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Evaluation of β-cyclodextrin-based chiral stationary phases for capillary column supercritical fluid chromatography

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Abstract

The chromatographic performance of two series of chiral stationary phases (CSPs) based on β-cyclodextrin, one copolymeric and one side-arm substituted, was investigated to improve the applicability of cyclodextrin-based CSPs in open-tubular column supercritical fluid chromatography. For the side-arm approach, the influence on performance of different amount of cyclodextrin in the CSP, attachment of the cyclodextrin at the wide or narrow opening, different substituents on the cyclodextrin, the structure of the spacer and the film thickness was studied. The immobilization of these CSPs was also investigated. In addition, the chromatographic performance in gas and supercritical fluid chromatography of one of these CSPs was compared at optimal conditions using test solutes having relatively large differences in size and functionality.

1. Introduction

Chiral stationary phases based on β-cyclodextrin are, today, the most widely applicable stationary phases available for chiral separations performed by open-tubular column gas chromatography (GC) [1–3] and supercritical fluid chromatography (SFC) [4]. The latter technique has proved to be able to separate the underivatized enantiomers of a wide range of compounds, e.g. alcohols, lactones, ketones, amines, carboxylic acids and steroids, including relatively large and polar compounds such as ibuprofen, flurbiprofen, warfarin, dihydrodiazepam, norgestrel and 10,2-camphorsultam, which are not readily analyzed by GC due to low volatility or thermal instability [5–13].

In previous work on CSPs with cyclodextrin functionalities for open-tubular column SFC, three approaches can be identified for the design of the CSPs: (i) a polysiloxane having permethylated β-cyclodextrin attached to aliphatic side-arms [5–8,10], (ii) a copolymer consisting of alternating β-cyclodextrin and polysiloxane units [9,11,12] and (iii) methylated β-cyclodextrin having short polysiloxane units as substituents [13,14].

In the present study the chromatographic performance of a number of β-cyclodextrin-based CSPs, synthesized according to the copolymeric (CSP **1–3**, Table 1) and side-arm approach (CSP **4–21**), was compared. For the side-arm approach, the influence on performance

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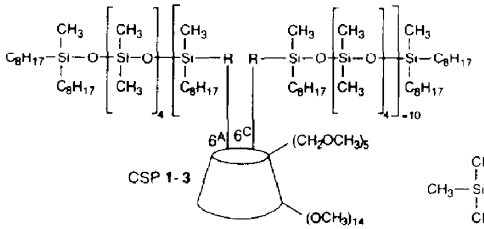
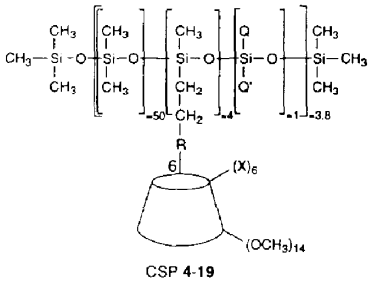
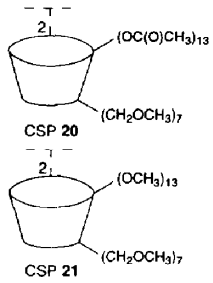
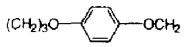
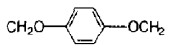
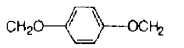
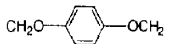
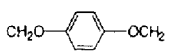
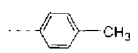
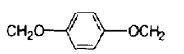
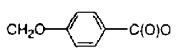
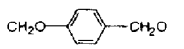
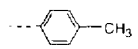
Table 1
Structures of the CSPs used in this study







CSP	R[a]	Q, Q'	X	% n/n[b]	% w/w[c]
1					56
2	$(CH_2)_3OCH_2$				59
3	$(CH_2)_{10}OCH_2$				57
4-11[d]		C_8H_{17}, CH_3	CH_2OCH_3	0.4-9.5	12-72
12		C_8H_{17}, CH_3	$CH_2OC(O)CH_3$	3.6	59
13		C_8H_{17}, CH_3	CH_3	3.6	50
14	None	C_8H_{17}, CH_3	CH_2OCH_3	3.6	56
15	CH_2CH_2	C_8H_{17}, CH_3	CH_2OCH_3	3.6	56
16	CH_2OCH_2	C_8H_{17}, CH_3	CH_2OCH_3	3.6	55
17	$CH_2(CH_2)_8OCH_2$	C_8H_{17}, CH_3	CH_2OCH_3	3.6	53
18			CH_2OCH_3	4.3	56
19[e]		C_8H_{17}, CH_3	CH_2OCH_3	4.0	53
20		C_8H_{17}, CH_3		3.6	59
21				4.3	56

[a] The left side of the spacer is attached to the siloxne.
[b] Amount of cyclodextrin expressed in % by substituent.
[c] Amount of cyclodextrin expressed in % by weight. The spacer is assigned to the achiral part of the CSP.
[d] Synthesized in eight versions with different amounts of cyclodextrin (0.4, 0.8, 1.5, 2.1, 2.7, 3.6, 5.4 and 9.5% by substituent, or 12, 21, 34, 42, 46, 53, 63 and 72 % w/w).
[e] Contains 4.0% cyclodextrin, 1.0% octyl and 5% cyanopropyl by substituent.

of different amounts of cyclodextrin in the CSP, attachment of the cyclodextrin at the wide or narrow opening, different substituents on the cyclodextrin, the structure of the spacer and the film thickness was studied. The possibility to immobilize these CSPs was also investigated. In addition, the chromatographic performance in GC and SFC of one of these CSPs was compared at optimal conditions for a number of test solutes having different size and functionality.

2. Experimental

2.1. Preparation of CSPs

The synthesis of the CSPs used in this study (Table 1) have previously been described (CSP **9, 12–17** [15]) or are in the process of being published (CSP **1–3, 4–8, 10–11** and **18–21** [16]).

2.2. Column preparation

The fused-silica capillaries, 5 m × 50 μm I.D. for SFC and 10 m × 100 μm I.D. for the comparison of SFC and GC (Polymicro Technologies, Phoenix, AZ, USA) were deactivated as previously described [17].

The deactivated capillaries were statically coated at 25°C using dichloromethane as solvent for the CSPs. The concentration of the coating solution, C_{CSP}, was calculated according to the following equation assuming a stationary phase density, ρ_{CSP}, of 1 g ml^{-1}:

$$C_{CSP} = \rho_{CSP}(r^2 - (r - d_f)^2)/r^2 \quad (1)$$

where r represents the internal radius of the capillary and d_f the approximate film thickness. In order to immobilize the CSPs by crosslinking, 5% (w/w, relative to the amount of CSP in the coating solution) dicumylperoxide (Aldrich, Steinheim am Albuch, Germany) was added as a free radical initiator.

The CSPs were immobilized by heating the coated capillaries from room temperature to 170°C at 4°C min^{-1} under a slow nitrogen purge (ca. 0.05 ml min^{-1}) and subsequently keeping the capillaries at 170°C for 40 min plus an additional 60 min at an increased nitrogen flow (ca. 0.20 ml min^{-1}). Prior to evaluation, the columns were rinsed with supercritical CO_2 for 60 min in a dynamic extraction (SFE) at temperatures and densities slightly above the testing conditions. The columns used in the evaluation of the immobilization procedure and stability test were subjected to repeated SFE at 415 atm and 120 and/or 60°C.

2.3. Samples

(±)-3,3-Dimethyl-1,2-butanediol, (±)-γ-phenyl-γ-butyrolactone, (±)-2-phenylcyclohexanone, (±)-pantolactone, (±)-diethyl tartrate, (±)-1-phenyl-1-ethanol, (±)-*trans*-1,2-cyclohexanediol and (±)-*trans*-2-phenylcyclohexanol were obtained from Aldrich (Steinheim am Albuch, Germany). (±)-Ibuprofen was obtained from Sigma Chemical Company (St. Louis, MO, USA). The following compounds were gifts from persons and companies listed below and are gratefully acknowledged: (±)-carboranylalanine derivative [(*N*-trifluoroacetyl)-propylester], S. Sjöberg (Uppsala University, Uppsala, Sweden); (±)-chlor-mezanone, Sanofi Winthorp Ltd. (Wythenshawe, Manchester, UK); (±)-dihydrodiazepam and (±)-γ-heptalactone, W. Walther (Hoffman-La Roche, Basel, Switzerland); (±)-2,8-di(hydroxyethyl)-6*H*,*H*-5,11-methanodibenzo-[*b*,*f*]-[1,5]-diazocine and (±)-1-(4-phenyl)phenylethanol, B.E. Rossiter (Brigham Young University, Provo, UT, USA) and (±)-glutethimide, K. Grolimund (Ciba-Geigy, Basel, Switzerland).

All racemates were dissolved in ethanol with the exception of the carboranylalanine derivative which was dissolved in dichloromethane.

2.4. Instrumentation

SFC–FID was performed with a Series 600-D SFC system (Dionex, Salt Lake City, UT, USA) equipped with a flame ionization detector (FID, 350°C). The injector consisted of a Model CI4W.5 high-pressure four port valve injector

with a 0.5 μl sample loop (Valco Instruments, Houston, TX, USA) combined with a splitter (300 μm I.D., SGE, Austin, TX, USA). The density of the mobile phase was kept approximately constant along the column using an in-house made deactivated integral [18] or frit restrictor (Dionex, Salt Lake City, UT, USA). SFC–MS was performed by coupling the system above to an API-III (SCIEX Perkin Elmer, Thornhill, Ontario, Canada) [19]. GC–FID was performed with a GC 6000 Vega Series 2 (Carlo Erba, Milan, Italy) equipped with a split injector (250°C) and an FID (350°C). Hydrogen was used as mobile phase at an average linear velocity of 75 cm s^{-1} at 100°C.

The chromatograms were registered with an SP4290 or ChromJet integrator (SpectraPhysics, San Jose, CA, USA), transferred to a Macintosh IIfx computer (Apple Computer, Cupertino, CA, USA) and subsequently decoded with an routine written in-house in Microsoft QuickBasic (Microsoft, Redmond, WA, USA). A program for general graphing and data analysis, Igor (Wave Metrics, Lake Oswego, OR, USA) was used for determination of peak widths, retention times etc.

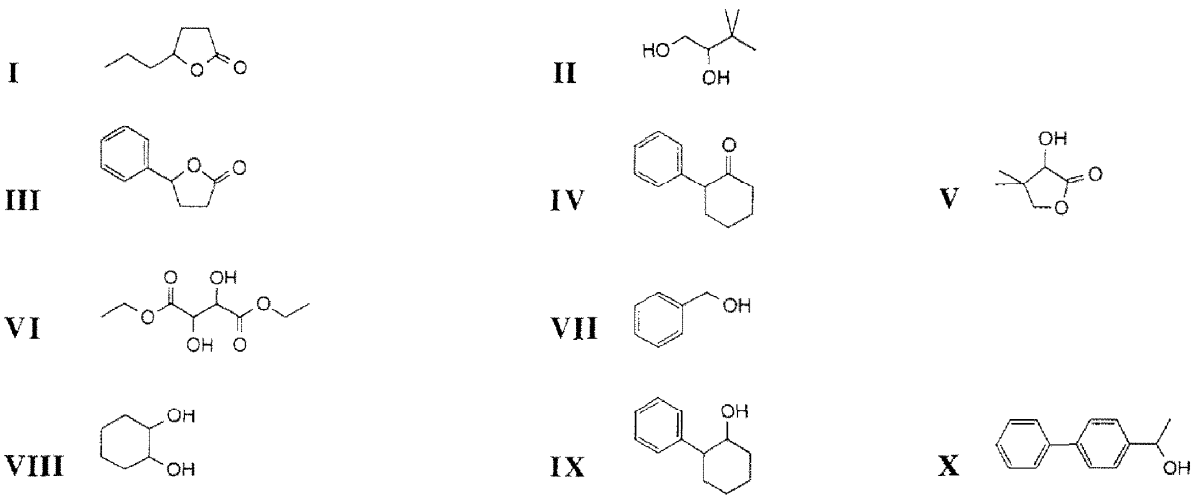
3. Results and discussion

3.1. Evaluation of CSP performance

The chromatographic performance of the CSPs in SFC was investigated by the injection of ten racemic test compounds **I–X** (Table 2) under isopycnic and isothermal conditions. The repeatability of the column preparation procedure was estimated by the preparation of five columns coated with CSP **18**. Based on injections of (±)-*trans*-2-phenylcyclohexanol **IX**, confidence intervals at the 95% level were calculated for the capacity factor, $k' \pm 0.17$, selectivity, $\alpha \pm 0.006$, and efficiency, $N \pm 380$ plates m^{-1}. The relatively large but quite normal variance in efficiency made it difficult to compare efficiency and resolution for different CSPs and emphasis has therefore been put on chiral selectivity instead.

As the densities of the different CSPs are unknown, the film thicknesses of columns coated with different CSPs may vary somewhat (Eq. 1) and consequently the comparison of retention may be somewhat biased. This bias should be most pronounced in the comparison of CSP **4–11**

Table 2
Compounds used to compare chromatographic performance on CSPs **1–21** in SFC



Conditions: **I** 60°C, 0.20 g ml^{-1}, **II** 50°C, 0.30 g ml^{-1}, **III–VII** 60°C, 0.30 g ml^{-1}, **VIII–IX** 60°C, 0.35 g m^{-1}, **X** 60°C, 0.40 g ml^{-1}.

as there is a large variation in cyclodextrin content between these CSPs. The selectivity should however not, according to theory, be influenced by differences in film thickness.

Initially it was decided to compare the chromatographic performance of the CSPs at equal chromatographic conditions, as well as equal retention. The relatively small changes in density required to achieve equal retention did, however, not cause any differences in chiral selectivity for (±)-2-phenylcyclohexanone **IV**, when chromatographed on the different CSPs. It was therefore decided that a comparison of selectivity and retention at identical conditions should suffice as this approach reduces the number of experiments.

3.2. Comparison of the copolymeric and the side-arm approach

Previously reported copolymeric CSPs based on cyclohexylenebisbenzamides showed, in contrast to the cyclodextrin-based copolymeric CSPs **1–3**, large differences in both retention and selectivity in response to small changes in the structure of the spacers between the chiral unit and the achiral siloxane block [17]. In the case of cyclodextrin-based CSPs it could, on the other hand, only be concluded that short and rigid spacers favour the selectivity somewhat (Fig. 1). These results indicate that the copolymeric approach require relatively small chiral units and not macrocycles like cyclodextrins for which the structure of the spacers does not seem to be critical (cf. section 3.6, Structure of the spacer).

As shown in Fig. 1, CSP **1**, representing the copolymeric approach, and CSP **9**, representing the side-arm approach [56 and 53% (w/w) cyclodextrin respectively], did not show any large differences in retention and chiral selectivity. The side-arm CSP gave, for all compounds in the test set, similar or somewhat better chiral selectivity, indicating that the secondary structure of the copolymers does not contribute to the chiral selectivity.

Considering these results and the fact that the copolymeric cyclodextrin-based CSPs are more complex to synthesize it was logical to focus further studies on the side-arm approach, CSP **4–21**.

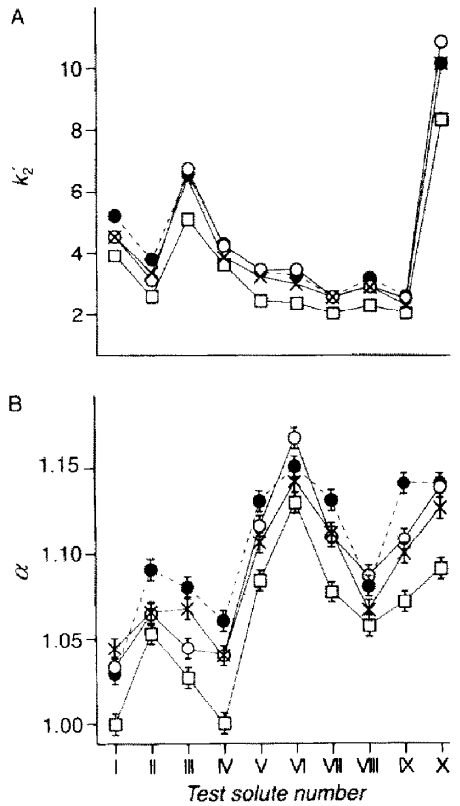


Fig. 1. Comparison of copolymeric (CSP **1** ○, **2** × and **3** □, solid line) and side-arm CSPs (CSP **9** ●, dashed line). (A) retention of the last eluting enantiomer and (B) chiral selectivity versus test solute number (SFC–FID cf. Table 2). Error bars correspond to confidence intervals at the 95% level ($k' \pm 0.17$ and $\alpha \pm 0.006$ estimated from 5 columns coated with CSP **18**).

3.3. Amount of cyclodextrin in the CSP

For CSPs similar to CSP **17** it has previously been found by Jung and Schurig [20] that the chiral selectivity in GC increases with increasing amount of cyclodextrin in the CSP (16–36%) up to a certain limit above which little or no chiral selectivity is gained, i.e. 20–30%. This phenomenon was explained on the basis of theoretical models for the relationship between the chiral selectivity and the amount of cyclodextrin in the CSP [21]. Comparisons of the chiral selectivity of CSP **4–11** (12–72% cyclodextrin) using SFC

showed similar trends for all compounds in the test set **I–X**. This suggest that the models can be applied also in SFC and for higher amounts of cyclodextrin than previously reported.

An increase of the amount of cyclodextrin in the CSP from 12 to 72% resulted in a significant increase of the capacity factor, often in the order of 300%. It is likely that this difference in retention is related to some extent to differences in the CSP density as mentioned above.

The influence of the amount of cyclodextrin on sample capacity was investigated by the injection of different amounts of (±)-γ-phenyl-γ-butyrolactone **III** on columns coated with CSP **8** and **11**, i.e. CSPs containing 46 and 72% cyclodextrin. By plotting the asymmetry factor for the first eluting enantiomer versus peak area it was found that the CSP having a higher content of cyclodextrin also had an enhanced sample capacity (Fig. 2). Large sample capacity is an important property of chiral columns as closely eluting enantiomers often have to be quantified at an enantiomeric impurity level of less than 1%. The sample capacity is usually low in comparison with achiral columns due to the relatively low number of chiral substituents. It may therefore be favourable to use a relatively large amount of cyclodextrin in the CSP, especially since this does not seem to influence the efficiency dramatically (CSP **4** 1400, **5** 1200, **6** 1900, **7** 600, **8** 2200, **9** 2200, **10** 1800 and **11** 1800 plates m^{-1}, (±)-*trans*-2-phenylcyclohexanol **IX**, SFC–FID cf. Table 2).

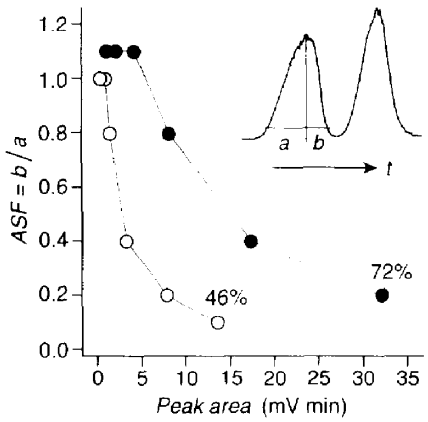


Fig. 2. A comparison of sample capacity for two columns (5 m × 50 μm I.D., $d_f \approx 0.25$ μm) coated with CSP **8** and **11** having different amounts of cyclodextrin (46 and 72% w/w). Asymmetry factor versus peak area was monitored in SFC–FID for (±)-γ-phenyl-γ-butyrolactone **III** at 60°C and 0.30 g ml^{-1}. The smallest and largest peak areas correspond to concentrations of 1.25 and 40.00 mg ml^{-1}, respectively, i.e. approximately 62 and 2000 ng on the columns.

3.4. Attachment to the siloxane backbone

The stationary phases **18** and **21** are structurally similar and differ only in that CSP **18** has the cyclodextrin attached at the narrow opening and CSP **21** at the wide opening. A comparison of the chromatographic performance (Fig. 3) shows that the attachment of the wide opening of the cyclodextrin to the siloxane backbone resulted in

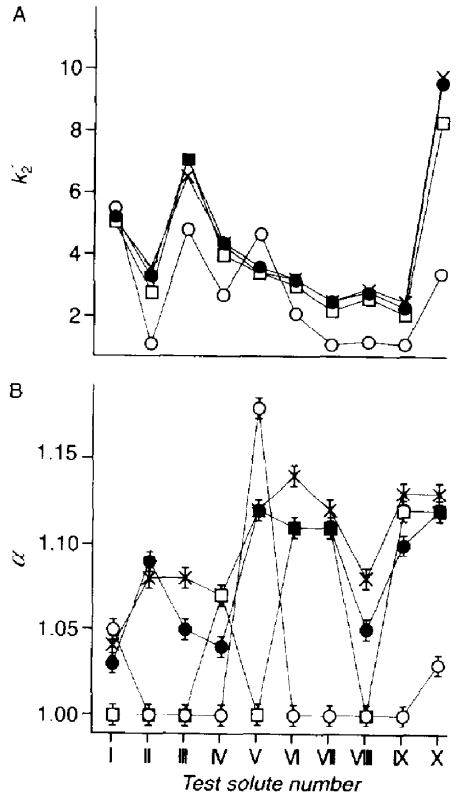


Fig. 3. Effect of attachment of the cyclodextrin on (A) retention of the last eluting enantiomer and (B) chiral selectivity versus test solute (SFC–FID cf. Table 2). Attachment at the narrow opening (CSP **12** □ and **18** ×) and the wide opening (CSP **20** ○and **21** ●).

a reduced chiral selectivity for 60% of the compounds in the test set. By comparing the performance of CSP **12** and **20** (Fig. 3) it is seen that such an attachment in combination with the acetylation of the hydroxyl groups at the opening on the side of attachment, i.e. narrow opening for CSP **12** and wide opening for CSP **20**, resulted in shorter retention and lower chiral selectivity for most compounds in the test set with exception of compound **V**. In other words, there was a loss of general selectivity but a gain in specific selectivity. Both CSP **12** and **20** showed a poorer general chiral selectivity than any other CSP in this study.

These results indicate that the acetylation of and/or the attachment of the wide opening of the cyclodextrin to the siloxane backbone will reduce the access to the cyclodextrin cavity and thereby hamper the chiral selectivity.

3.5. Substitution at the narrow opening of the cyclodextrin

The replacement of the hydroxyl groups at the narrow opening of the cyclodextrin with hydrogen, CSP **13**, resulted in a somewhat shorter retention and a chiral selectivity that was different from that of the permethylated versions (Fig. 4). It is, with the present number of test compounds, not possible to state which CSP, **9** or **13**, that would be most widely applicable.

The acetylation of the hydroxyl groups at the narrow opening, i.e. CSP **12**, resulted in a significant decrease in chiral selectivity in comparison to the permethylated versions, e.g. CSP **9** (Fig. 4). One explanation for this loss in selectivity could be a disturbance of the interactions at the narrow opening, while another explanation is a change of the shape of the cyclodextrin torus. Molecular modelling studies [22] indicate that a derivatization of the narrow opening of the cyclodextrin may influence, i.e. distort, the shape of the wide opening.

3.6. Structure of the spacer

The different spacers connecting the cyclodextrin to the siloxane backbone, evaluated in this

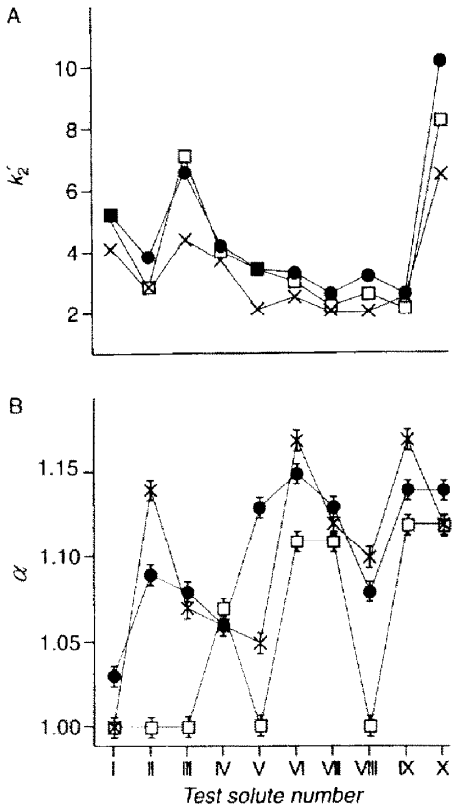


Fig. 4. Substitution at the narrow opening of the cyclodextrin, (A) retention of the last eluting enantiomer and (B) chiral selectivity versus test solute (SFC–FID cf. Table 2). CSP **9** ●, **12** □ and **13** ×.

study, CSP **9**, **14–17**, showed similar retention and selectivity. It is therefore not possible to distinguish between the influence of a long and short spacer or between an aromatic and aliphatic spacer. Thus it is reasonable to believe that the structure of the spacer is not critical for this type of CSPs.

3.7. Film thickness

Five capillaries (5 m × 50 μm I.D.) were coated with CSP **9** to give columns having film thicknesses of approximately 0.125, 0.25, 0.375, 0.50 and 0.625 μm, respectively. The peak asymmetry was then monitored for (±)-γ-phenyl-γ-butyrolactone **III** in analogy with the study of the influence of substitution ratio above.

An increasing film thickness is expected to

give both increased sample capacity and retention whereas the selectivity should remain constant. While retention and selectivity behaved as expected, the sample capacity, measured in terms of peak asymmetry, did not differ significantly between columns with different film thicknesses. This unexpected result, for which we have no reasonable explanation, indicates that it is not advisable to increase the film thickness of these CSPs above ca. 0.25 μm to increase the sample capacity as this would mainly increase the retention and decrease the efficiency.

3.8. Substitution of the siloxane backbone

CSPs having octyl, CSP **9**, and tolyl, CSP **18**, groups attached to the siloxane backbone to enhance immobilization did not differ significantly with respect to chromatographic performance (Fig. 5). The addition of a cyanopropyl group, CSP **19**, did, however, result in increased retention as well as chiral selectivity for most compounds in the test set. It must consequently be concluded that the polar cyano groups participate in and improve the chiral recognition of certain solutes. It is therefore not unlikely that the presence of other small polar groups on the siloxane backbone, or the rim(s) of the cyclodextrin, could enhance the chiral selectivity.

3.9. Immobilization and column stability

Due to the solvating power of the supercritical mobile phase, it is necessary to immobilize the stationary phase in order to obtain a stable column and allow the analysis of non-volatile compounds. In open-tubular column SFC, the CSP is generally immobilized by thermal or radical initiated crosslinking, as has successfully been done for achiral columns [23]. In the presence of the bulky chiral selector, however, radical initiated crosslinking is often disturbed and it becomes necessary to incorporate groups in the non-chiral part of the CSP that facilitate crosslinking. A small amount of octyl, CSP **1–17**, **19** and **20**, or tolyl, CSP **18** and **21**, groups (ca. 1% by substituent) was therefore incorporated in the siloxane for this purpose. The use of dicumylperoxide as a free radical initiator was found to reduce the α values for CSP **9** and **18** with about 3% in comparison with the non-immobilized CSPs. A rinsing with supercritical CO_2 at 60°C and 0.89 g ml^{-1} (415 atm, the upper pressure limit of the instrument) for five hours followed by a rinsing at 120°C and 0.71 g ml^{-1} (415 atm) for another five hours resulted, for the dicumylperoxide treated CSP **9**, in a 30% decrease in k', a 37% decrease in N but no decrease in α. For the dicumylperoxide treated CSP **18** the same rinsing procedure resulted in a 22% decrease in k', a 14% decrease in N and no decrease in α, a result promoting the use of tolyl substitution of the CSP. Tolyl substituted polysiloxanes should have a solubility that is more compatible with cyclodextrin than the corresponding octyl substituted polysiloxanes, an im-

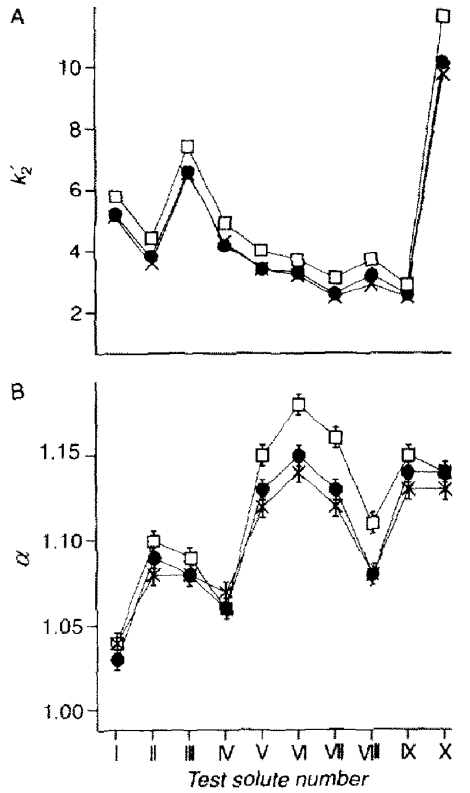


Fig. 5. Substitution of the siloxane backbone, (A) retention of the last eluting enantiomer and (B) chiral selectivity versus test solute (SFC–FID cf. Table 2). CSP **9** ●, **18** × and **19** □.

portant consideration for reproducible and uniform synthesis.

The relatively large decrease in k' indicates that the CSPs might contain a low-molecular mass fraction that can not be immobilized. In an attempt to decrease the loss of stationary phase during rinsing, 10 mg of CSP **18** was coated on the walls of a piece of glass tubing and subsequently subjected to dynamic SFE to remove low-molecular-mass material from the CSP prior to the coating of columns. Even though the CSP was extracted at 60°C and a density of 0.80 g ml^{-1} for two hours no decrease in mass could be observed which indicates that the CSP should have a uniform molecular mass. However, a column coated with the extracted CSP did not show any improved degree of immobilization. One explanation for this could be that the relatively thick polymer film on the glass prevented an efficient extraction. During the SFE treatment it was realized that the CSP becomes milky and non-transparent after a rapid density decrease in contrast to its otherwise colorless and transparent appearance. This observation stresses the need to avoid drastic density changes in order to maintain good column performance.

Column stability was investigated by subjecting a column coated with CSP **18** to extremely rapid density programs to the maximal density allowed by the instrument. The chromatographic performance was monitored after 0, 25, 50, 75 and 100 density programs respectively and, as is illustrated in Fig. 6, no degradation could be observed.

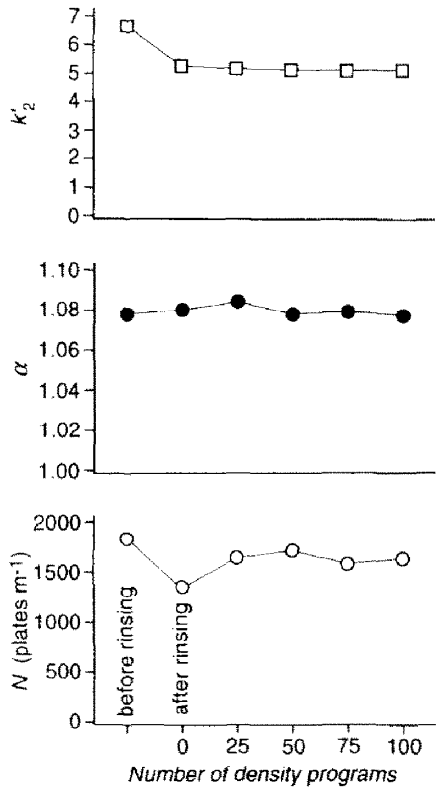


Fig. 6. Immobilization and column stability, chromatographic performance of a column (5 m × 50 μm, $d_f \approx 0.25$ μm) coated with CSP **18** monitored by the injection of (±)-γ-phenyl-γ-butyrolactone **III** (SFC–FID 60°C and 0.30 g ml^{-1}) before and after rinsing as well as after 25, 50, 75 and 100 extremely rapid density programs to the maximal density allowed by the instrument (from 0.20 to 0.89 and 0.20 g ml^{-1} at ± 0.20 g ml^{-1} min^{-1} and 60°C).

3.10. Chromatographic performance with different mobile phases

According to our observations and previous studies by Schurig and co-workers [7], CSPs based on cyclodextrins give lower α values in SFC than in GC *at the same temperature* due to solvation effects. A comparison of efficiencies also revealed that GC provides higher efficiencies (250 μm I.D. columns, ca. 3000 plates m^{-1}) than SFC (50 μm I.D. columns, ca. 2000 plates m^{-1}).

However, the fact that the chiral selectivity increases with decreasing temperature can in some cases be used to compensate for the lower chiral selectivities and efficiencies in SFC. As the retention is usually controlled by the density of the mobile phase the temperature thus becomes a free variable. In addition, the use of low temperatures reduces the risk of thermal decomposition and racemization.

In most comparisons of the behaviour of a CSP in SFC and GC, retention, selectivity, resolution (R_s) and efficiency have been monitored at the same temperatures. In the present study, the separations of ten compounds with different polarity and molecular mass were com-

pared at "iso-conditions" yielding an R_s value of 1.5 within the shortest possible time or, if this was not obtainable, at a retention time of 60 min with as large resolution as possible (Table 3). Deviations of ±0.1 R_s units and ±5 min between predicted and experimental values were accepted, as the predictions were made by *extrapolation* from a small number of experiments [24–28]. A maximum working temperature of 200°C was used in order to prevent degradation of the column. From these comparisons it can be concluded that when using the same column, 10 m × 100 μm I.D., in both techniques, GC provides significantly shorter retention times than SFC for volatile compounds such as 1-phenylethanol and diethyl tartrate. However, the enantiomers of more polar compounds or compounds of higher molecular mass, e.g. 2,8-di(2-hydroxyethyl) - 6*H*,12*H* - 5,11 - methanodibenzo-[*b*,*f*]-[1,5]-diazocine, ibuprofen and dihydrodiazepam, are often difficult or even impossible to separate with GC.

It should be pointed out that this comparison was performed with a 10 m × 100 μm I.D. column which is theoretically favourable for GC and unfavourable for SFC. If instead a 50 μm I.D. column is used in SFC it is possible to decrease the retention times significantly. For example, with the 10 m × 100 μm I.D. column it was, for (±)-*trans*-2-phenylcyclohexanol **IX**, only possible to obtain baseline resolution at a retention time of 56 min whereas the use of a 5 m × 50 μm I.D. column allowed baseline resolution within 14 minutes, one minute shorter than in the GC separation (Table 3). Other examples of separations of the compounds listed in Table 3 using 5 m × 50 μm I.D. columns are shown in Figs. 7 and 8.

3.11. Examples of separations

The broad applicability of CSP **9** and **18** is exemplified in Figs. 7 and 8 in which the enantiomers of non-volatile compounds of pharmaceutical interest have been efficiently separated by SFC.

Fig. 7A shows the separation of the racemic (*N*-trifluoroacetyl)propylester of carboranylalanine, a compound which is not readily analyzed by either GC or LC as it requires a relatively

Table 3
Comparison between SFC and GC at optimal conditions using the same 100 μm I.D. column coated with CSP **18**

Compound	SFC 5 m × 50 μm I.D. [a,b]				SFC 10 m × 100 μm I.D. [a,c]				GC 10 m × 100 μm I.D. [a,d]		
	T (°C)	ρ (g ml^{-1})	t_{R2} (min)	R_s	T (°C)	ρ (g ml^{-1})	t_{R2} (min)	R_s	T (°C)	t_{R2} (min)	R_s
(±)-*trans*-2-Phenylcyclohexanol **IX**	60	0.35	14.1	1.6	40	0.20	56.1	1.6	120	15.3	1.5
(±)-1-Phenyl-1-ethanol **VII**					48	0.17	31.8	1.6	114	2.7	1.5
(±)-Pantolactone **V**					50	0.16	56.8	1.3	130	2.5	1.6
(±)-Diethyl tartrate **VI**					50	0.18	55.6	1.4	140	4.6	1.6
(±)-Glutethimide Fig. 7D					58	0.27	63.0	1.1	144	61.6	0.9
(±)-1-(4-Phenyl)phenylethanol **X**					51	0.29	59.9	1.6	139	60.2	1.0
(±)-Ibuprofen Fig. 7B					60	0.30	62.3	1.0	200	>60	–
(±)-Dihydrodiazepam Fig. 8					58	0.52	14.2	1.6	200	>60	–
(±)-2,8-Di(2-hydroxyethyl)-6H,-12H-5,11-methanodibenzo-[*b*,*f*]-[1,5]-diazocine (Fig. 7E)					53	0.61	64.9	1.5	200	>60	–
(±)-Carboranylalanine as its (N-trifluoroacetyl)propylester (Fig. 7A)					56	0.32	55.2	1.2	143	61.1	0.0

[a] For both the 50 and 100 μm I.D. columns the film thickness was ca. 0.25 μm.
[b] CO_2 at an average linear velocity of 1.9 cm s^{-1} at 60°C and 0.30 g ml^{-1}.
[c] CO_2 at an average linear velocity of 2.5 cm s^{-1} at 60°C and 0.30 g ml^{-1}.
[d] H_2 at an average linear velocity of 75 cm s^{-1} at 100°C.

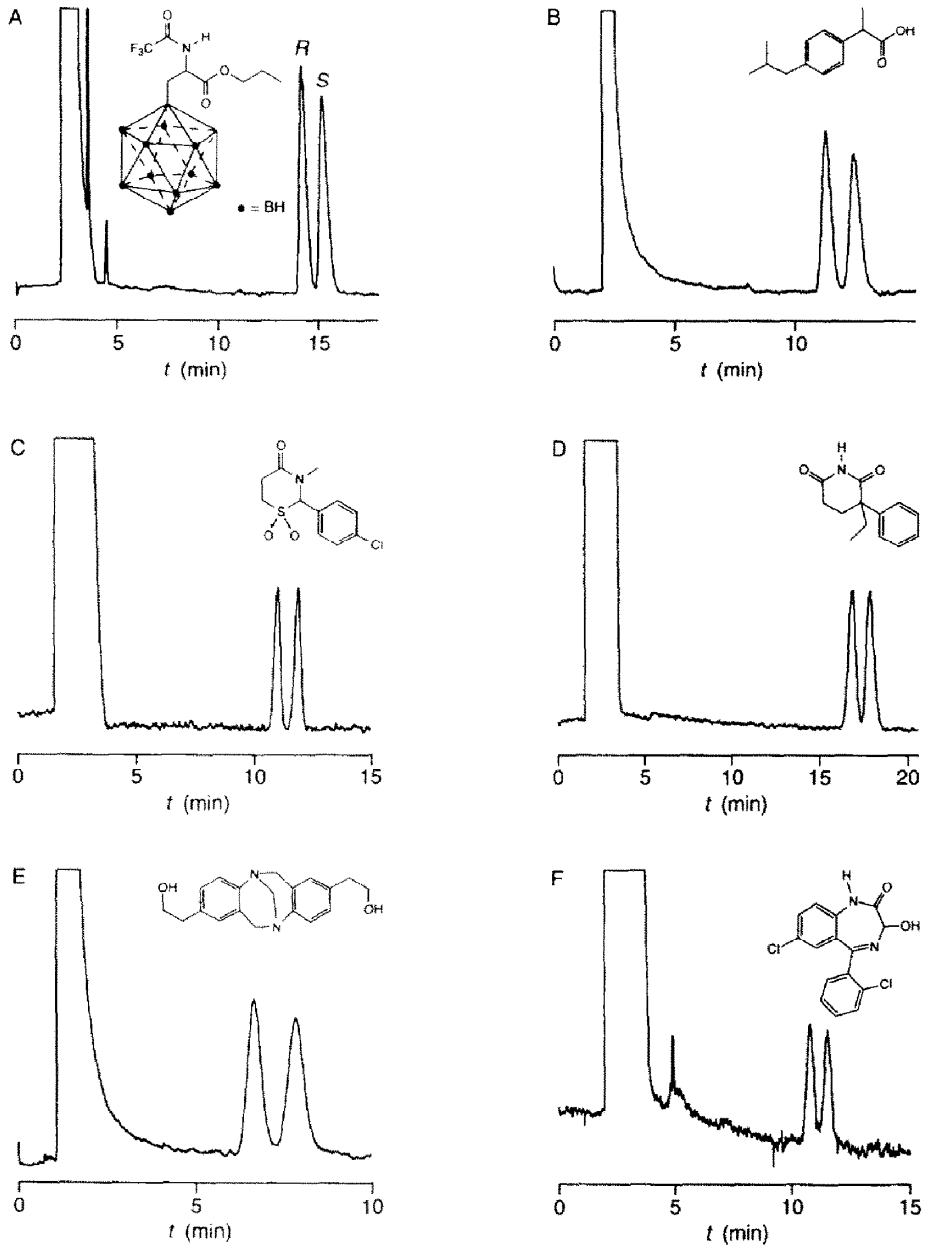


Fig. 7. Examples of separations performed by SFC–FID and columns coated with CSP **9** (A,B) and **18** (C–F), respectively. Column: 5 m × 50 μm I.D., $d_f \approx 0.25$ μm. Conditions: CO_2, 60°C, (A) (±)-carboranylalanine as its (N-trifluoroacetyl) propylester density programmed from 0.20 to 0.485 g ml^{-1} at 0.20 g ml^{-1} min^{-1} after a 2 min isopycnic period, (B) (±)-ibuprofen density programmed from 0.18 to 0.375 g ml^{-1} at 0.20 g ml^{-1} min^{-1} after a 2 min isopycnic period, (C) (±)-chlormezanone density programmed from 0.18 to 0.51 g ml^{-1} at 0.20 g ml^{-1} min^{-1} after a 2 min isopycnic period, (D) (±)-glutethimide density programmed from 0.18 to 0.375 g ml^{-1} at 0.20 g ml^{-1} min^{-1} after a 2 min isopycnic period, (E) (±)-2,8-di(2-hydroxyethyl)-6H,12H-5,11-methanodibenzo-[*b*,*f*]-[1,5]-diazocine isopycnic at 0.74 g ml^{-1} and (F) (±)-lorazepam density programmed from 0.20 to 0.79 g ml^{-1} at 0.20 g ml^{-1} min^{-1} after a 2 min isopycnic period.

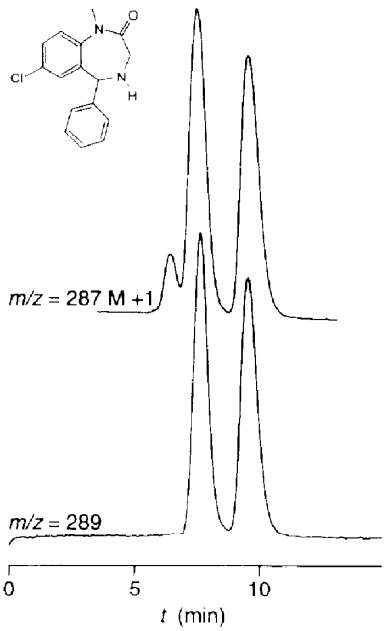


Fig. 8. SFC–MS, selected ion monitoring allowing the elimination of the signal from a compound coeluting with the analyte (±)-dihydrodiazepam. Column: CSP **18**, 5 m × 50 μm I.D., $d_f \approx 0.25$ μm. Conditions: CO_2 isopycnic at 0.48 g ml^{-1} and 85°C.

high temperature to elute in the former technique and only absorbs light at short wavelengths making LC detection difficult [27].

Also benzodiazepines, such as dihydrodiazepam, lorazepam and oxazepam [28] can be separated with this type of CSPs. Unfortunately, the FID response is quite small for lorazepam and oxazepam in comparison with other compounds (Fig. 7F), but this problem can probably be circumvented by the use of a UV detector, electron capture detector or mass spectrometer. The latter solution has several advantages as it is, for example, possible to eliminate the signals from coeluting compounds by employing selected-ion monitoring, as is illustrated in Fig. 8 for the enantiomers of dihydrodiazepam and an unknown impurity.

4. Conclusions

The copolymeric approach for the construction of CSPs seems to have no benefits over the side-arm approach when the chiral selector consists of macrocycles like β-cyclodextrin. Consequently, further development of cyclodextrin-based CSPs should be focused on the latter approach as this approach requires a less complicated synthesis.

Among the side-arm substituted CSPs evaluated in this study, the permethylated β-cyclodextrin and the permethylated β-cyclodextrin having the hydroxyl groups at the narrow opening replaced with hydrogen proved to be the most widely applicable CSPs. This result is well in line with previous observations in GC for alkyl and acetyl substituted cyclodextrins dissolved in polysiloxanes [2]. The structure of the spacer between the cyclodextrin and the siloxane part of the CSP does not seem to be critical. A small amount of cyanopropyl groups attached to the siloxane backbone improved the chiral selectivity. This indicates that a small number of polar groups on the siloxane backbone or the rim(s) of the cyclodextrin could be beneficial.

Since the sample capacity increased and the efficiency did not decrease dramatically with increasing substitution ratio it should be favourable to utilize relatively large amounts of cyclodextrin in the CSP, e.g. 70%.

Even though the present approach does not allow a complete immobilization of the CSPs (ca. 20% was lost in terms of decrease in k'), the stability study clearly shows that the obtained columns are stable enough to allow a more widespread use.

As illustrated in the comparison between open-tubular column GC and SFC, SFC should be a valuable complement to GC for the analysis of non-ionic compounds of low to medium volatility.

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