Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1042 (2004) 55–60
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

High-performance liquid chromatographic enantioseparations on monolithic silica columns containing a covalently attached 3,5-dimethylphenylcarbamate derivative of cellulose

Bezhan Chankvetadze [a], Tomoyuki Ikai [b], Chiyo Yamamoto [b], Yoshio Okamoto [b,*]

[a] *Molecular Recognition and Separation Science Laboratory, School of Chemistry, Tbilisi State University, Chavchavadze Avenue 1, 380028 Tbilisi, Georgia*
[b] *Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan*

Received 29 January 2004; received in revised form 19 April 2004; accepted 6 May 2004

Abstract

Covalent immobilization of 3,5-dimethylphenylcarbamate derivative of cellulose was performed in situ onto native silica monoliths cladded in a 50 mm × 4.6 mm polyether ether ketone high-performance liquid chromatographic (HPLC) column. The covalent attachment of cellulose derivative in the range of 16–19% (w/w) was performed via an epoxide moiety. The column obtained by this technique combines the high enantiomer-resolving ability of the polysaccharide derivative with favourable dynamic properties of monolithic HPLC columns. The covalent attachment of the cellulose derivative enables this column to be used in combination with the mobile phases which are incompatible with coated-type polysaccharide columns due to solubility of chiral selector in some organic solvents.

Keywords: Enantiomer separation; Monolithic columns; Cellulose dimethylphenylcarbamate; Trifluoroanthrylethanol; Cobalt tris(acetylacetonate); Dihydroxy dimethylbiphenyl

1. Introduction

Monolithic separation columns may offer significant advantages for high-performance liquid chromatography (HPLC) from the viewpoint of peak efficiency and speed of analysis [1–3]. The potential advantages of monolithic columns were recognized more than 30 years ago (see ref. [1] in ref. [3]). However, a preparation of common size and capillary columns containing silica-based [1–7] or organic [8–11] monoliths became possible only few years ago. At present, the HPLC columns with 4.6 mm internal diameter containing native- and octadecyl-modified silica monoliths [12], as well as the capillary column containing octadecyl-modified silica monoliths [13] are commercially available. There is no commercially available monolithic column either of common or capillary size for enantioseparations although first examples of chiral modification of monolithic columns containing native silica monoliths [14] or silica monoliths with octadecyl- [14,15] or amino-functionalities [16] have been published.

Polysaccharide phenylcarbamates represent one of the most successful chiral selectors for HPLC enantioseparations [17–19]. These materials can be used not only for analytical but also for preparative and product scale enantioseparations of chiral pharmaceuticals, chemicals, agrochemicals, etc. One of the most important advantages of polysaccharide based chiral stationary phases (CSP) is that these materials can effectively be used in combination with various mobile phases such as less polar organic mobile phases (for example, *n*-hexane–alcohol mixtures) [17,18] in aqueous-organic (reversed-phase) [17,18,20] and in so-called polar organic (alcohols or acetonitrile) mode [19,21–24]. The latter is especially attractive for preparative and product scale enantioseparations due to common higher solubility of samples in these solvents and easier removal of the (residual) mobile phase from the product.

* Corresponding author. Tel.: +81 52 7894600; fax: +81 52 7893188.
E-mail address: okamoto@apchem.nagoya-u.ac.jp (Y. Okamoto).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.05.011

A combination of powerful chiral recognition ability of polysaccharide derivatives with favourable dynamic properties of monolithic silica is promising for both, analytical and preparative scale enantioseparations. For example, as illustrated recently the baseline enantioseparation with analysis time less than 30 s can be achieved with cellulose tris(3,5-dimethylphenylcatbamate) coated on octadecyl-modified silica monoliths [14,15].

A drawback of coated-type polysaccharide-type CSPs for enantioseparations is solubility or swelling of many of these materials in solvents such as tetrahydrofuran, acetone, chlorinated solvents, etc. As it has been shown by several groups [25–27], these solvents may be advantageous not only from the viewpoint of higher sample solubility but also from the viewpoint of expanded or alternative chiral recognition ability of polysaccharide derivatives.

Although covalent attachment of polysaccharide derivative to silica surface involves additional steps compared to the modification by simple coating, it may result the column with higher peak efficiency. Thus, during in situ modification of monolithic silica columns with polysaccharide derivatives, inhomogeneous film may be created on the silica surface inside the column. This problem becomes especially severe when the modification is performed with concentrated solution of polysaccharides possessing high viscosity. The inhomogeneous distribution of polysaccharide derivative inside the column may unfavourably affect both, the peak symmetry and efficiency. In contrast to this, the silanising bifunctional reagents are attached to the surface of silica from rather diluted solutions that may result in homogenous distribution of activated functional groups on the surface of silica monoliths, where a polysaccharide derivative is attached on the following step. This may appear advantageous from the viewpoint of peak symmetry and efficiency.

The present work describes a covalent in situ modification of monolithic silica column (Chromolith Performance Si) with cellulose 3,5-dimethylphenylcarbamate derivative and preliminary evaluation of this column for HPLC enantioseparations.

2. Experimental

2.1. Chemicals and reagents

Microcrystalline cellulose (Avicel) was from Merck (Darmstadt, Germany). 3,5-Dimethylphenyl isocyanate, trityl chloride, boron trifluoride etherate, γ-glycidoxypropyltrimethoxysilane, pyridine, acetone, methanol, dry toluene, chloroform, 2-propanol and *n*-hexane were from Tokyo Kasei (Tokyo, Japan). Most of racemic analytes (Fig. 1) were from Aldrich (Sigma–Aldrich Japan K.K., Tokyo, Japan). 1,2,2,2-Tetraphenylethanol and 2,2′-dihydroxy-6,6′-dimethylbiphenyl were obtained from different sources and used without further purification.

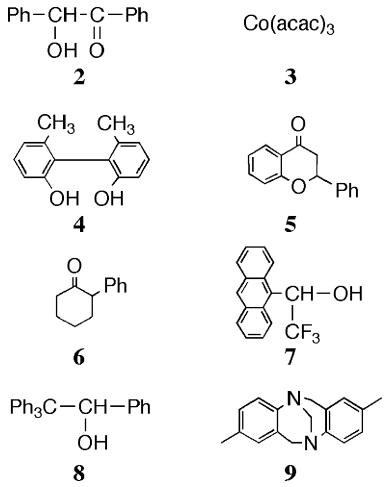

Fig. 1. Structure of chiral analytes—**2**: benzoin; **3**: cobalt(III) tris(acetylacetonate); **4**: 2,2′-dihydroxy-6,6′-dimethylbiphenyl; **5**: flavanone; **6**: 2-phenylcyclohexanone; **7**: 2,2,2-trifluoro-1-(9-anthryl)ethanol; **8**: 1,2,2,2-tetraphenylethanol; **9**: Tröger's base.

2.2. Equipments

Chromatographic separations were performed using a Jasco PU 980 Intelligent HPLC pump in combination with a Jasco DG-980 50 in-line degasser (Jasco, Tokyo, Japan), a Rheodyne 7125 injector with a 20 μl loop (Rheodyne, Cotati, CA, USA) and a Jasco UV-970 Intelligent UV–Vis detector which was connected on-line with Jasco OR-990 polarimetric detector. The samples were dissolved in the mobile phase in a concentration 0.1 mg/ml.

2.3. Preparation of regioselectively and partially derivatized cellulose 3,5-dimethylphenylcarbamate

Cellulose 3,5-dimethylphenylcarbamate derivative containing ca. 30% of primary hydroxyl groups in position 6 free (**1**) was obtained by following way [28]. The primary hydroxyl groups in microcrystalline cellulose Avicel were protected by reaction with trityl chloride in dry pyridine at 80 °C for 48 h. The product was isolated as methanol-insoluble fraction and dried in vacuum at 60 °C for 12 h.

On the next step the 6-tritylcellulose was reacted with excess of 3,5-dimethylphenylisocyanate in dry pyridine at 80 °C for 24 h. The cellulose derivative was isolated as methanol-insoluble fraction and dried in vacuum at 60 °C for 12 h and deprotected by hydrolytic removal of trityl groups with 1% HCl in methanol at room temperature. Cellulose bis(3,5-dimethylphenylcarbamate) was washed with methanol to remove HCl and then dried at 60 °C for 12 h.

On the final step cellulose bis(3,5-dimethylphenylcarbamate) was reacted with calculated amount of 3,5-dimethylphenylisocyanate in dry pyridine at 80 °C for 24 h in order to obtain the 3,5-dimethylphenylcarbamate of cellulose

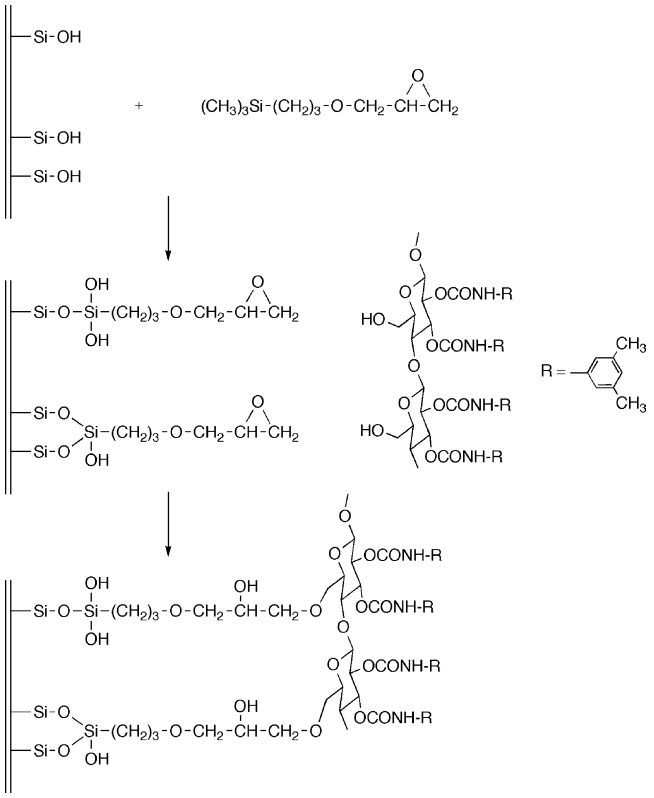

Fig. 2. Immobilization schema of cellulose derivative (**1**) on monolithic silica.

containing ca. 30% of primary hydroxyl groups in the position 6 free. The structure of final material was confirmed by the ^{1}H NMR spectrum taken in [$^{2}H_6$]dimethyl sulfoxide (DMSO-d_6) at 80 °C with Varian Gemini-2000 400 MHz NMR equipment.

2.4. Preparation of monolithic column containing covalently attached 3,5-dimethylphenylcarbamate derivative of cellulose

The activation of silica monolith with epoxide groups was performed according the schema shown in Fig. 2. This technique is similar to that described by Liao et al. [29] for the covalent attachment of methylcellulose onto the surface of fused silica capillary. In particular, monolithic column Chromolith Performance Si 100-4.6 (Batch No. UM2069, Rod. No. UM2069/058) was cut in two parts with the length of 50 mm each part. Both parts were used for enantioseparations after appropriate modification separately. The column was rinsed with ca. 20 ml acetone and dried in the vacuum oven at 60 °C overnight. After that the column was rinsed with 10% (v/v) γ-glycidoxypropyltrimethoxysilane in chloroform for 20 min, then both ends of the column were sealed and the column was left at the room temperature for 12 h. The solution was removed and the column was dried in vacuum oven at 60 °C for 3 h. The weight of the column after this treatment increased for 36.3 mg.

The above-mentioned monolithic column was filled with 100 mg/ml solution of (**1**) in acetone by pumping the solution at 40 bar pressure. The column was left without end-fittings at the room temperature for 12 h in order to evaporate acetone. On the final step the column was placed in vacuum oven at 40 °C for 12 h. After this treatment the mass of the column increased for 73.2. The column was evaluated for enantioseparation of some chiral analytes.

After evaluation the mobile phase was removed from the column. The column was filled with the solution of 10% (v/v) BF_3 etherate in dry toluene, sealed in order to avoid the evaporation of the reagent from the column and left overnight at room temperature. After this treatment the column was flashed with 50 ml acetone in order to remove unreacted cellulose derivative and the column was dried in vacuum oven at 60 °C for 12 h.

The amount of the cellulose carbamate which was washed out from the column was 23 mg. Thus, the column contained 50.2 mg immobilized cellulose derivative. Based on the amount of silica monolith in the column declared by the manufacturer (250 mg), the degree of coating might be 16.7%. This column was evaluated again in HPLC.

On the final step the column was dried in the vacuum at 60 °C for 12 h, filled with the solution of 300 μl 3,5-dimethylphenylisocyanate in 700 μl of pyridine, sealed on both ends and left for 12 h at 80 °C. Unreacted isocyanate was removed from the column by successive washing with pyridine and methanol and the column was dried at 80 °C for 12 h in vacuum oven. After this treatment the column was evaluated again in HPLC.

3. Results and discussion

3.1. Enantioseparations on epoxide-attached silica monoliths coated with 3,5-dimethylphenylcarbamate derivative of cellulose

Enantiomer resolving ability of cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) and (**1**) may differ substantially. Therefore, the enantiomer resolving ability of monolithic column was evaluated on the intermediate step just after coating with (**1**). Basically, most of the investigated analytes have been slightly better resolved with CDMPC material compared to (**1**). However, in few cases (**1**) showed superior enantiomer resolving ability. Thus, for example, the enantiomers of cobalt(III) tris(acetylacetonate) and the enantiomers of 2,2′-dihydroxy-6,6′-dimethylbiphenyl can be better resolved with **1** compared to CDMPC containing column (data not shown). The free hydroxyl groups present in (**1**) are responsible for improved enantioselectivity for few chiral analytes. This short monolithic column

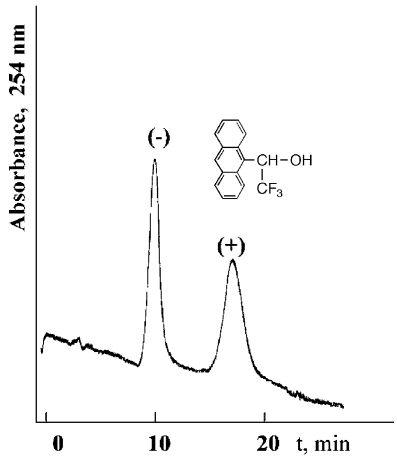

Fig. 3. Enantioseparation of 2,2,2-trifluoro-1-(9-anthryl)ethanol on monolithic silica coated with 22.6% (w/w) of (**1**). Mobile phase: *n*-hexane-2-propanol (90:10, v/v); flow rate: 0.2 ml/min.

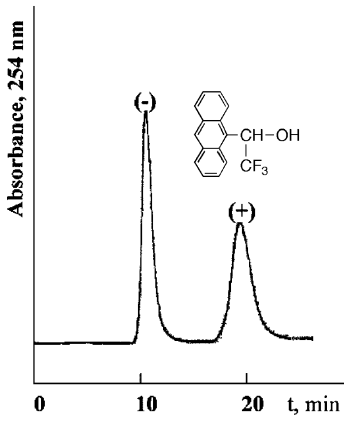

Fig. 4. Enantioseparation of 2,2,2-trifluoro-1-(9-anthryl)ethanol on monolithic silica after covalent immobilization of 16.7% (w/w) of (**1**). Chromatographic conditions were the same as in the experiment shown in Fig. 3.

exhibited superior selectivity also towards the enantiomers of 2,2,2-trifluoro-1-(9-anthryl)ethanol (Fig. 3).

In general, enantiomer resolving ability of (**1**) did not appear to be notably inferior compared to CDMPC. This result indicated that in the case of successful covalent immobilization column could be quite useful for HPLC enantioseparations.

3.2. Enantioseparations on monolithic silica column after covalent immobilization of 3,5-dimethylphenylcarbamate derivative of cellulose

The main goal on this step was at first to evaluate the efficacy of epoxide moiety for covalent immobilization of polysaccharide derivative on monolithic silica, and the second, to investigate the effect of covalent immobilization on the enantiomer resolving ability of the chiral selector. Careful washing with acetone after immobilization removed ca. 31% of the coated polysaccharide derivative from the monolithic column. After this treatment the enantiomer resolving ability of the monolithic column reduced for some analytes. Thus, for example, very low enantioselectivity of the column containing coated polymer (**1**) towards few chiral analytes (for example, *trans*-cyclopropan-dicarboxylic acid dianilide) disappeared almost completely after covalent immobilization and washing the excess cellulose derivative out of the column. However, the separation results of the enantiomers shown in Table 1 and Fig. 4 indicate that the monolithic column still retains the adequate overall enantiomer resolving ability. The plate number increased for several analytes without significant decrease of separation factor after covalent immobilization and washing non-immobilized polysaccharide derivative out of the column. In addition, as shown in Figs. 5 and 6 the enantiomer resolving ability of monolithic column containing covalently attached (**1**) for some analytes exceeds that of the column containing CDMPC [30].

Thus, it is obvious that activation with epoxide group represents useful way for efficient covalent immobilization of regioselectively carbamoylated polysaccharide derivatives onto the silica surface without notable decrease of enantiomer resolving ability.

3.3. Enantioseparations on monolithic silica column after additional treatment with 3,5-dimethylphenylisocyanate

On the final step the aforementioned monolithic column was treated with the excess of 3,5-dimethylphenylisocyanate in order to convert the hydroxyl groups of cellulose

Table 1
Enantioseparation of test racemic analytes on monolithic silica column containing 16.7% (w/w) covalently immobilized cellulose derivative (**1**)[a]

Chiral analyte	t_1 (min)	t_2 (min)	k'_1	k'_2	α	N_1	N_2	Rs
Benzoin (**2**)	7.00	8.00	3.38	4.00	1.19	1410	1540	1.1
Cobalt(III) tris(acetylacetonate) (**3**)	3.80	4.28	1.13	1.68	1.48	1400	1300	0.9
2,2′-Dihydroxy-6,6′-dimethylbiphenyl (**4**)	6.00	16.40	2.94	9.79	3.33	1250	910	6.2
Flavanone (**5**)	4.0	4.4	1.50	1.75	1.17	–	–	–
2-Phenylcyclohexanone (**6**)	3.05	3.33	0.60	0.75	1.25	1100	1080	0.8
2,2,2-Trifluoro-1-(9-anthryl)ethanol (**7**)	4.30	6.30	1.69	2.94	1.74	1520	1400	3.0
1,2,2,2-Tetraphenylethanol (**8**)	4.52	5.00	1.82	2.13	1.17	1170	1080	0.8
Tröger's base (**9**)	3.80	4.52	1.13	1.82	1.61	1025	980	1.0

[a] Column: 50 mm × 4.6 mm; mobile phase: *n*-hexane-2-propanol (95:5, v/v) with the flow rate 0.5 ml/min.

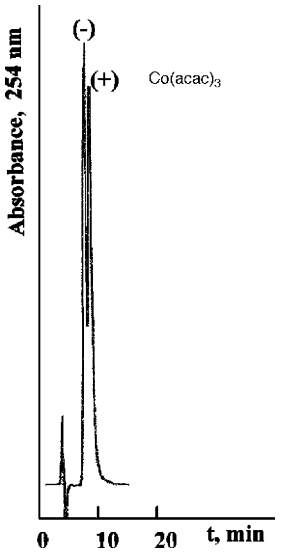

Fig. 5. Enantioseparation of cobalt(III) tris(acetylacetonate) on monolithic silica after covalent immobilization of 16.7% (w/w) of (**1**). Chromatographic conditions were the same as in the experiment shown in Fig. 3.

(apparently also free hydroxyl groups formed due to catalytic cleavage of epoxide moieties), remaining free after covalent immobilization of (**1**) into carbamate moieties. The idea was to maximally approach by this treatment the properties of the monolithic column to those of the column modified with CDMPC. Interestingly, no significant improvement of the enantiomer resolving ability of monolithic column was observed towards the analytes of this study after this final treatment. In opposite, the enantiomers of cobalt(III) tris(acetylacetonate) were not resolved on the monolithic column after this treatment (Fig. 7). The most likely reason for this observation is the conversion of free hydroxyl groups present in (**1**) into carbamate moieties after this final treatment. As mentioned above some amount of free hydroxyl groups favour the enantioseparation of few chiral analytes and among them of cobalt(III) tris(acetylacetonate) on cellulose 3,5-dimethylphenylcarbamate.

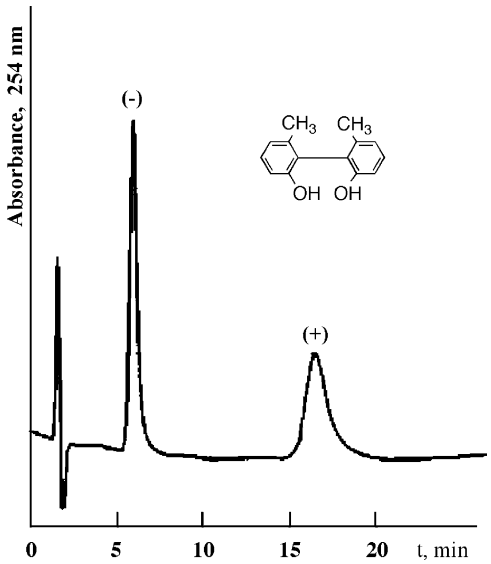

Fig. 6. Enantioseparation of 2,2′-dihydroxy-6,6′-dimethylbiphenyl on monolithic silica after covalent immobilization of 16.7% (w/w) of (**1**). Chromatographic conditions were the same as in the experiment shown in Fig. 3.

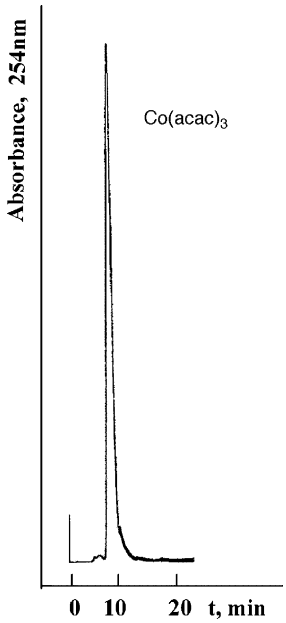

Fig. 7. Enantioseparation of cobalt(III) tris(acetylacetonate) on monolithic silica after additional treatment with the excess of 3,5-dimethylphenylisocyanate. Chromatographic conditions were the same as in the experiment shown in Fig. 3.

In conclusion, this study shows for the first time that polysaccharide phenylcarbamate derivatives containing a suitable amount of free hydroxyl groups can successfully be attached in situ to the surface of the silica monoliths cladded into the polyether ether ketone (PEEK) HPLC columns. These columns combine high enantiomer resolving ability of polysaccharide based CSPs with favourable dynamic properties of monolithic silica columns and appear to be very promising for analytical and preparative scale chromatographic enantioseparations. The backpressure on the chiral monolithic column did not exceed 1 bar with the linear flow rates of the mobile phase in the range 0.5–1.0 ml/min.

Acknowledgements

This work was partially supported by Grants-in-Aid for Scientific Research on Priority Areas No. 13355033 from the Ministry of Education, Science, Sports and Culture, and the Venture Business Laboratory Project “Advanced Nanoprocess Technologies” at Nagoya University. Bezhan Chankvetadze thanks the Venture Business Laboratory at Nagoya University for financial support of his stay at the Department of Applied Chemistry, Nagoya University.

References

[1] N. Tanaka, H. Nagayama, H. Kobayashi, K. Hosoya, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Cabrera, D. Lubda, J. High Resolut. Chromatogr. 23 (2000) 111.
[2] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, Anal. Chem. 68 (1996) 3498.
[3] M. Kelle, G. Guiochon, J. Chromatogr. A 960 (2002) 19.
[4] B. Bidlingmeyer, K.K. Unger, N. Von Doehren, J. Chromatogr. A 832 (1999) 11.
[5] K. Cabrera, D. Lubda, H.-M. Eggenweiler, H. Minakuchi, K. Nakanishi, J. High Resolut. Chromatogr. 23 (2000) 93.
[6] D. Wistuba, V. Schurig, Electrophoresis 21 (2000) 4136.
[7] D. Wistuba, V. Schurig, Electrophoresis 21 (2000) 3152.
[8] S. Hjerten, J.-L. Liao, R. Zhang, J. Chromatogr. 473 (1989) 273.
[9] F. Svec, J.M. Frechet, Anal. Chem. 64 (1992) 820.
[10] M. Lammerhoefer, E.C. Peters, C. Yu, F. Svec, J.M. Frechet, W. Lindner, Anal. Chem. 72 (2000) 4614.
[11] T. Koide, K. Ueno, J. Chromatogr. A 893 (2000) 177.
[12] Product Catalogue, Merck, Darmstadt, Germany.
[13] Chromolith CapRod (Product Information), Merck, Darmstadt.
[14] B. Chankvetadze, C. Yamamoto, N. Tanaka, Y. Okamoto, Presented at ISCD, Abstract OA-12, Shizuoka, Japan, 2003, p. 85.
[15] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Chem. Lett. 32 (2003) 250.
[16] G. Massolini, E. Calleri, A. Lavecchia, F. Loiodice, D. Lubda, C. Temporini, G. Fracchiolla, P. Tortorella, E. Novellino, G. Caccialanza, Anal. Chem. 75 (2003) 535.
[17] Y. Okamoto, E. Yashima, Angew. Chem. Int. Edn. Engl. 37 (1998) 1020.
[18] E. Yashima, C. Yamamoto, Y. Okamoto, Synlett (1998) 344.
[19] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Comb. Chem. High Throughput Scr. 3 (2000) 497.
[20] K. Tachibana, A. Ohnishi, J. Chromatogr. A 906 (2001) 127.
[21] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Chem. Lett. 29 (2000) 1176.
[22] B. Chankvetadze, C. Yamamoto, Y. Okamoto, J. Chromatogr. A 922 (2001) 127.
[23] B. Chankvetadze, I. Kartozia, C. Yamamoto, Y. Okamoto, J. Pharm. Biomed. Anal. 27 (2002) 467.
[24] A.M. Krstulovic, G. Rossey, J.P. Porziemsky, D. Long, I. Chekrum, J. Chromatogr. 411 (1987) 461.
[25] F. Vögtle, A. Hünten, E. Vogel, S. Buschbeck, O. Safarowsky, J. Becker, A.H. Parham, M. Knott, W.M. Müller, U. Müller, Y. Okamoto, T. Kubota, W. Lindner, E. Francotte, S. Grimme, Angew. Chem. Int. Ed. 40 (2001) 2468.
[26] C. Yamamoto, T. Hayashi, Y. Okamoto, S. Ohkubo, T. Kato, Chem. Commun. (2001) 925.
[27] E. Francotte, Presented at Molecular Chirality, Abstract IL-7, Shizuoka, Japan, October 2003, p. 11.
[28] Y. Kaida, Y. Okamoto, Bull. Chem. Soc. Jpn. 66 (1993) 2225.
[29] J.-L. Liao, J. Abramson, S. Hjertén, J. Cap. Electrophor. 2 (1995) 191.
[30] Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986) 173.