 26°C, 1.12 bar dihydrogen [134]. *Right*: Stepwise enantioselective surface plasmon resonance (SPR) signals with Lipodex E as sensor coating on exposure to single enantiomers of "compound B" in a concentration range between 0 and 140 μg/L at 30°C [141]

selector followed by competitive displacement by the selectand. A clue to this proposal, i.e., self-inclusion of one 6-*O*-pentyl group into Lipodex E, has been obtained by NMR measurements and molecular dynamics (MD) calculations [135]. The molecular associations of single enantiomers of "compound B" obtained by preparative GC and octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) and heptakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-β-cyclodextrin were also studied by ^{19}F-NMR spectroscopy in apolar d_{12}-cyclohexane [136]. Association constants of the interaction of the two enantiomers of "compound B" with Lipodex E and its β-cyclodextrin analogue were determined by NMR chemical shift titration and showed large differences in agreement with the GC results. Heteronuclear NOE measurements proved that inclusion complex formation takes place with "compound B" located inside the cavity of the cyclodextrin moiety. The study could not rationalize the striking difference in enantioselectivity between the β-CD and its γ-CD congener observed by enantioselective GC.

A comprehensive thermodynamic study has been performed for the enantiomeric differentiation of "compound B" by Lipodex E diluted in polysiloxane PS 255 (30 wt%) which exhibited the unexpectedly high enantioseparation factor α of 10.6 at 26°C [134]. Since the selector was diluted in the polysiloxane solvent S, the retention-increment R' method (Sect. 8) has been employed. The use of (9) in

comparing the retention factors k of the enantiomers on the reference column and the enantioselective column would require identical column dimensions and coating parameters. In order to overcome this obstacle, relative retentions $r = t'_R/t'_{Rref}$ were used according to (10) in which the adjusted retention time t'_R (= total retention time t_R minus unretained-peak holdup time t_M) of the enantiomers was related to an inert reference standard. For α-amino-acid selectors and metal-complex selectors, *n*-alkanes have been used as reference standards as they undergo neither hydrogen-bonding nor complexation with transition metal ions [112, 114]. However, in the case of cyclodextrin selectors, minor molecular association with *n*-alkanes has been detected [24]. This is also corroborated by the observation that chiral branched alkanes, devoid of any functionality, can be enantioseparated on modified cyclodextrins by GC [137, 138]. A theoretical treatment of the retention-increment R' approach has been put forward for *n*-alkane reference standards which undergo a definite, albeit negligible, interaction with modified cyclodextrins [24]. Indeed, *n*-alkanes could previously not be used as reliable reference standards in a thermodynamic study of derivatized amino acids and modified cyclodextrins [112]. However, due to the very large retention factors k involved for "compound B" and Lipodex E, the use of *n*-alkanes as references standards was straightforward [134]. Three columns were used in the study. The reference column (30 m × 0.25 mm i.d.) was coated statically with pure polysiloxane PS 255 while the enantioselective columns (10 m × 0.25 mm i.d.) were coated statically with 0.5 μm Lipodex E in polysiloxane PS 255 (5 wt% and 10 wt%). "Compound B," methane (as holdup time marker), and the *n*-alkanes (the reference standards *n*-pentane, *n*-hexane, *n*-heptane, and *n*-octane) were split-injected together (1:100). Highly precise data for the true enantioselectivity $-\Delta_{SR}\Delta G^{true}$ (the (*S*)-enantiomer is eluted after the (*R*)-enantiomer) were obtained [134] irrespective of the choice of the reference standards (C5–C8) and the concentration of the selector in the polysiloxane (5 wt% vs 10 wt%), thus underlining the validity of (10) and the need to separate rigorously achiral from chiral contributions to retention via the concept of the retention-increment R' (Fig. 23). The measured value of $-\Delta_{SR}\Delta G^{true}_{303} = 5.7\ (0.05)$ kJ/mol (at 30°C) is very large and beyond the estimated intrinsic error of molecular modeling calculations [139].

The question arose why the (*S*)-enantiomer of "compound B" underwent an unprecedented high molecular interaction with the γ-cyclodextrin selector vis-à-vis the negligible interaction of the (*R*)-enantiomer and why this strong bias is lost for the corresponding α-cyclodextrin selector (see above). GC temperature-dependent data for the true enantioselectivity $-\Delta_{SR}\Delta G^{true}$ obtained at 11 temperatures between 30°C and 80°C and measured at both concentrations of the selector (5 wt% and 10 wt%) furnished the following thermodynamic parameters via the strictly linear van't Hoff plots according to (13) [134]:

$$\begin{aligned} -\Delta_{SR}\Delta H^{true} &= 20.1(0.64)\ \text{kJ/mol} \\ \Delta_{SR}\Delta S^{true} &= -47.4(2.0)\text{J/mol K} \\ T_{iso} &= 424(30)\ \text{K}. \end{aligned}$$

These data represent the highest figures ever found in enantioselective GC. The high value of T_{iso} (~150°C) prevented its experimental verification, i.e., peak coalescence and inversion of the elution order [134]. All thermodynamic data were obtained from the ratio of the retention-increments R'_S/R'_R according to (11) and were independent of the concentration (5 wt% and 10 wt%) of the selector A in the polysiloxane S for both columns. Indeed, with the retention-increment R' approach (Sect. 8) only the enantioselective contribution to retention of the selector A is quantified and $-\Delta_{SR}\Delta G^{true}{}_{303}$ does not refer to the total CSP (i.e., A in S). Therefore only the ratio of the retention-increments R'_S/R'_R and not the ratio of retention factors k_S/k_R is appropriate to quantify the true enantioselectivity for diluted CSPs (Fig. 23).

Enantiomers cannot be distinguished on the reference column containing the achiral solvent matrix S. Therefore $r^\circ{}_i$ is identical for the enantiomers (*S*) and (*R*) of "compound B." $r^\circ{}_i$ need not be determined separately but can be extrapolated from two sets of data of the relative retention, r_i, for the (*S*)- and (*R*)-enantiomers at two (arbitrary) activities a_i of the CD selector A in the solvent S of the columns (1) and (2) as a consequence of the following expression [140]:

$$r^\circ = \frac{\left(r_S{}^{(1)} r_R{}^{(2)} - r_R{}^{(1)} r_S{}^{(2)}\right)}{\left(r_S{}^{(1)} + r_R{}^{(2)}\right) - \left(r_R{}^{(1)} + r_S{}^{(2)}\right)} \quad . \tag{14}$$

This expression, which directly follows the theorem of intersecting lines of Thales (Fig. 23), can be used to assess the non-enantioselective contributions to retention when r° is not readily accessible due to the unavailability of the solvent S to prepare a reference column (e.g., for Chirasil-type CSPs). It is enough to collect retention data from two columns of different activities (or concentrations in dilute systems) of the selector A in S. Thus values for r° of "compound E" on Lipodex E were calculated using two columns coated with Lipodex E in polysiloxane PS 255 (5 wt% and 10 wt%) with four reference *n*-alkanes standards (C5–C8) at 11 temperatures and a satisfactory agreement between measured and extrapolated values of r° were obtained [134]. This finding reinforced the validity of the retention-increment R' approach which relies on some experimental conditions, i.e., use of traces of the selectand (10^{-8} g) to guarantee a true 1:1 association equilibrium and the presence of a dilute solution of the selector A in the solvent S (typically 0.05–0.1 molal). The method has also been adopted in enantioselective LC in order to separate achiral and chiral contributions to retention [108].

The highly enantioselective supramolecular selectand-selectant system "compound B" and Lipodex E has been selected as a versatile model system to study the enantiomeric differentiation of enantioselective sensor devices based on quartz thickness shear mode resonators (TSMR), surface acoustic wave sensors, surface plasmon resonance (SPR) (Fig. 27, right), and reflectometric interference spectroscopy [141]. For all enantioselective sensor devices the (*S*)-enantiomer of "compound B" showed the stronger interaction with Lipodex E as was found in enantioselective GC. Based on the distinction between enantioselective and non-enantioselective

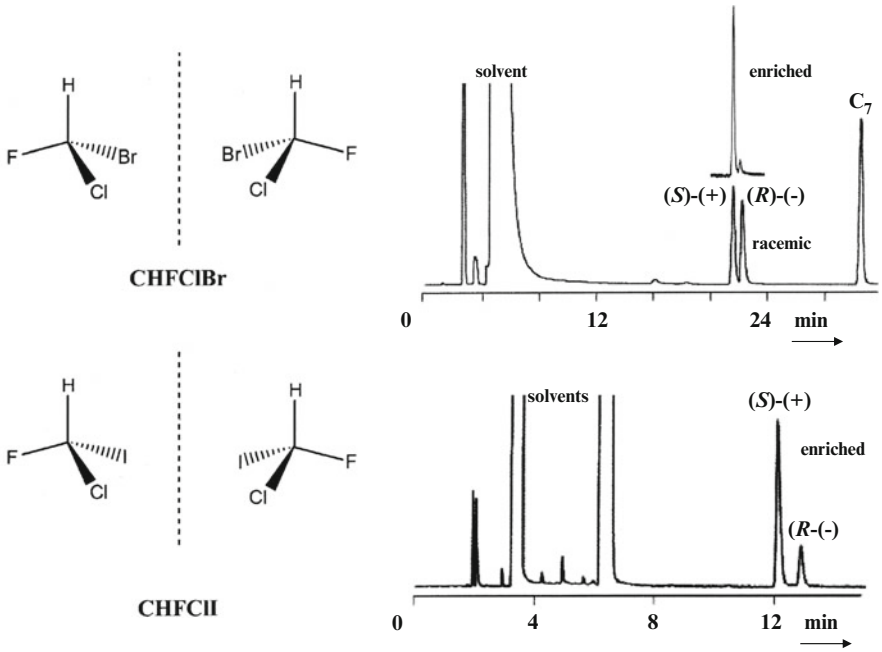

Fig. 28 *Left*: Enantiomers of C*HFClBr and C*HFClI. *Right*, *top*: Analytical gas-chromatographic enantioseparation of racemic and enantiomerically enriched C*HFClBr. 40 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.25 μm immobilized Chirasil-γ-Dex at −20°C and 100 kPa dihydrogen [147]. *Right*, *bottom*: Analytical gas-chromatographic enantioseparation of enantiomerically enriched C*HFClI. 40 m × 0.25 mm (i.d.) fused silica capillary column coated with 0.25 μm immobilized Chirasil-γ-Dex at 15°C and 100 kPa dihydrogen [145]

interactions (see above), thermodynamic association constants of the single enantiomers of "compound B" with Lipodex E were determined by a quartz TSMR and by surface plasmon resonance at 30°C and the observed enantioselectivity $-\Delta_{SR}\Delta G^{\text{true}}{}_{303} = 5.7$ kJ/mol (TSMR) and $-\Delta_{SR}\Delta G^{\text{true}}_{303} = 5.9$ kJ/mol (SPR) [141] agreed well with that determined by enantioselective GC, i.e., $-\Delta_{SR}\Delta G^{\text{true}}_{303} = 5.7$ kJ/mol [134].

12 Analytical Gas-Chromatographic Enantioseparation of Bromochlorofluoromethane and of Chlorofluoroiodomethane

Enflurane possesses a stereogenic center at a carbon atom which is substituted by three different atoms (Fig. 2). When the organic ether residue in enflurane is replaced by either bromine or iodine, the smallest penta-atomic (non-isotopically labelled) chiral molecules, i.e., the enantiomers of bromochlorofluoromethane, C*HFClBr, and chlorofluoroiodomethane, C*HClFI, are obtained (Fig. 28, left)

(incidentally, the smallest hypothetical isotopically labelled chiral molecule is $C^{*1}H^2H^3HX$ (X = halogen) which is not considered here).

Theoretically, true enantiomers are based on the non-superposability of matter and antimatter. Earthbound chiral D and L molecules, e.g., proteinogenic 2-amino acids, which both possess negative charged electrons, are actually diastereomers (quasi-enantiomers) and not true enantiomers as the charge is not mirrored. The resulting parity violation energy difference (PVED) of the weak force between enantiomers is often linked with the evolution of homochirality on primordial Earth [142–144]. The penta-atomic chiral halomethanes C*HFClBr and C*HClFI containing heavy atoms represent ideal target molecules for spectroscopic PVED measurements [143]. However, the search for subtle differences in the ultrahigh infrared spectra of the right- and left-handed tri(hetero)halogenomethanes requires the availability of large amounts of single enantiomers. Preparative access to the enantiomers of C*HFClBr and C*HClFI relies on the decarboxylation (with retention of configuration [70, 71], Fig. 14, right) of the corresponding pre-resolved tri(hetero)halogenoacetic acids [143, 145]. However, only incomplete enantiomeric excesses ee have been obtained thus far. Also the direct preparative GC enantioseparation is as yet elusive. However, following a preliminary report on the GC enantioseparation of C*HFClBr by König [16], the quantitative analytical enantioseparation of C*HFClBr and C*HFClI has systematically been explored on Chirasil-γ-Dex (octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin linked to poly(dimethylsiloxane)) [51, 145–147]. The Chirasil-γ-Dex CSP [32] exhibits the advantages inherent to polysiloxanes in GC, i.e., high resolution, inertness, and susceptibility to high and low GC operating temperatures. The CSP can even be used under cryoscopic conditions without loss of column efficiency down to −20°C which was required for the quantitative enantioseparation of C*HFClBr (Fig. 28, top right) whereas C*HFClI could be enantioseparated at 15°C (Fig. 28, bottom right). The analytical tool allowed the determination of the enantiomer excess ee of the tri(hetero)halogenomethanes obtained by decarboxylation of enantiomerically enriched tri(hetero)halogenoacetic acids (Fig. 28, bottom right) [145]. The (+)-(*S*)- and (–)-(*R*) configurations of C*HFClI and C*HFClBr were assigned by quantum mechanical calculations [145, 148].

The following thermodynamic data of enantioselectivity (Sect. 8) were obtained for the tri(hetero)halogenomethanes and Chirasil-γ-Dex [147] (D and L denote enantiomers irrespective of the true absolute configurations):

$$C * HFClBr:$$
$$\Delta_{D,L}\Delta H = -0.46\ \text{kJ/mol}$$
$$\Delta_{D,L}\Delta S = -1.37\ \text{J/mol K}$$
$$T_{iso} = 336\ \text{K},$$

$$C * HFClI:$$
$$\Delta_{D,L}\Delta H = -1.52 \text{ kJ/mol}$$
$$\Delta_{D,L}\Delta S = -4.57 \text{ J/mol K}$$
$$T_{iso} = 333 \text{ K}.$$

Thus the enantioselectivity difference of the haloethers on the CSP as expressed by thermodynamic data is threefold for the iodo compound. However, the linearity of the plot of $\Delta_{D,L}\Delta H$ vs $\Delta_{D,L}\Delta S$ is indicative of a compensation effect [105] and infers an identical inclusion mechanism. Moreover, the low isoenantioselective temperature T_{iso} (~60°C), which is due to a strong entropic contribution to enantiomeric differentiation, implies that very low temperatures are beneficial to increase enantioselectivity [147] and that cryogenic temperatures are required for any attempted preparative enantioseparation of tri(hetero)halogenomethanes on Chirasil-γ-Dex as a prerequisite of PVED measurements. Unfortunately, the reversal of the elution order above T_{iso} [111–114] could not be verified as the retention times were too short at ambient temperatures. The mechanistic rationalization of enantioselectivity between the smallest chiral molecules and a modified γ-cyclodextrin with the largest molecular cavity ($\alpha < \beta < \gamma$) remains a challenge for the future.

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