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Gas chromatographic separation of the enantiomers of volatile fluoroether anesthetics by derivatized cyclodextrins. III. Preparative-scale separations for enflurane

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Abstract

Preparative-scale gas chromatographic separation of the enantiomers of enflurane, a volatile anesthetic, has been achieved with a 1 m long, 10 mm I.D. column, packed with 25% (w/w) trifluoroacetyl-γ-cyclodextrin-coated 60/80 mesh Chromosorb A, installed in a custom-designed preparative gas chromatograph. An effluent-sampling interface valve, located between the exit of the preparative column and the fraction collector, takes 10-μl samples of the effluent every 15 s and sends them onto a short, efficient analytical capillary column providing on-line enantiomeric analysis of the eluting bands. This permits the precise calculation of product purity, recovery and production rate for the preparative separation and leads to aggressive, yet safe, fraction pooling schemes.

1. Introduction

Prompted by the first successful capillary GC separation of the enantiomers of fluoroether anesthetics [1], in Part I of this series we have determined the operating parameters which lead to maximized separation selectivities and reasonable capacity factors for all the commercially available fluoroether anesthetics on all the commercially available cyclodextrin-based chiral GC stationary phases [2]. In Part II, we have demonstrated how this information can be used to develop a preparative GC separation for the enantiomers of one of the fluoroether anesthetics, isoflurane [3]. In the present paper we will detail the preparative scale GC separation of the enantiomers of another fluoroether anesthetic, enflurane ($CHFClCF_2OCHF_2$), that became possible due to the use of an improved packing material and a custom-designed preparative GC system that affords on-line enantiomeric analysis of the effluent of the preparative column.

2. Experimental

A custom-designed preparative gas chromatographic system has been constructed according to Fig. 1 from a modified Model 439 GC apparatus (Chrompack, Middelburg, The Netherlands), equipped with a septumless split/splitless injector, a flame ionization detector, and a Model 4270 integrator (Varian, Walnut Creek, CA,

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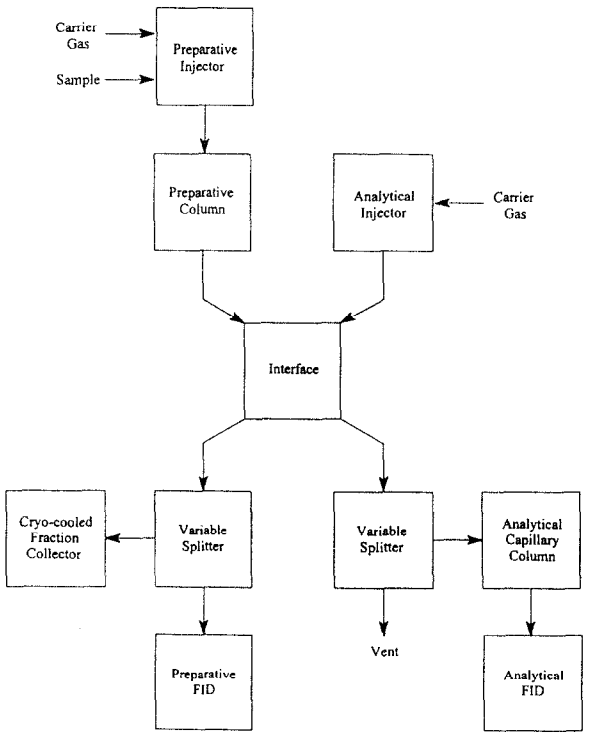

Fig. 1. Schematic of the preparative chromatographic system.

USA) for data acquisition, a modified Model FE thermostatted circulating oil-bath system (Science Electronics, Dayton, OH, USA), modified HP 5982 MS interface heated transfer lines (Hewlett-Packard, Avondale, PA, USA), a modified, heated Series 7000 (Rheodyne, Cotati, CA, USA) switching valve-based effluent sampling interface, a modified variable split injector (Tracor, Houston, TX, USA) and a custom-made analytical capillary column installed in an HP 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA), a septumless split/splitless injector, a flame ionization detector, and a CHEMSTATION data collection/analysis system.

The preparative scale GC separations were completed on 1.0 m × 10.0 mm I.D. stainless-steel preparative columns, which were packed as described in Ref. [3] with 60/80 mesh (approximately 175–250 μm) Chromosorb A (Supelco, Bellefonte, PA, USA), coated with 25% (w/w) trifluoroacetyl-γ-cyclodextrin, GTA (ASTEC, Whippany, NJ, USA). The preparative column was operated isothermally at 40°C.

The effluent of the preparative column was analyzed on-line for enantiomeric purity using a 7.0 m × 0.25 mm I.D. fused-silica capillary column, statically coated [4] with a 0.25 μm thick film of GTA. Hydrogen was used as carrier gas at a linear velocity of 124 cm/s (methane was used as unretained compound). The capillary column was operated isothermally at 38°C.

Enflurane was obtained from Anaquest, a division of BOC Health Care (Murray Hill, NJ, USA).

3. Results and discussion

3.1. Column packing

Chromosorb A was found to yield a better packing material than Chromosorb W AW used in Part II of this work [3], both in terms of mechanical strength and column efficiency, and it could be coated with as much as 25% (w/w) of the GTA stationary phase, just as Chromosorb W AW. With hydrogen as carrier gas, the efficiency maximum of the 1 m × 10 mm I.D. column was found at a linear velocity of 5 cm/s, yielding a height equivalent to a theoretical plate (HETP) value of 1.0 mm.

3.2. The custom-designed preparative chromatographic system

Our objective has been to create a preparative GC system which, in addition to the preparative scale separations, can be used to determine, on-line, prior to fraction collection, the enantiomeric purity of the effluent of the preparative column. The system, shown in Fig. 1, has been built using commercially available, easily modified components. The Chrompack 439 gas chromatograph is used to supply the carrier gas for the preparative column and facilitate the injection of the racemic enflurane feed via a gas-tight syringe. The preparative column is equipped with an oil jacket and is operated

isothermally using a circulating thermostatted oil bath. This arrangement allows the use of widely different column sizes and configurations, unconstrained by the GC oven.

Since fairly large selectivity values ($\alpha > 1.3$) are required for a successful preparative separation, a capillary column providing only a few thousand theoretical plates is all that is necessary for the on-line enantiomeric analysis of the effluent of the preparative column. These few thousands of plates can be generated easily with very short capillary columns (3 to 10 m) which are coated with a thin film (0.25 μm) of the same stationary phase as the preparative column, and operated at very high carrier gas linear velocities.

At the exit of the preparative column there is a heated interface, constructed from a programmable injection valve (e.g. a pneumatically operated, electronically activated Rheodyne Model 7000 valve), which samples the effluent of the preparative column and injects these samples (in our case 10 μl of the effluent gas) onto the analytical capillary column installed in the HP 5890 Series II gas chromatograph. As shown in Fig. 2, the cycling frequency of the interface valve is limited by the sum of the following time intervals: the duration of the pressure pulse that is caused by the turning of the interface valve to the inject position and back to the refill position (A), the duration of the flat baseline section before the peak of the less retained enantiomer (B), the duration of the first peak (C), the duration of the second peak (D), both determined at the highest effluent concentration expected during the preparative separation, and the duration of the flat baseline section after the peak of the more retained enantiomer necessary for reliable peak area determination (E). Additionally, the separation time on the analytical capillary column has to be an integer number multiple of the cycle time. Of the components of the cycle time, segments C and D can be adjusted most readily by changing the temperature of the capillary (to adjust separation selectivity), the film thickness (phase ratio) of the capillary to adjust the capacity factor (peak end) of the second component, and a combination of the

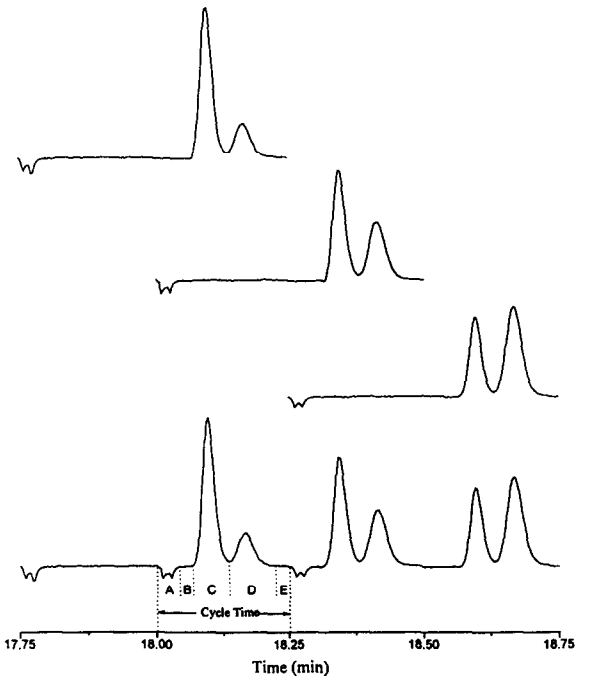

Fig. 2. Determination of the cycling time of the effluent sampling interface valve using the analytical chromatograms of three successive fractions of the preparative effluent. For details, see text. Top trace: individual analytical chromatogram of the first fraction injected onto the capillary column. Second trace: individual analytical chromatogram of the second fraction injected onto the capillary column. Third trace: individual chromatogram of the third fraction injected onto the capillary column. Bottom trace: actual detector trace that results from the three successively injected fractions. Analytical column: 7.0 m × 0.25 mm I.D. fused-silica capillary, coated with a 0.25-μm film of GTA, operated isothermally at 38°C with a H_2 carrier gas velocity of 124 cm/s.

length of the capillary and the linear velocity of its carrier gas to provide the necessary efficiency. In our case the actual separation time on the analytical capillary column is 30 s and the cycle time is 15 s, which results in 75 to 80 enantiomeric composition data points during an average preparative separations. As an example, the signal of the flame ionization detector in the HP 5890 gas chromatograph [analytical flame ionization detection (FID)] is shown in Fig. 3 as a function of time during the preparative-scale separation of a 50-mg enflurane sample.

Due to the finite switching time of the inter-

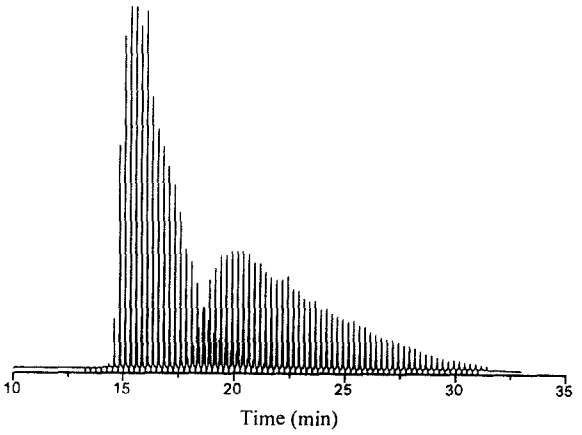

Fig. 3. Analytical detector trace during the preparative separation of a 50-mg enflurane sample. Preparative column: 25% (w/w) GTA on 60/80 mesh Chromosorb A packed into a 10 mm I.D., 1 m long stainless-steel column operated isothermally at 40°C with H_2 carrier gas at a linear velocity of 5.0 cm/s. Effluent-sampling interface valve cycle time: 15 s. Analytical column: 7.0 m × 0.25 mm I.D. fused-silica capillary, coated with a 0.25-μm film of GTA, operated isothermally at 38°C with a H_2 carrier gas velocity of 124 cm/s.

face valve, a variable splitter (fashioned from parts of a Tracor variable split injector) is installed between the exit of the interface valve and the analytical capillary column. The split ratio can be changed over a broad range to prevent overload of the analytical capillary and hence provide the required number of plates for the analytical separation. The original injector of the HP 5890 gas chromatograph can be used for the quantitative calibration of the entire analytical system, because it is located upstream of the interface valve.

The other exit of the interface is connected to a second variable splitter. The minor stream from the splitter is taken to the detector [either thermal conductivity detection (TCD) or FID] of the preparative GC apparatus to monitor the actual effluent concentration histories (envelope chromatograms). Since in our system the fraction collection (cut-point selection) is governed by the results of the on-line enantiomeric purity analysis, there is no absolute need for an additional bulk detector on the preparative GC apparatus. Nevertheless, we decided to record these chromatograms as well (using FID of the Chrompack GC apparatus) to obtain information that could corroborate the results of the on-line enantiomeric analysis. The envelope chromatograms shown in Fig. 4 were obtained for the increasing enflurane loads of 1, 3, 6, 20, 32, 50, 75 and 120 mg. Regardless of sample size, the concentration of the more retained enantiomer is below the detection limit after 35 min.

The major stream from the variable splitter is connected to the fraction collector (e.g., a custom-modified cryo-cooled PSGC 10-40 fraction collector, VAREX, Burtonsville, MD, USA, used in Part II of this study [3]).

Naturally, all system components, modules, and transfer lines (fused-silica or stainless-steel capillaries) are thermostatted to prevent undesirable wall adsorption and memory effects.

The stability and reproducibility of the entire preparative system is demonstrated in Fig. 5 by a 1-h segment of a single day's production campaign (50 mg enflurane injections, 35 min preparative cycle time, 15 s interface valve cycling time), and in Fig. 6 by one separation segment from each of two successive production campaigns completed on two consecutive days (50 mg enflurane injections, 35 min preparative cycle time, 15, and 30 second interface valve cycling time).

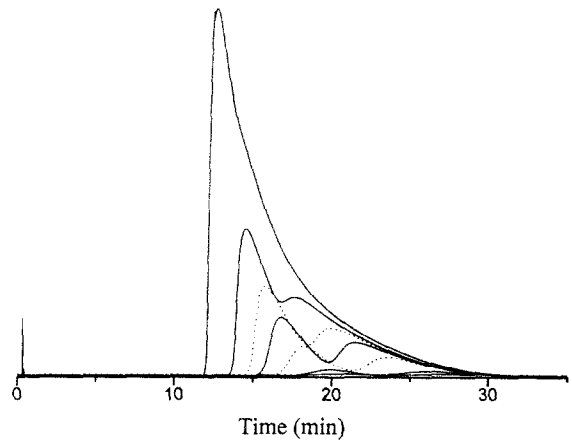

Fig. 4. Preparative FID traces (envelope chromatograms) for enflurane samples of increasing size. Preparative column: as in Fig. 3. Enflurane sample loads: 1, 3, 6, 20, 32, 50, 75, and 120 mg, respectively, increasing as peak height.

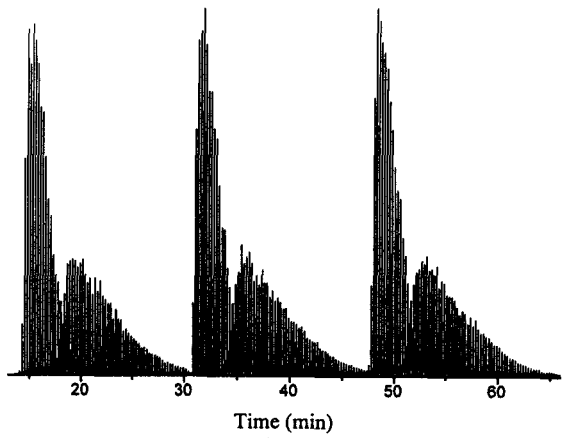

Fig. 5. Stability and reproducibility of the preparative system: a 1-h segment of the analytical FID trace during a single day's production campaign (50-mg enflurane injections, 35-min preparative cycle time, 15-s interface valve cycling time). Conditions as in Fig. 3.

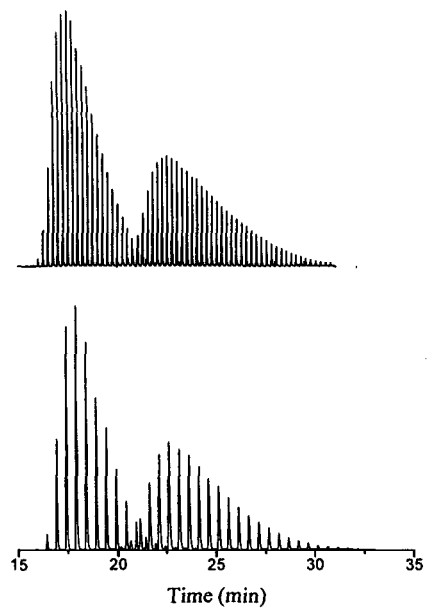

Fig. 6. Stability and reproducibility of the preparative system: one separation segment each of two successive production campaigns completed on two consecutive days (50 mg enflurane injections, 35-min preparative cycle time, 15-, and 30-s interface valve cycling time). Conditions as in Fig. 3.

3.3. Preparative results

In order to obtain an idea of the loading capacity of the 1 m × 10 mm I.D. column, a series of enflurane injections were made in the 3 to 250 mg load range. (The envelope chromatograms for some of these separations are shown in Fig. 4.) The on-line effluent enantiomer concentration data were obtained in each case (as shown, for example, for the 50 mg load in Fig. 5) and the peak areas of the enantiomers were used to calculate the enantiomeric purity vs. production and enantiomeric purity vs. % recovery curves as presented, for example, in Fig. 7 for the 120 mg injection. The inset in Fig. 7 shows the reconstructed chromatogram of the enantiomers and reveals that at the 120 mg load level there is already a significant interference between the less and the more retained enantiomers. The reconstructed chromatogram also reveals that at this load the isotherm effects, rather than the "sorption effect", dominate the peak shape [5].

Using the production vs. enantiomeric purity curves (such as in Fig. 7), the production vs. feed relationships can be calculated for various product purities as shown in Fig. 8 for the less

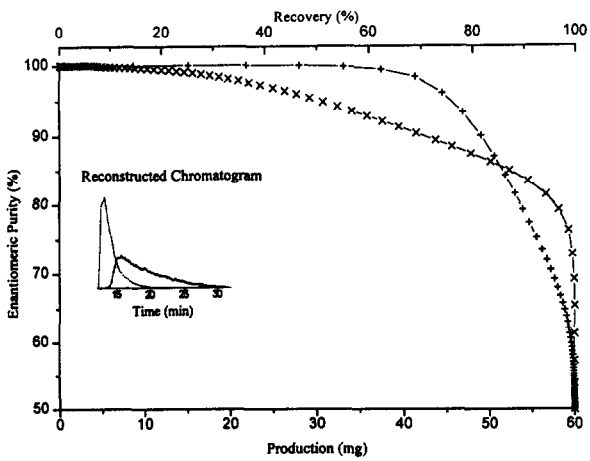

Fig. 7. Enantiomeric purity as a function of production and % recovery for a 120-mg injection calculated from the reconstructed chromatogram shown in the inset. Conditions as in Fig. 3. + = less retained enantiomer; × = more retained enantiomer.

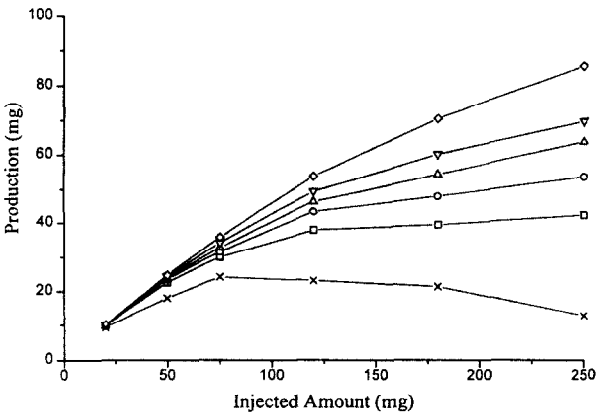

Fig. 8. Production for the less retained enflurane enantiomer as a function of the injected sample amount at various levels of product purity. Conditions as in Fig. 3. ◇ = 80%; ▽ = 90%; △ = 95%; ○ = 97%; □ = 99%; × = 100% purity, respectively.

retained enantiomer and Fig. 9 for the more retained enantiomer. These graphs allow us to determine the maximum injection sizes that should be used in the production campaigns for a particular product purity level.

If the less retained enantiomer is to be produced at 100% purity, the maximum injection size should be 75 mg, leading to 24 mg of the pure product; at higher loads the production decreases. If the purity requirement is relaxed to 99%, the production increases significantly (to 35 mg) as the injection size is increased to 120 mg, but then levels off. At 97% or lower purity levels the production of the less retained enantiomer increases continuously as the injected amount is increased in the range tested (up to 250 mg feed).

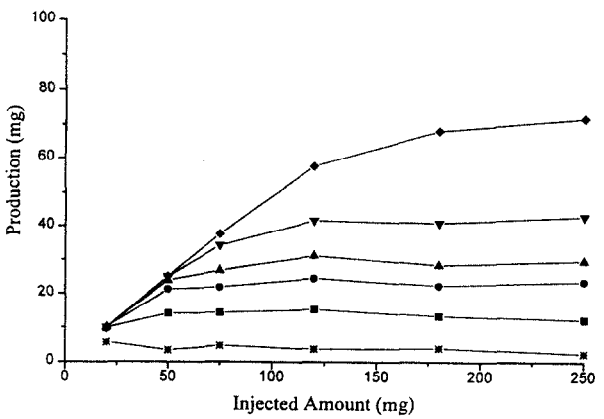

Fig. 9. Production for the more retained enflurane enantiomer as a function of the injected sample amount at various levels of product purity. Conditions as in Fig. 3. ◆ = 80%; ▼ = 90%; ▲ = 95%; ● = 97%; ■ = 99%; * = 100% purity, respectively.

The picture is quite different for the more retained enantiomer. As shown in Fig. 9, only about 6 mg can be produced at 100% purity, and it requires an injection size of 20 mg. Above this load, production drops slowly. At 99% enantiomeric purity the production plateau of 13 mg is reached with an injection size of 50 mg. In the load range tested, the production plateau does not disappear until the purity requirement is relaxed to 80%.

3.4. Production results

Figs. 8 and 9, which are based on single-injection separations, underestimate the production levels that can be achieved if the separations are repeated round-the-clock. This is because in a production campaign the repetitive injections can be timed such that the end of the second peak in a separation coincides with the start of the first peak in the subsequent preparative separation. Since the front of the first peak moves forward as the load is increased while the tail end of the second peak (representing infinite dilution) remains constant, the elution width of the peak pair becomes important as more cycles of lighter load can be carried out in a 24-h production day than heavier load (e.g. 90 cycles at 16 min each for 25 mg load, 65 cycles at 22 min each for 250 mg load).

Assuming that both enantiomers are to be recovered at the same purity level, Fig. 10 shows the daily production levels (and the amount of material that has to be recycled because its enantiomeric purity is below the specified level) as a function of the specified product purity and the amount of racemate feed injected in each cycle. The daily production surface for the more retained enantiomer is shown by the thickest line, the surface for the less retained enantiomer by the moderately thick line, while the daily

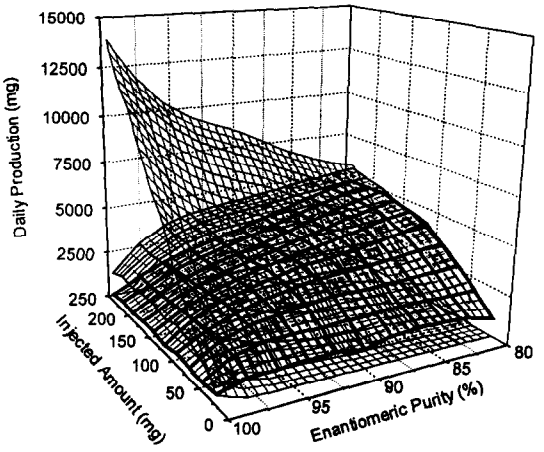

Fig. 10. Daily production as a function of sample loads and enantiomeric purity of product (symmetric purity regime). Conditions as in Fig. 3. Thickest line: more retained enantiomer; medium line: less retained enantiomer; thinnest line: daily recycle.

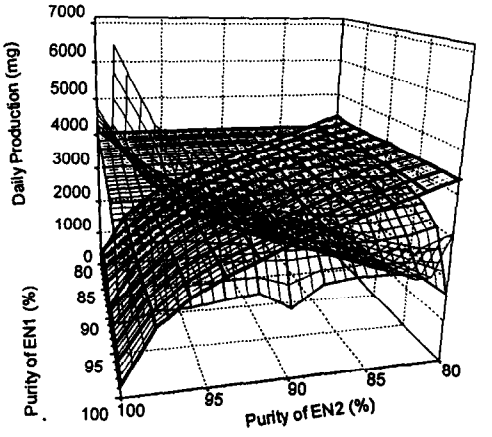

Fig. 11. Daily production as a function of the enantiomeric purity of both products for 120 mg enflurane injections. Conditions as in Fig. 3. Thickest line: more retained enantiomer; medium line: less retained enantiomer; thinnest line: daily recycle.

recycle surface is shown by the thinnest line. For 100% enantiomeric purity in both products, 75 mg injections should be used resulting in about 1900 mg of the less retained and 375 mg of the more retained enantiomers, and the recycle burden is about 3700 mg. Larger injections greatly increase the recycled amount and lead to slightly lower productions for both enantiomers. At 99% purity levels the trends are the same. However, when the purity requirement is relaxed to below 98%, the production surface for the less retained enantiomer loses its local maximum as the injected amount is increased (i.e. production continues to increase as load is increased, though at a decelerating rate), while that of the more retained enantiomer retains its local maximum. Thus, depending on the objectives of the campaign (production of larger, equal or lesser quantities of the first or second enantiomers), different operating conditions can be selected. The local maximum in the production surface of the more retained enantiomer remains, even at as low a product purity as 80%.

If the production campaign calls for the production of enantiomers of different purity, a three-dimensional daily production vs. product purities graph can be created for each injection level. As an example, the surface belonging to the 120-mg injection campaign is shown in Fig. 11. Once the required purities are set, the feed size that leads to the highest daily productions can be easily selected from the successive graphs.

4. Conclusions

A custom-designed preparative gas chromatographic system has been assembled from easily modified commercially available components. In addition to the preparative separation, the system accomplishes the on-line enantiomeric analysis of the effluent of the preparative column. On-line enantiomeric analysis is achieved by the use of a short, efficient capillary column, which is connected to a programmable effluent-sampling interface valve. For best characterization of the preparative column effluent, the analysis time on the capillary column has to be an integer number multiple of the cycle time of the sampling interface valve. This crucial condition can be met by the simultaneous adjustment of the operation temperature, length and phase ratio of the capillary column, as well as the linear velocity of its carrier gas. The on-line enantiomeric analysis permits the use of aggressive, yet safe production regimes.

Using this system and a 1 m long, 10 mm I.D. column packed with a chiral stationary phase, a successful preparative separation scheme has been developed for the production of gram/day quantities of the pure enantiomers of the volatile fluoroether anesthetic, enflurane. Further work is underway in our laboratory to use the system and extend this method development scheme for the production of other enantiomers of value or interest.

Acknowledgements

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