



ELSEVIER

Journal of Chromatography A, 769 (1997) 119–127

JOURNAL OF  
CHROMATOGRAPHY A

# Preparative enantiomer separation of the inhalation anesthetics enflurane, isoflurane and desflurane by gas chromatography on a derivatized $\gamma$ -cyclodextrin stationary phase

Markus Juza, Eberhard Braun, Volker Schurig\*

*Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany*

## Abstract

The preparative enantiomeric separation of the inhalation anesthetics enflurane (**1**) and isoflurane (**2**) in very high chemical (>99.5%) and enantiomeric excess (ee>99%) by gas chromatography (GC) on octakis(3-O-butanoyl-2,6-di-O-n-pentyl)- $\gamma$ -cyclodextrin (**4**), dissolved in the apolar polysiloxane SE-54 and coated on Chromosorb P AW DMCS, is described. Up to 1 g of each enantiomer of **1–2** can be obtained per diem. The enantiomers of the highly volatile desflurane (**3**) can also be separated, albeit with diminished ee. The enantiomeric excess of **1–3** was checked by analytical GC on **4** and the absolute configuration of **2** and **3** has been determined via anomalous X-ray diffraction.

**Keywords:** Enantiomer separation; Preparative chromatography; Desflurane; Isoflurane; Enflurane; Anesthetics; Octakis(3-O-butanoyl-2,6-di-O-n-pentyl)- $\gamma$ -cyclodextrin

## 1. Introduction

The first preparation of diethyl ether is generally attributed to Valerius Cordus in 1540 and was described for the first time by Frobensius, who coined the name “ether” [1]. Its anesthetic properties were adopted in the 16th century by Paracelsus [2]. Diethyl ether was introduced into surgery as a human inhalation anesthetic by W.E. Clarke from Rochester, NY, USA [3] and by C.W. Long from Jefferson, GA, USA in 1842 [4], and into dentistry by W.T.G. Morton, in Boston, MA, USA in 1846 [5,6]. By substituting hydrogen for halogen, improved narcotic gases were developed with reduced toxicity and flammability, increased volatility and potency. The compounds essentially satisfy the six important properties of an inhalation anesthetic;

muscle relaxation, analgesia, hypnosis, sedation, as well as rapid onset and recovery [7]. At present, the haloethers (cf. Fig. 1) enflurane (**1**) and isoflurane (**2**) are the most frequently administered inhalation anesthetics. The highly volatile narcotic gas desflurane (**3**) has now been approved in selected countries.

The inhalation anesthetics **1–3** contain a chiral centre in the molecule that was obviously introduced unintentionally. At present, **1–3** are produced and administered clinically as racemic mixtures. In view of the recent realization that the enantiomers of volatile anesthetics may have different or improved pharmacological properties compared to the racemate [7–10], it became of great importance to provide sufficient amounts of pure enantiomers for biological and medical trials. Even with the prospective view that the costly introduction of single enantiomers into medicine is eventually not warranted, investigations

\*Corresponding author.

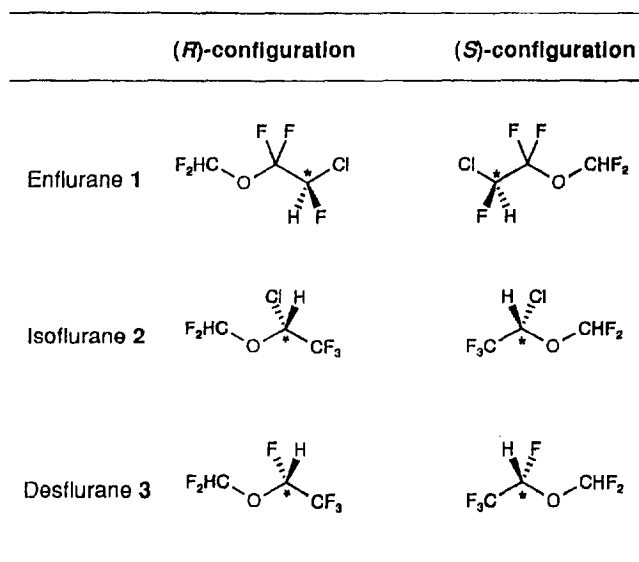
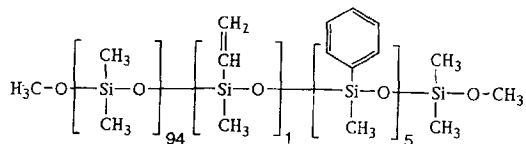
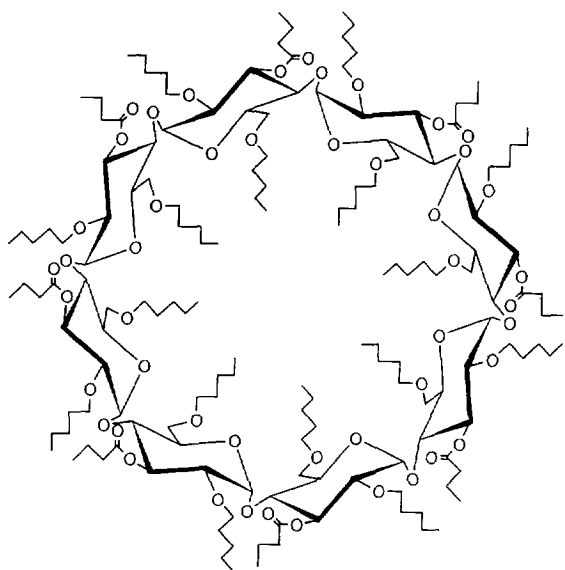


Fig. 1. Haloethers employed as human inhalation anesthetics.

Fig. 2. The chiral selector octakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)- $\gamma$ -cyclodextrin (4) [14] and the polysiloxane solvent, SE-54.

involving pure enantiomers should shed light on the biological action of inhalation anesthetics and should help to unravel the inherent mechanisms of anesthesia. With the advent of cyclodextrin-containing stationary phases in gas chromatography [11,12], racemic halogenated hydrocarbons and haloethers could successfully be resolved. Thus, Meinwald et al. [13] reported the first enantiomeric separation of 1, 2 and halothane [ $C^*HClBr(CF_3)$ ] with hexakis(2,3,6-tri-*O-n*-pentyl)- $\alpha$ -cyclodextrin and with octakis(6-*O*-methyl-2,3-di-*O-n*-pentyl)- $\gamma$ -cyclodextrin, respectively, at 30°C. We found that octakis(3-*O*-butanoyl-2,6-di-*O-n*-pentyl)- $\gamma$ -cyclodextrin (4, cf. Fig. 2) [14], when diluted in polysiloxanes such as OV-1701 or SE-54 [15], resolves 1–3 and halothane [16–18].

The large chiral separation factors  $\alpha > 2$  for 1 and  $\alpha > 1.3$  for 2 and 3 observed on 4 (cf. Fig. 3) permitted their semi-preparative gas chromatographic enantiomeric separation in high chemical and enantiomeric purity [16–18]. Shitangkoon et al. [19] and Staerk et al. [20,21] separated the enantiomers of 1 and 2 on undiluted pertrifluoroacetylated  $\gamma$ -cyclodextrin, a commercially available synthetic mixture of isomers and homologues.

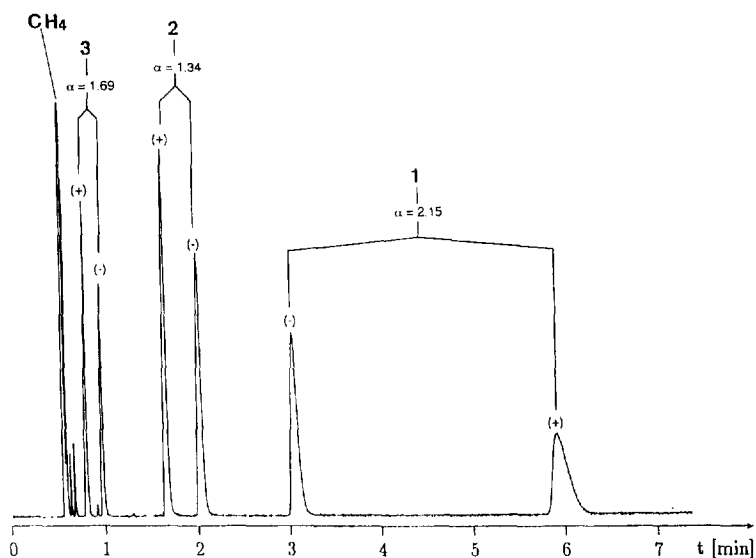


Fig. 3. Simultaneous analytical GC enantiomer separation of the inhalation anesthetics 1–3. Fused-silica capillary column (25 m $\times$ 0.25 mm I.D.) coated with **4** [14] dissolved in SE-54, film thickness 0.5  $\mu$ m, at 26°C. Carrier gas: 1.1 bar helium.

## 2. Experimental

The necessary precautions when working with volatile inhalation anesthetics (ventilation) were observed.

### 2.1. Materials

Racemic **1** and **2** were obtained from a local hospital. Racemic **3** was kindly provided by Pharmacia (Erlangen, Germany).  $\gamma$ -Cyclodextrin was courtesy of the Consortium für Elektrochemische Industrie (Munich, Germany).

### 2.2. Synthesis of octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)- $\gamma$ -cyclodextrin (**4**)

An 11-g amount of **4**, introduced by König et al. [14], was synthesized according to their procedure, except that the acylation was accomplished in triethylamine at 50°C. The unpurified crude product, which was only filtered over a small amount of silica gel in order to remove the catalyst and non-reacted cyclodextrin, was dried at 60°C and 0.008 Torr for

16 h (1 Torr=133.322 Pa). Access to 11 g of highly purified **4** is not practical at present because of serious losses in the purification steps. However, as demonstrated previously [18], the use of crude rather than highly purified **4** in fact increases the separation factor,  $\alpha$ , of **2** dramatically, while it reduces that of **1** and **3** by an acceptable level.

### 2.3. Preparation of the packing

A 90.5-g amount of polysiloxane SE-54 (WGA, Düsseldorf, Germany) was dissolved in 1300 ml of dry chloroform under reflux in a round-bottomed flask and approximately 10.75 g of **4** were added to the solution. A 398-g amount of Chromosorb P AW DMCS (80–100 mesh; Alltech, Arlington Heights, IL, USA) was poured into the mixture. The slurry was manually agitated until it became homogeneous. The solvent was carefully evaporated using a rotary evaporator at 40°C at a moderate vacuum for several hours, followed by drying at 70°C and 0.004 Torr for 10 h. The coated Chromosorb (approximately half a kilogram) prepared in this way contains 20.3% (w/

w) stationary phase, which consists of 10.6% (w/w) **4** dissolved in SE-54.

#### 2.4. Production and testing of the packed semi-preparative columns

The three semi-preparative columns, I, II and III, were obtained as described previously [22] and installed into a Fractovap 2101 gas chromatograph (Thermoseparations, formerly Carlo Erba, Mainz, Germany). All sample volumes were measured with a Hamilton syringe (Hamilton, Bonaduz, Switzerland). In the case of the highly volatile **3**, both the sample and the syringe were cooled to  $-18^{\circ}\text{C}$  to prevent boiling.

#### 2.5. Production of the packed preparative columns

Before the columns were filled, the packing was sieved carefully (mesh 100) to exclude lumps and to ensure a homogeneous flow through the column. Three preparative columns made of stainless steel [1 m $\times$ 24 mm I.D. (column A) and two columns (1 m $\times$ 6 mm I.D.) combined to form column B] were packed as described previously [22]. The custom-made column A was equipped with two sieves of sintered PTFE at the in- and outlet to establish a continuous flow profile and to hold back the column packing. These sieves cannot be contaminated with the racemate or the other enantiomer. The special design of the screw cap, B, prevents the creation of dead volumes, which would lead instantaneously to a mixing of the two separated enantiomers (cf. Fig. 4).

The twin column B consisted of two identical commercial columns (formerly Dr. Hupe Apparatebau, Karlsruhe, Germany). Column A proved to be superior in performance compared to the longer twin column B, due to the lower pressure drop along the homogeneous flow profile and the greater amount of packing employed.

#### 2.6. Preparative chromatography of enflurane

The preparative chromatography of **1** has been described previously [22].

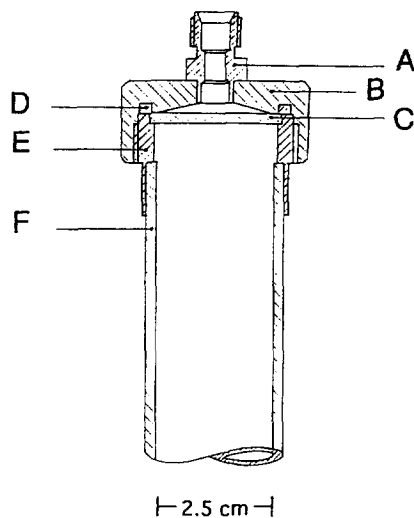


Fig. 4. Schematic representation of column A. Designations: A=column inlet; B=screw cap; C=sintered PTFE sieve; D=PTFE sealing ring; E=screw socket and F=column, containing stationary phase.

#### 2.7. Preparative chromatography of isoflurane and desflurane

The preparative columns made of stainless steel were installed into a slightly modified prototype of a Hupe APG 402 automated preparative gas chromatograph built in the 1960s (formerly Dr. Hupe Apparatebau). The injector and the flame ionization detection (FID) system were held at  $100^{\circ}\text{C}$ . Nitrogen (99.996%) was used as the carrier gas at 1 bar (column A) or 3 bar (column B). The Hupe gas chromatograph permitted the automatic injection of **2** and **3** from a storage vessel that is connected to the vaporizer via a flexible PTFE capillary, and the automatic switching of the permanently attached cooling traps. The original storage vessel and the original cooling traps were replaced with vessels of reduced size, thus facilitating the handling of the small volumes of liquid. The collection process was controlled by the signal level obtained from the flame ionization detector (approximately 5% of the total carrier gas stream). This allowed a reliable and reproducible switching of the cooling traps regardless of fluctuating retention times. These specially designed cooling traps (cf. Fig. 5) were equipped with a condensation coil section with a continuous

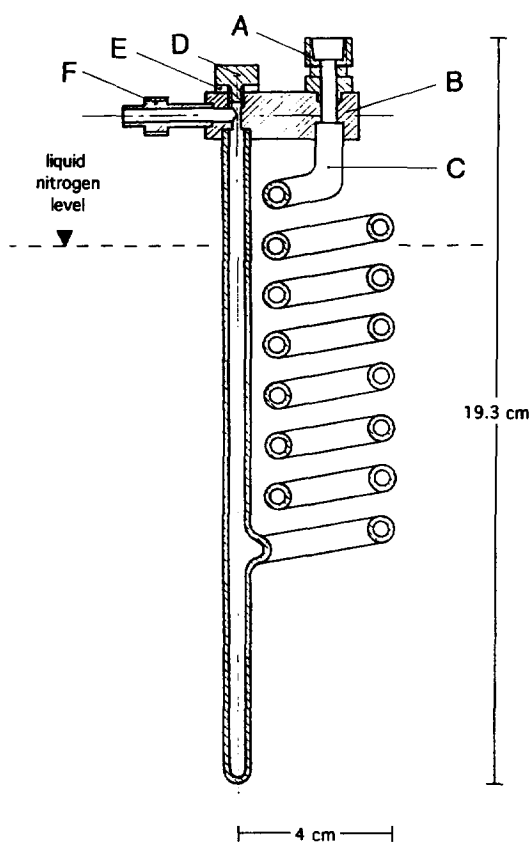


Fig. 5. Schematic representation of specially designed cooling traps. Designations: A=inlet; B=top plate; C=condensation coil (glass); D=screw cap; E=silicon sealing ring and F=outlet.

slope. From there, the liquid enantiomer drops into the collection section and crystallizes, whereas the carrier gas stream leaves the trap via the end section through a magnetic valve. This end section is closed by a removable screw cap, which allows easy sample transfer.

The cooling traps were immersed into a Dewar container filled with liquid nitrogen and covered with a tight-fitting HD-polystyrene block to prevent excessive evaporation. The liquid nitrogen level was continuously adjusted by a custom-made monitoring unit, consisting of two semiconductor diodes, a relais block, a pressure-regulating pump and an insulated storage tank for the supply of liquid nitrogen. After thawing, the liquid enantiomers were collected with two (different!) precooled syringes and sealed in PTFE-faced silicon septa crimp vials. The amount of

the enantiomers after this transfer was used to determine the recovered material. The isolated enantiomers were characterized by  $^1\text{H}$  NMR and chiral headspace capillary gas chromatography (GC).

### 3. Results and discussion

The high separation factor,  $\alpha > 2$ , for **1** can still be increased by lowering the column temperature. While the semi-preparative enantiomer separation can be performed with an analytical packed column (2.3 m  $\times$  3 mm I.D.), the preparative separation of 50 mg of injected **1** has been achieved in one run with a packed column (4 m  $\times$  7 mm I.D.). This packed column consisted of a laboratory-made, modified condenser of a rotary evaporator filled with the stationary phase (Chromosorb P AW DMCS, 80–100 mesh, which was coated with 20.3% (w/w) of the mixture of 10.0% (w/w) pure **4** in the polysiloxane SE-54). Manual repetitive injections allowed the collection of 250 mg of each enflurane enantiomer of high enantiomeric (99.9%) and chemical purity (99.5%) [18,22] that was suitable for chiroptical or biological trials in one day.

In 1971, Pescar [23] wrote that in preparative chromatography "even the most optimized column represents a compromise between speed of analysis, component resolution, and column sample capacity". This basic rule also governs preparative GC enantiomer separation and two parameters can only be optimized at the expense of the third. Thus, the variables time, enantiomeric excess and isolated quantity of sample have to be carefully balanced.

Preparative enantiomer separations depend critically on the amount of chiral selector in the stationary phase. In order to investigate the influence of the concentration of the cyclodextrin derivative **4** on the separation factor,  $\alpha$ , three semi-preparative analytical columns (cf. Table 1), containing 10, 20 and 50% of **4**, were compared (cf. Fig. 6). As can be seen from Fig. 6, the  $\alpha$  vs. the weight percent cyclodextrin derivative curves level off at approximately 20%, as expected from a thermodynamic study that was conducted previously [24].

There is obviously no need to employ the valuable (and costly!) chiral selector in a high concentration or in an undiluted form. Another factor influencing

Table 1  
The three semi-preparative columns

Column	Loading of support (w/w)	Amount of stationary phase (sp) (cyclodextrin derivative (cd))	Ratio of sp-cd
I	22.1%	2.84 g (0.284 g)	10:1
II	13.4%	1.42 g (0.284 g)	5:1
III	22.1%	1.42 g (1.420 g)	1:1

efficiency in preparative enantiomer separations is the loading of the support material compared to the maximum sample volume that can be baseline-separated. Chromosorb P AW DMCS permits a loading with a liquid stationary phase of up to 30%. Thus, we choose a safety margin of 22% stationary phase loading. A comparison of the three semi-preparative columns (cf. Table 2) shows that albeit the stationary phase in column II contains the same amount of chiral selector as column I, up to 60% more of **1** could be baseline-separated, whereas no influence on the separation of **3** could be observed. Nevertheless, the retention time of the second eluted enantiomer of **1** was almost doubled.

This effect is caused by the high volatility of **3** in comparison to **1**, which results in very low retention factors,  $k$ , for the enantiomers. The amount of the chiral haloethers that could be baseline-separated with column III is considerably increased in comparison to columns I and II, but the retention times also increase dramatically, which is detrimental to the desired up-scaling, since several fast repetitive injections will yield better productivity. Thus, the composition of the stationary phase of column I offered several advantages over columns II and III, in regard to fast separations, good stability of the stationary phase (over several months) and sufficient

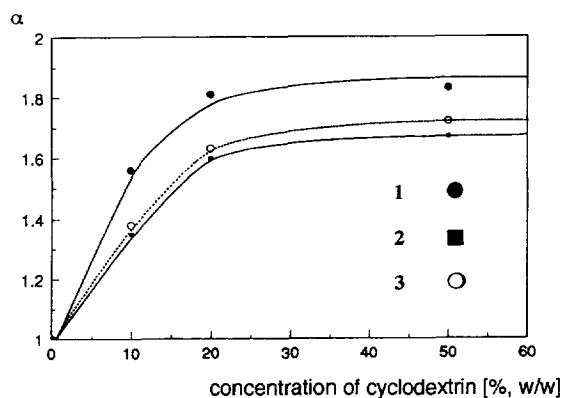


Fig. 6. Comparison of the  $\alpha$ -values for **1**–**3**, depending on the concentration of the chiral selector **4** [14] in the stationary phase.

sample loading at a relatively low amount of the selector **4**. These promising results prompted us to automate the process for injection and collection of the enantiomers and to extend our investigations to the preparative up-scaling of the enantiomer separation of **2** and **3**.

The preparative enantiomer separation of **2** was performed isothermally at room temperature (varying from 26 to 31°C) with the commercial preparative gas chromatograph as described in Section 2. Lowering the temperature in order to increase the separation factor,  $\alpha$ , [24] and, as a consequence, to increase the isolated yield of the enantiomers, was, unfortunately, not feasible for **3** with the present instrumental set-up. The packed column A consisted of a custom-made stainless-steel (1 m×24 mm I.D.) column filled with the stationary phase [Chromosorb P AW DMCS, 80–100 mesh coated with 20.3% (w/w) of the mixture of 10.6% (w/w) unpurified **4** in the polysiloxane SE-54]. As mentioned before, the chiral separation factor,  $\alpha$ , for **2** is increased from 1.3 to 1.6 (at 30°C) when using the unpurified **4** (i.e.

Table 2  
Maximum obtainable baseline separations

Column <sup>a</sup>	Ratio of stationary phase-cyclodextrin	Enflurane ( $\mu$ l)	Isoflurane ( $\mu$ l)	Desflurane ( $\mu$ l)
I	10:1	0.6	0.5	0.2
II	5:10	1.0	0.7	0.2
III	1:1	2.8	2.4	1.8

<sup>a</sup>All columns: 2.3 m×3 mm I.D.; support material, Chromosorb P AW DMCS, 80–100 mesh; 7.6 g of total stationary phase.

containing under- and over-alkylated/acetylated congeners and regioisomers) as the chiral selector [18]. Repetitive injections allowed the collection of 300 mg of each isoflurane enantiomer per diem (130 automated runs) in high enantiomeric (>99.9%) and chemical purity (>99.5%) that was suitable for

chiroptical, NMR spectroscopic or biological trials [24–26]. Injection and switching of the three trapping devices was executed automatically. The first and the third fractions of the elution profile (cf. Fig. 7a) of **2** were collected in laboratory-made cooling traps via condensation with liquid nitrogen as the

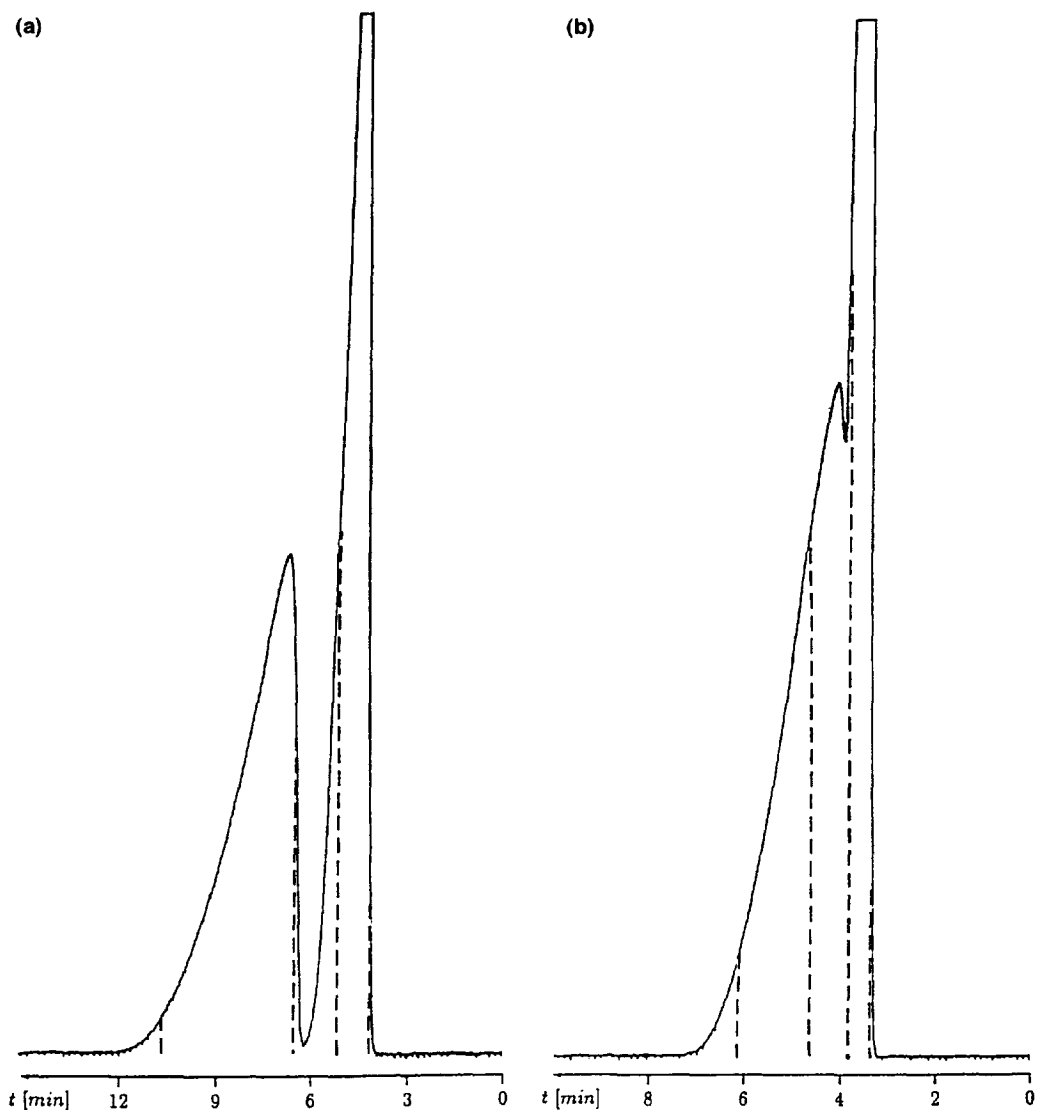


Fig. 7. Preparative GC enantiomer separation of **2**. Column: 1 m×24 mm I.D. (column A) filled with Chromsorb P AW DMCS, 80–100 mesh, coated with 20.3% (w/w) of the mixture of 10.6% (w/w) unpurified **4** [14] in the polysiloxane, SE-54; carrier gas, 1 bar nitrogen; 27°C. Dotted lines indicate cut-points for fractions. (b) Preparative GC enantiomer separation of **3**. Column: two connected columns, 1 m×24 mm I.D. (column B) filled with Chromsorb P AW DMCS, 80–100 mesh, coated with 20.3% (w/w) of the mixture of 10.6% (w/w) unpurified **4** [14] in the polysiloxane, SE-54; carrier gas, 3 bar nitrogen; 26°C. Dotted lines indicate cut-points for fractions.

cooling medium, since our attempts to collect enantiomers of **2** as liquids at  $-60^{\circ}\text{C}$  were unsuccessful, due to the high volatility of the compound and the formation of “mist”-droplets (cf. Table 3).

Desflurane, a new generation human inhalation anesthetic [7], contains a fluorine atom instead of a chlorine atom at the chiral centre, compared with isoflurane (cf. Fig. 1). Unfortunately, as can be seen from Table 3, **3** is gaseous at room temperature. Thus, the preparative enantiomeric separation of **3** was carried out under overlapping band conditions, contrary to the resolution of **2**, which was performed with baseline-separation. In preparative gas–solid chromatography [27,28], as well as in preparative liquid chromatography, the former strategy has proved to yield higher production rates [10]. The first and the last fractions of the elution profile of **3**, albeit resembling an unresolved peak, were collected while the middle fraction was recycled or discharged (cf. Fig. 7b).

As an alternative to column A, two  $1\text{ m}\times 6\text{ mm}$  I.D. stainless steel columns (column B) operated in series were fitted into the preparative gas chromatograph. Using the cut-off points indicated in Fig. 7b, in 170 runs (total time, approximately 24 h), 500 mg of (+)-desflurane (ee=91%) and 450 mg of (–)-desflurane (ee=68%) were isolated. A higher enantiomeric excess can be obtained at the expense of the isolated amount. The less retained enantiomer is obtained with a higher production rate and enantiomeric excess than the more retained enantiomer. This is in agreement with results reported for liquid chromatography [10] and GC [21] using the overlapping band mode.

Due to the high rubber–gas partition coefficients

for halogenated ethers [7], it turned out to be necessary to prevent any contact of the isolated enantiomers with usual rubber or styrene–butadiene rubber septa, seals, O-rings and connections, which would instantaneously lead to a decrease in ee by contamination with previously absorbed racemate or, even worse, the other enantiomer. Therefore, all transfer-lines and sealings were either made of PTFE or stainless steel. The cooling traps and syringes used for sample collection were extremely prone to contamination by the other enantiomer and had to be heated for several hours to  $60^{\circ}\text{C}$  to desorb all residues of anesthetics that had been collected earlier.

The isolated enantiomers were characterized by  $^1\text{H}$  NMR and chiral headspace capillary GC. The pure enantiomers of **2** have been distributed to several laboratories for pharmacological studies. The interaction of the pure enantiomers of **1** and **2** with **4** was studied by  $^1\text{H}$ ,  $^{19}\text{F}$  NMR and  $^1\text{H}$ -NOE NMR [25]. Also, the anomalous X-ray diffraction technique [26] was used to determine the absolute configuration of **2** and **3**. Both dextrorotatory enantiomers possess the (*S*)-configuration. Thus, the chiroptical assignment of dextrorotatory isoflurane as (+)-(*S*)-**2** was confirmed, while the chiroptical assignment of dextrorotatory desflurane as (+)-(*R*)-**3** had to be revised as (+)-(*S*)-**3**.

#### 4. Conclusion

The preparative GC enantiomer separation of the three inhalation anesthetics **1–3** employing crude **4** in SE-54 provides easy access to small amounts (1–5

Table 3  
Physical properties of the inhalation anesthetics **1**, **2** and **3**

	m.p. ( $^{\circ}\text{C}$ )	b.p. ( $^{\circ}\text{C}$ )	Vapour pressure (Torr)
Enflurane		56.5	$\log p_{\text{vap}} = 6.988 - \frac{1107.84}{T(^{\circ}\text{C}) + 213.1}$
Isoflurane	–98	48.5	$\log p_{\text{vap}} = 8.056 - \frac{1664.58}{T(^{\circ}\text{C}) + 273.16}$
Desflurane	–126	22.8	$\log p_{\text{vap}} = 8.515 - \frac{1665.9}{T(^{\circ}\text{C}) + 273.16}$



g) of samples of enriched or pure enantiomers. It is demonstrated that an old-time preparative GC instrument is suited for the purpose of preparative enantiomer separation of volatile halocarbons. A further technical scale-up of this process via simulated moving bed (SMB) chromatography [29] should be possible and should allow the preparation of larger quantities of the pure enantiomers that are needed for clinical trials (5–50 g). A prerequisite for this application is the determination of adsorption isotherms in the spirit of the pioneering work of Rouchon et al. [27] and Roles and Guiochon [28].

The preparative enantiomeric separation of **1–3** allows investigations of the biological mode of action of these important inhalation anesthetics. Furthermore, the absolute configuration of the enantiomers of isoflurane and desflurane could be confirmed and reassigned, respectively, which had a considerable impact on investigations into the reaction pathways of stereochemical reactions involving **2** and **3** [16,26].

### Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie. We thank the Consortium für Elektrochemische Industrie GmbH, Munich, Germany, for the generous gift of  $\gamma$ -cyclodextrin. The authors wish to thank G.J. Nicholson, University of Tübingen, Germany, for assistance in the maintenance of the preparative gas chromatograph, A. Riefert, University of Tübingen, Germany, for technical drawings and Dr. H. Grosenick, BASF, Ludwigshafen, Germany, for valuable discussions.

### References

- [1] J.A.S. Frobensius, *Phil. Trans. R. Soc. Lond.*, 36 (1739) 283.
- [2] D.F. Halpern, *Chem. Tech.*, 19 (1989) 304.
- [3] A. Bigelow, *J. Am. J. Med. Sci.*, 141 (1876) 164.
- [4] J. Jeffereys, *Lancet*, 2 (1872) 241.
- [5] C.W. Long, *Sth. Med. J.*, 5 (1849) 705.
- [6] A. Zbinden and D. Thomson, in A. Doenicke, D. Kettler, W.F. List, J. Tarnow and D. Thomson (Editors), *Anästhesiologie*, Springer Verlag, Berlin, 1992, Ch. 2.4, p. 116.
- [7] D.F. Halpern, in R. Filer, Y. Kobayashi and L.M. Yagupolskii (Editors), *Organofluorine Compounds in Medicinal Chemistry and Biomedical Applications (Studies in Organic Chemistry 48)*, Elsevier, Amsterdam, 1993, pp. 101–133.
- [8] B. Harris, E. Moody and P. Skolnick, *Eur. J. Pharmacol.*, 217 (1992) 215.
- [9] N.P. Franks and W.R. Lieb, *Nature*, 367 (1994) 607.
- [10] E.J. Moody, B.D. Harris and P. Skolnick, *Trends Pharmacol. Sci.*, 15 (1994) 387.
- [11] V. Schurig and H.-P. Nowotny, *Angew. Chem., Int. Ed. Engl.*, 29 (1990) 939.
- [12] W.A. König, *Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins*, Hüthig, Heidelberg, 1992.
- [13] J. Meinwald, W.R. Thompson, D.L. Pearson, W.A. König, T. Runge and W. Francke, *Science*, 251 (1991) 560.
- [14] W.A. König, R. Krebber and P. Mischnick, *J. High Resolut. Chromatogr.*, 12 (1989) 732.
- [15] V. Schurig and H.-P. Nowotny, *J. Chromatogr.*, 441 (1988) 155.
- [16] V. Schurig, H. Grosenick and B.S. Green, *Angew. Chem., Int. Ed. Engl.*, 32 (1993) 1662.
- [17] V. Schurig and H. Grosenick, *J. Chromatogr. A*, 666 (1994) 617.
- [18] V. Schurig, M. Juza and H. Grosenick, *Recueil Trav. Chim. Pays-Bas*, 114 (1995) 211.
- [19] A. Shitankoon, D.U. Staerk and G. Vigh, *J. Chromatogr. A*, 657 (1993) 387.
- [20] D.U. Staerk, A. Shitankoon and G. Vigh, *J. Chromatogr. A*, 663 (1994) 79.
- [21] D.U. Staerk, A. Shitankoon and G. Vigh, *J. Chromatogr. A*, 677 (1994) 133.
- [22] V. Schurig, B.S. Green and H. Grosenick, *German Offenlegungsschrift*, P 4317139.7, 1994.
- [23] R.E. Pescar, in A. Zlatkis and V. Pretorius (Editors), *Preparative Gas Chromatography*, Wiley-Interscience, New York, London, Sidney, Toronto, 1971, p. 75.
- [24] V. Schurig and M. Juza, *J. Chromatogr. A*, 757 (1997) 119.
- [25] H. Grosenick, M. Juza, J. Klein and V. Schurig, *Enantiomer*, 1 (1996) 314.
- [26] V. Schurig, M. Juza, B.S. Green, J. Horakh and A. Simon, *Angew. Chem., Int. Ed. Engl.*, 35 (1996) 1680.
- [27] P. Rouchon, M. Schoenauer, P. Valentin, C. Vidal-Madjar and G. Guiochon, *J. Phys. Chem.*, 89 (1985) 2076.
- [28] J. Roles and G. Guiochon, *J. Chromatogr.*, 591 (1992) 233, 245, 267.
- [29] M. Mazzotti, R. Baciocchi, G. Storti and M. Morbidelli, *Ind. Eng. Chem. Res.*, 35 (1996) 2313.