ELSEVIER

Journal of Chromatography A, 694 (1995) 237–243

JOURNAL OF CHROMATOGRAPHY A

Gas chromatographic enantiomer separation of pharmaceuticals on capillary columns coated with novel chiral polysiloxanes

Iwao Abe[a,*], Takako Nishiyama[a], Taketoshi Nakahara[a], Hartmut Frank[b]

[a]*Department of Applied Chemistry, College of Engineering, University of Osaka Prefecture, 1-1 Gakuen-Cho, Sakai, Osaka 593, Japan*

[b]*Institute for Toxicology, University of Tübingen, Wilhelmstrasse 56, D-72074 Tübingen, Germany*

Abstract

Six chiral polysiloxanes were prepared by block condensation of 3-(dichloromethylsilyl)-2-methylpropionic acid 2′,2′,2′-trifluoroethyl ester with disodium tetramethyldisiloxane1-,3-diolate and subsequent nucleophilic displacement with chiral amines. The polysiloxanes were coated on to capillaries and the capillary columns were used for the gas chromatographic separation of pharmaceutical enantiomers. The columns showed sufficient enantioselectivity and thermal stability to separate various pharmaceuticals into enantiomeric pairs within a reasonable time. (*S*)-Valine-(*R*)-1-(α-naphthylethyl)amide-modified polysiloxane showed better enantioselectivity, and (*S*)-valine-(*d*)-menthylamide-modified polysiloxane gave a higher coating efficiency than the other phases.

1. Introduction

Chiral stationary phases in gas chromatography (GC) afford enantiomer separations with a mechanism due to solute–solvent enantioselective hydrogen bonding interactions; they are still indispensable for the separation of amino acid and amine enantiomers. Since the first successful report of this type of phase by Gil-Av et al. [1], significant developments in increasing the thermal stability and enantioselectivity of the phases have made [2–6]. In 1977, Chirasil-Val, a chiral polysiloxane with (*S*)-valine-*tert.*-butylamide anchored to carboxypropyl-modified dimethylpolysiloxane was reported [7]. Since then, polysiloxanes have received increasing attention as suitable liquid matrices for anchoring and dispersing chiral moieties [8–11]. The Chirasil-Val capillary column can be obtained commercially. Polysiloxanes with different chiral moieties have also been reported [12–14].

In previous papers [15,16], we described a novel method for the synthesis of functionalized polysiloxanes by block condensation followed by nucleophilic displacement of active ester groups for chiral amines such as (*S*)-valine-*tert.*-butylamide or (*S*)-α-naphthylethylamine in the presence of imidazole. The method was found to yield chiral polysiloxanes with high thermal stability and enantioselectivity.

In this paper, we report on the synthesis of various types of chiral polysiloxanes by nucleophilic displacement introduction of chiral amines to functionalized polysiloxanes prepared by block condensation, and their application to the enantiomer separation of pharmaceuticals.

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)00835-3

The GC enantiomer separation of pharmaceuticals on a conventional-type Chirasil-Val capillary column has been described [17]. For pharmaceuticals, one enantiomer is usually more effective than the other. In a few instances the less effective enantiomer even has toxic properties. Therefore, we tried to develop a fast and inexpensive analytical method for the separation of pharmaceutical enantiomers by capillary GC with chiral polysiloxane stationary phases prepared by rigorously regulated processes.

2. Experimental

2.1. Materials

(*S*)-α-Naphthylethylamine, (*R*)-α-naphthylethylamine, N-*tert.*-butoxycarbonyl (BOC)-(*S*)-valine, dicyclohexylcarbodiimide (DCC) and imidazole were obtained from Wako (Osaka, Japan), *l*-menthol, *d*-menthol and pentafluoropropionic anhydride (PFPA) from Tokyo Kasei Organic Chemicals (Tokyo, Japan) and pharmaceuticals from Aldrich (Milwaukee, WI, USA). All solvents were obtained from Wako and were distilled once before use.

2.2. Synthesis

Trifluoroethyl ester-functionalized polysiloxane

The polysiloxane was prepared according to the literature [16] by block condensation of 3-(dichloromethylsilyl)-2-methylpropionic acid 2',2',2'-trifluoroethyl ester with disodium tetramethyldisiloxane-1,3-diolate by use of dichlorodimethylsilane as a diluent to adjust the ratio of functionalized ester to unsubstituted dimethylsiloxane units to at least 1:2, with an average of 1:4.

d- and l-menthylamine

As *d*- and *l*-menthylamine were not available commercially, we prepared them for the corresponding menthols. First, 1.5 *M* H_2SO_4 (600 ml) was placed in a round-bottomed flask and $K_2Cr_2O_7$ (11.8 g, 0.4 mol) was added with stirring at room temperature. Menthol was slowly added to the solution in three or four portions. The solution was heated at 60°C for 1 h and then cooled. The oxidized product of menthone was extracted with 600 ml of diethyl ether. The ether layer was washed three times with 200 ml of 5% NaOH and three times with 200 ml of water, successively, followed by drying over anhydrous Na_2SO_4. After filtration from Na_2SO_4, the ethereal solution was condensed to about 100 ml on a Rotavapor and then distilled under reduced pressure to yield 72 g (0.47 mol) of menthone (b.p. 88–92°C/17 mmHg; yield 80%).

Menthone (30 g, 0.19 mol) and hydroxylamine hydrochloride (21 g, 0.3 mol) were dissolved in 84 ml of ethanol–water (5:1, v/v) and cooled in an ice-bath with stirring. After addition of ground NaOH (38 g), the solution was refluxed for about 1 h. The solution was cooled to room temperature and poured into 1.8 *M*- HCl (800 ml). A white precipitate formed, which was filtered off. The solution was evaporated almost to dryness on a Rotavapor and the residue was recrystallized from methanol. After drying the crystal over P_2O_5 under vacuum, 24 g of menthoneoxime were obtained (yield 75%).

Next, 200 ml of freshly distilled ethanol and menthone oxime (15 g) were placed in a round-bottomed flask equipped with a reflux condenser. The ethanol solution was heated until it began to boil, the heating was stopped instantaneously, and 25 g of sodium (1.1 mol) were added immediately. Soon after completion of the reaction of the sodium, the solution was cooled to room temperature, 250 ml of water were added and the mixture was distilled. The distillates were poured into 600 ml of 6 *M* HCl. After the reaction mixture had almost distilled off, about 150 m of water were added and the distillation was continued for complete azeotropic distillation of the required compound. The distillate of HCl solution was evaporated on a Rotavapor to remove HCl, water, alcohol and unchanged menthone oxime. A white precipitate of menthylamine hydrochloride salt was obtained, which was transferred into a round-bottomed flask (100-ml volume) fitted with a reflux condenser. KOH (40%) (50 ml) was added and the mixture was refluxed for about 1 h to isolate

free *d*- or *l*-menthylamine. At room temperature, the solution was transferred into a separating funnel, 100 ml of diethyl ether were added to extract menthylamine and the lower aqueous layer was discarded. About 50 ml of saturated KOH were added to the ethereal solution and the lower aqueous layer was discarded. After repeating this procedure three times, anhydrous Na_2SO_4 was added to dry the ethereal solution. The Na_2SO_4 was removed by filtration and the solution was distilled twice (b.p. 94–94.5°C) to yield 12 g of menthylamine (yield 77%). ^1H NMR (270 MHz, $CHCl_3$); δ (ppm) = 2.45–2.53 (m, 1H), 2.04–2.12 (m, 1H), 0.86–0.92 (q, 6H), 0.74–0.77 (d, 3H).

(S)-Valine-tert.-butylamide, (S)-valine-(S)-1-(α-naphthylethyl)amide, (S)-valine-(R)-1-(α-naphthylethyl)amide, (S)-valine-(l)-menthylamide and (S)-valine-(d)-menthylamide

(*S*)-Valine-*tert.*-butylamide was prepared as described previously [16]. The other (*S*)-valineamides were prepared in a similar manner.

Chiral polysiloxanes

Chiral polysiloxanes were synthesized almost according to the previously published procedure [16]. The polysiloxanes were prepared by nucleophilic displacement of trifluoroethyl ester-functionalized polysiloxane for chiral amines in the presence of imidazole. Imidazole works as an accelerating catalyst of the reaction rate. For example, trifluoroethyl ester-functionalized polysiloxane (200 mg), (*S*)-valine-*d*-menthylamide (120 mg, 0.47 mmol) and imidazole (30 mg, 0.44 mmol) were dissolved in 50 μl of freshly distilled dioxane and placed in a Pyrex test-tube (100 × 13 mm) equipped with a Teflon-lined screw-cap and small magnetic stirrer. Dry argon was slowly bubbled through the solution for 10 min at room temperature to remove dissolved oxygen. The tube was capped tightly and heated at 120°C for 24 h with continuous stirring. The tube was cooled and 3 ml of dichloromethane were added. The solution was washed with 1 *M* HCl and three times with water and dried over anhydrous Na_2SO_4. After removal of the Na_2SO_4 by centrifugation, the solvent was evaporated on a Rotavapor and the residue was redissolved in a 5 ml of *n*-hexane. The solution was cooled in ice and centrifuged again to remove undissolved material. The supernatent was brought to dryness under high vacuum (below 2 mmHg) on a water-bath at 90°C for 1 h. A colourless, transparent, viscous, oily liquid was obtained in a yield of 245 mg (95%).

A schematic diagram of the synthesis of the chiral polysiloxane is shown in Fig. 1. A representative Fourier transform (FT) IR spectrum of the chiral polysiloxane prepared from (*S*)-valine-(*R*)-1-(α-naphthylethyl)amide is shown in Fig. 2.

2.3. Column preparation

Pyrex glass capillaries (0.25 mm I.D.) were leached with 6 *M*- HCl, dehydrated at 300°C under vacuum and silylated with diphenyltetramethyldisilazane at 420°C [18]. The capillaries were coated with 0.25% solutions of the chiral polysiloxane stationary phases in *n*-pentane–dichloromethane (2:1, v/v) by means of a static method.

2.4. Apparatus

Measurements were performed on a Shimadzu (Kyoto, Japan) Model 9AM gas chromatograph with flame ionization detection in the split mode. A Shimadzu C-R7A data processor was used for the determination of retention times, separation factors and resolution. The carrier gas was helium. The columns were first conditioned by programming from 60 to 230°C at 4°C/min and holding at 230°C for 3 h, after which they were ready for use.

2.5. Sample derivatization

The pharmaceuticals used were synephrine, penicillamine, octopamine, metanephrine, isoproterenol, norepinephrine, β-3,4-dihydroxyphenylalanine (DOPA) and propranolol. These samples were acylated with PFPA prior to GC injection, except penicillamine and DOPA, which were esterified with 2-propanol before acylation.

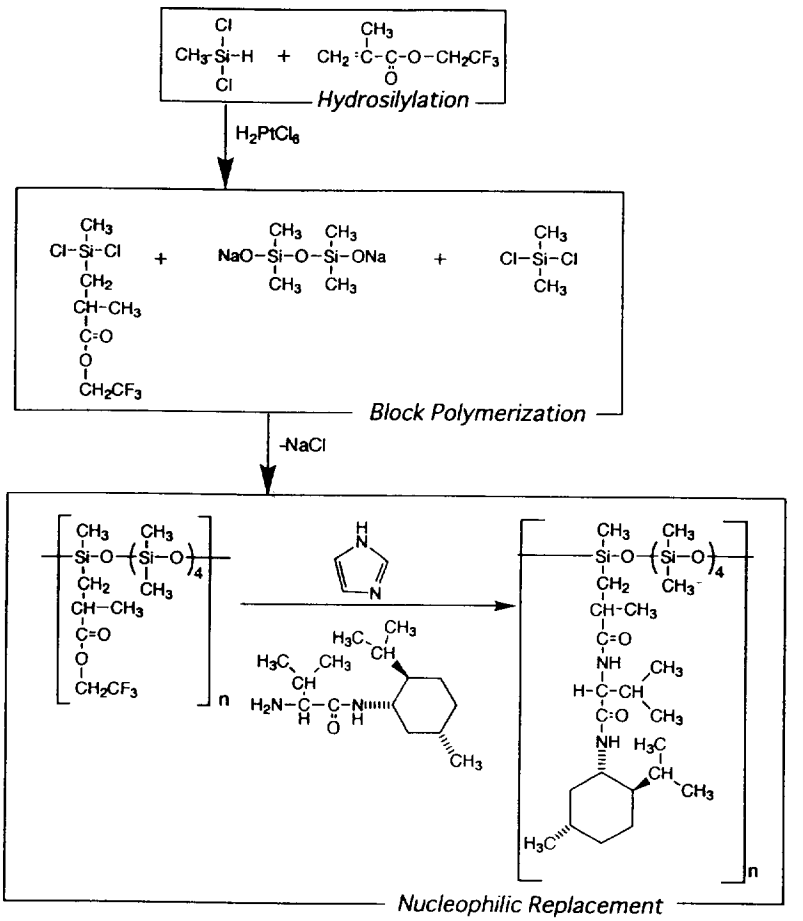

Fig. 1. Scheme of the synthesis of (*S*)-valine-*d*-menthylamide-linked polysiloxane.

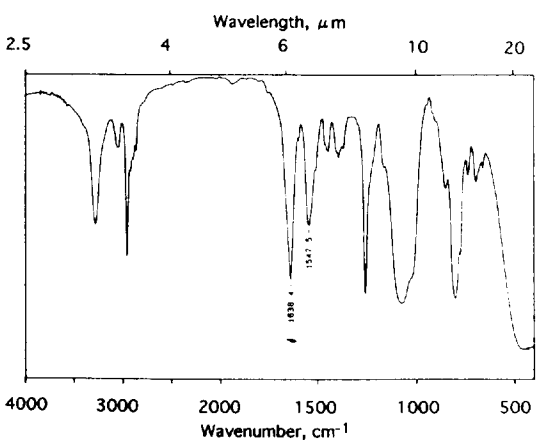

Fig. 2. FT-IR spectrum of (*S*)-valine-(*R*)-1-(α-naphthylethyl)amide-linked polysiloxane.

3. Results and discussion

Nucleophilic displacement of trifluoroethyl ester groups with chiral compounds was found to be efficient for the introduction of various types of chiral amines. The trifluoroethyl group linked to the polysiloxane chain is completely displaced with chiral amines with a molar ratio of only 10–20% excess of amines, as shown in Fig. 2; this is particularly important when precious chiral amines are employed. The chiral polysiloxanes are stable for use at least up to 210°C with continuous operation. The enantioselectivities of the six chiral stationary phases were characteristic and it was possible to separate the pharmaceutical enantiomers.

Table 1
GC separation factors and resolutions for N,O,S-pentafluoropropionyl derivatives of pharmaceutical enantiomers on chiral polysiloxanes with various selectors coated on 20 m × 0.25 mm I.D. glass capillaries

Selector	Pharmaceutical						
	Synephrine (140°C)[a]	Penicillamine[b] (160°C)[a]	Octopamine (160°C)[a]	Metanephrine (160°C)[a]	Isoproterenol (160°C)[a]	Norepinephrine (180°C)[a]	DOPA[b] (180°C)[a]
(S)-Val-amides							
-*tert.*-Butyl	1.011(0.60)	1.000	1.043(2.51)	1.025(1.34)	*1.024(1.32)*[c]	1.024(1.32)	1.079(3.78)
-(*S*)-1-(α-Naphthylethyl)	1.000	1.139(6.27)	1.026(1.28)	1.000	1.000	1.000	*1.114*(5.06)
-(*R*)-1-(α-Naphthylethyl)	*1.053(2.72)*	*1.337(9.76)*[c]	*1.200(9.15)*	1.016(0.81)	1.000	*1.097(4.58)*	1.050(2.47)
-*l*-Menthyl	1.016(1.03)	1.172(8.20)[c]	1.082(5.00)	1.000	1.000	1.049(2.89)	1.036(2.09)
-*d*-Menthyl	1.000	1.195(9.02)	1.034(2.08)	*1.026(1.52)*	1.000	1.000	1.113(*6.10*)
Monoamide							
(*S*)-1-(α-Naphthylethyl)	1.000	1.089(2.94)	1.035(1.73)	1.014(0.68)	1.000	1.000	1.031(1.41)

The highest separation factor and resolution of each compound are in italics. For each enantiomeric pair, the *d*-enantiomer eluted faster. Data in parentheses are resolutions, calculated with the equation [19] $R = 1.18(t_{R_2} - t_{R_1})/(W_{1/2,1} + W_{1/2,2})$, where R = resolution, t_{R_1} = retention time of the first-eluted enantiomer, t_{R_2} = retention time of the second-eluted enantiomer, $W_{1/2,1}$ = peak width at half-height of the first-eluted enantiomer and $W_{1/2,2}$ = peak width at half-height of the second-eluted enantiomer.
[a] Column temperature.
[b] Isopropyl ester.
[c] Reversal of elution order of enantiomers.

Chromatographic data obtained for seven pharmaceutical enantiomers are given in Table 1. The highest separation factors and resolution obtained with six chiral polysiloxane stationary phases are given in italics. DOPA enantiomers could be separated completely on all chiral polysiloxanes. Similarly, penicillamine showed complete separation on all phases except (*S*)-

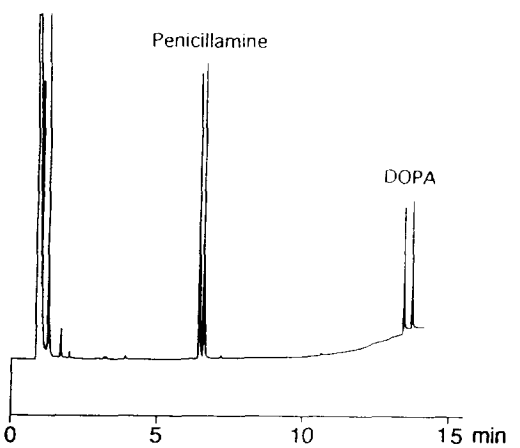

Fig. 3. GC enantiomer separation of pharmaceuticals. Column, glass capillary (20 m × 0.25 mm I.D.) coated with (*S*)-valine-*tert.*-butylamide-functionalized polysiloxane (Chirasil-Val); column temperature, 120°C for 2 min, then increased at 3°C/min to 130°C, 5°C/min to 150°C and 10°C/min to 170°C. For more details, see text.

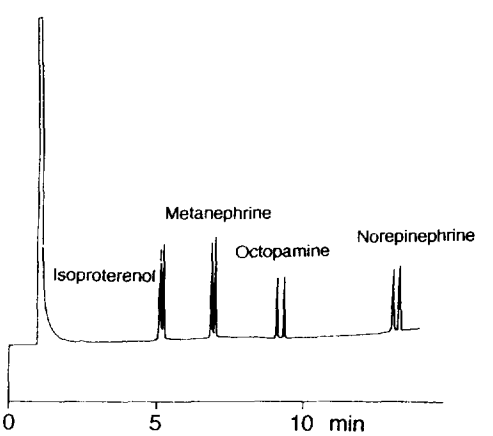

Fig. 4. GC enantiomer separation of pharmaceuticals. Column, Chirasil-Val; column temperature, 150°C for 2 min, then increased at 2°C/min to 170°C and 4°C/min to 200°C. For other conditions, see Fig. 3 and text.

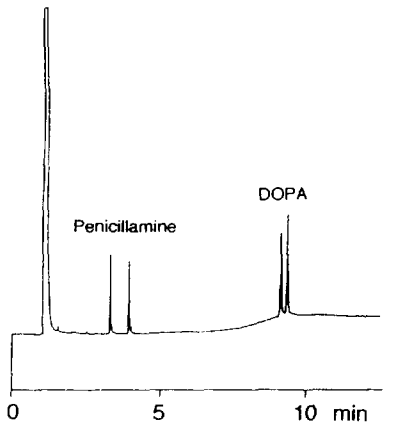

Fig. 5. GC enantiomer separation of pharmaceuticals. Column, glass capillary (20 m × 0.25 mm I.D.) coated with (*S*)-valine-(*R*)-1-(α-naphthylethyl)amide-linked polysiloxane; column temperature, 160°C for 2 min, then increased at 3°C/min to 170°C and 6°C/min to 190°C.

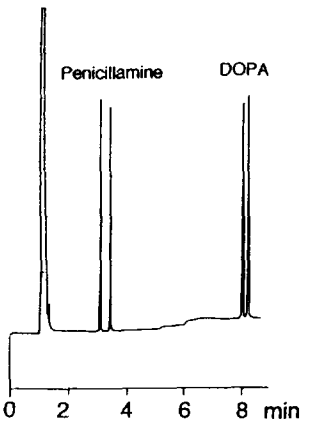

Fig. 6. GC enantiomer separation of pharmaceuticals. Column, glass capillary (20 m × 0.25 mm I.D.) coated with (*S*)-valine-*l*-menthylamide-linked polysiloxane; column temperature, 160°C for 2 min, then increased at 3°C/min to 180°C.

valine-*tert*.-butylamide-anchored polysiloxane (Chirasil-Val). However, Chirasil-Val could separate penicillamine enantiomers completely at a lower column temperature, as shown in Fig. 3. The enantioselectivity of Chirasil-Val was not very high towards these pharmaceuticals, but all of them could be separated into enantiomeric pairs (Fig. 4) Overall, (*S*)-valine-(*R*)-1-(α-naphthylethyl)amide-linked polysiloxane offered the highest separation factors. Figs. 5 and 6 show typical chromatograms.

The enantioselectivities of the polysiloxanes functionalized with (*S*)-valine-*l*-menthylamide and (*S*)-valine-*d*-menthylamide were unexpectedly low. However, the capillary columns coated with these chiral polysiloxanes showed fairly high column efficiencies, which may be due to their low polarities. The highest separation factors coincide with the highest resolutions, except for DOPA, which showed the highest resolution on (*S*)-valine-*d*-menthylamide-linked polysiloxane.

Table 2 represents the coating efficiencies of the capillary columns with different types of chiral polysiloxanes. The efficiencies obtained from the capillaries coated with (*S*)-valine-*d*-

Table 2
Efficiencies of the capillary columns coated with polysiloxanes with different selector groups

Selector	Capillary I.D. (mm)	Film thickness (μm)	HETP for methyl *n*-decanoate at 80°C (mm)	Coating efficiency (%)
(*S*)-Valine-*tert*.-butylamide	0.25	0.14	0.30	74
(*S*)-Valine-(*S*)-1- -(α-naphthylethyl)amide	0.25	0.14	0.37	60
(*S*)-α-Naphthylethylamine	0.25	0.14	0.43	52
(*S*)-Valine-*d*-menthylamide	0.25	0.14	0.25	89
(*S*)-Valine-*d*-menthylamide	0.15	0.11	0.14	94

The capillaries were all made of Pyrex glass and deactivated as described in the text. Carrier gas, helium; linear gas velocity, 32 cm/s.

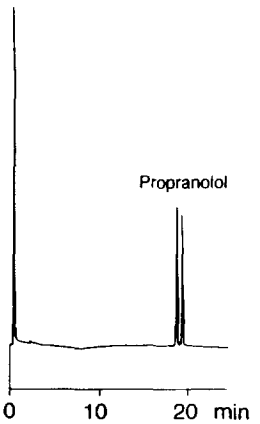

Fig. 7. GC enantiomer separation of propranolol. Column, glass capillary (10 m × 0.15 mm I.D.) coated with (*S*)-valine-*d*-menthylamide-linked polysiloxane; column temperature, 180°C isothermal.

menthylamide-functionalized polysiloxane were found to be the highest. This phase is particularly useful for the separation of the less volatile propranolol enantiomers (Fig. 7).

4. Conclusions

Chiral polysiloxane stationary phases for capillary GC prepared by block condensation and nucleophilic displacement are applicable to the preparation of chiral polysiloxanes with a wide variety of selectors. The phases were found to be efficient for the separation of pharmaceutical enantiomers of the amino acid and amino alcohol types. This method for the preparation of functional polysiloxanes is considered to be useful in other areas of research.

Acknowledgement

This work was supported partially by the Ministry of Education of Japan, Culture and Science: Project No. 05835009.

References

[1] E. Gil-Av, B. Feibush and R. Charles-Sigler, *Tetrahedron Lett.*, (1966) 1009.
[2] U. Beitler and B. Feibush, *J. Chromatogr.*, 123 (1976) 149.
[3] W.A. Konig, W. Parr, H.A. Lichtenstein, E. Bayer and J. Oro, *J. Chromatogr. Sci.*, 8 (1970) 183.
[4] B. Feibush, *Chem. Commun.*, (1971) 544.
[5] R. Charles, U. Beitler, B. Feibush and E. Gil-Av, *J. Chromatogr.*, 112 (1975) 121.
[6] R. Charles and E. Gil-Av, *J. Chromatogr.*, 195 (1980) 317.
[7] H. Frank, G.J. Nicholson and E. Bayer, *J. Chromatogr. Sci.*, 15 (1977) 174.
[8] H. Frank, G.J. Nicholson and E. Bayer, *Angew. Chem.*, 90 (1978) 396.
[9] T. Saeed, P. Sandra and M. Verzele, *J. Chromatogr.*, 186 (1979) 61.
[10] T. Saeed, P. Sandra and M. Verzele, *J. High Resolut. Chromatogr.*, 3 (1980) 35.
[11] W.A. Konig and I. Benecke, *J. Chromatogr.*, 209 (1981) 91.
[12] M. Schleimer and V. Schurig, *J. Chromatogr.*, 638 (1993) 85.
[13] M. Jung and V. Schurig, *J. Microcol. Sep.*, 5 (1993) 11.
[14] C.Y. Wu, J.S. Cheng and Z.R. Zeng, *Chromatographia*, 35 (1993) 33.
[15] H. Frank, I. Abe and G. Fabian, *J. High Resolut. Chromatogr.*, 15 (1992) 444.
[16] I. Abe, T. Nishiyama and H. Frank, *J. High Resolut. Chromatogr.*, 17 (1994) 9.
[17] H. Frank, G.J. Nicholson and E. Bayer, *J. Chromatogr.*, 146 (1978) 197.
[18] K. Grob, *Making and Manipulating Capillary Columns for Gas Chromatography*, Hüthig, Heidelberg, 1986.
[19] L.R. Snyder, *J. Chromatogr. Sci.*, 10 (1972) 200.