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Effect of urea addition on chiral separation of dansylamino acids by capillary zone electrophoresis with cyclodextrins

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Abstract

The chiral separation ability of unmodified and di- and trimethylated α-, β- and γ-cyclodextrins (CDs) as chiral selectors in capillary zone electrophoresis was investigated in the presence of urea derivatives using twelve dansylamino acids as model solutes. The addition of these urea derivatives (unsubstituted, methyl-, ethyl- and 1,3-dimethylureas) produced dramatic enhancement in the enantioselectivity of unmodified β-CD but also reduced the enantioselectivities of the other CDs.

1. Introduction

Capillary zone electrophoresis (CZE) is a technique for the chiral separation of a wide range of ionic and ionizable compounds undergoing rapid development at the present time owing to its rapid run-times, extremely high separation efficiencies, low sample requirements, etc. [1,2]. In order to perform chiral separations, various chiral selectors are added to the CZE running buffers. Unmodified and chemically modified cyclodextrins (CDs) have been successfully utilized for these purposes. Among the various kinds of chemically modified CD derivatives, methylated ones have been widely used as chiral selectors for CZE [3–15].

In previous papers, we reported the chiral separation of dansylamino acids by CZE using unmodified α-, β- and γ-CDs or 2,6-di-methylated and 2,3,6-trimethylated α- and β-CDs [16] and selectively methylated β-CDs [17] as chiral selectors. The chemical modifications of α- and β-CDs produced remarkable changes in their enantioselectivities for the dansylamino acids. In these experiments, the chiral selector concentration was fixed at 10 m*M*, considering the low aqueous solubility of β-CD. The CD concentration is one of the very important factors which affect the CD enantioselectivity [18]. Urea has been utilized to increase the β-CD solution concentration further above its water solubility (ca. 14–16 m*M*) [19,20].

Recently, we found that the chiral separation of the dansylamino acid enantiomers is greatly enhanced by the addition of urea to a running-buffer solution containing unmodified β-CD as the chiral selector. In this paper, we describe the addition of unsubstituted, methylated or ethylated urea to running-buffer solutions for the chiral separation of dansylamino acids as model

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solutes by CZE with unmodified and di- and trimethylated α-, β- and γ-CD derivatives.

2. Experimental

2.1. Apparatus

An Applied Biosystems (Foster City, CA, USA) Model 270A fully automated CZE system was used, with a 72 cm long (50 cm from inlet to detector) × 50 μm I.D. fused-silica capillary. On-column UV detection at 220 nm was performed at the cathodic end of the capillary. The temperature and applied voltage were held constant at 30°C and 20 kV, respectively, unless otherwise specified. Sample solutions (0.2 m*M*) were injected by a vacuum technique (12.7 cmHg pressure difference for 1.0 s) after introducing methanol as a neutral marker to estimate the osmotic flow. Before each run, the capillary was rinsed successively with 0.1 *M* NaOH and the running buffer. Electropherograms were recorded with a Hitachi (Hitachi, Japan) D-2500 Chromato-integrator. All experiments were run in duplicate to ensure reproducibility.

2.2. Reagents

Unmodified α-, β- and γ-CDs were purchased from Ensuiko Seito (Yokohama, Japan), and their 2,6-di-O-methylated and 2,3,6-tri-O-methylated derivatives were prepared by well-known methods [21,22]. After isolation, crude methylated CD derivatives were fractionated by silica-gel column chromatography, using chloroform–methanol as eluents. The methylated CD derivatives thus obtained were characterized by ^{1}H and ^{13}C NMR spectroscopy and fast atom bombardment mass spectrometry (FAB-MS). The di- and trimethylated CDs obtained are denoted by prefixing the unmodified CDs with DM- and TM-, respectively. The composition of the methylated CD derivatives used here for CZE was estimated by FAB-MS and is given in Table 1. Dansylamino acids were obtained from Sigma (St. Louis, MO, USA) and others from Wako (Osaka, Japan).

Table 1
Composition of methylated CD derivatives

CD derivative	Composition (%)[a]			
	$-CH_3$	O	$+CH_3$	$+2CH_3$
DM-γ-CD	0	60.6	30.9	8.5
TM-α-CD	7.5	92.5	0	0
TM-β-CD	8.2	91.8	0	0

[a] O = desired methylated CD; − = under-methylated CD derivative; + = over-methylated CD derivative.

Running buffers were prepared by dissolving each CD at 10 m*M* in 0.1 *M* sodium borate–0.05 *M* sodium phosphate buffer (ionic strength 0.143 *M*). Its pH was fixed at 9.0 in order to run the solutes in their fully ionized forms. The buffer solutions were filtered through a membrane filter after ultrasonication for 10 min prior to use.

3. Results and discussion

3.1. Addition of urea to unmodified β-CD-containing buffers

The extent of separation of the two peaks of a racemate is usually represented by the well-known factor of R_s. However, this R_s does not efficiently give the extent of separation for the poorly resolved peaks, because their width cannot be precisely measured. Therefore, the resolution was expressed as $R' = 100(H - H')/H$, where H and H' are the height of the first peak and that of the valley between the two peaks, respectively. In this definition, the greater the R' value, the better the resolution, and $R' = 100$ represents a baseline separation of the two peaks.

The separation of the enantiomers in this CZE method is based on their inclusion complex formation with CDs. Therefore, it is essential to examine the effect of CD concentration in the running buffer. Since the diameter of β-CD is similar to that of naphthalene, β-CD tightly encapsulates dansylamino acids [23]. Consequently, the effect of the β-CD concentration on

the chiral separation of the dansylamino acids was examined. β-CD could be dissolved even at 40 m*M* in the above-mentioned buffer at pH 9.0. Table 2 gives the chiral separation of the twelve pairs of dansylamino acid enantiomers in the presence of β-CD over a range of 0–40 m*M*. On the whole, the optimum β-CD concentration seems to be in the range of 10–15 m*M*, though dansyl-D,L-α-amino-*n*-butyric acid and -phenylalanine exhibit different behaviours.

The applied voltage is also considered to affect the chiral separation. Therefore, the *R'* values for the dansylamino acids were evaluated by increasing the applied voltage from 10 kV in steps of 5 kV. When 25 kV was applied to the buffer (pH 9.0) in the presence of 10 m*M* β-CD, the electrical current exceeded 80 μA. Based on the *R'* values, this is too high, and a current below ca. 60 μA is preferred. A linear decrease in current with an increase in urea concentration was reported in micellar electrokinetic chromatography [24]. The addition of urea (7 *M*) to the buffer containing 10 m*M* β-CD decreased the current to ca. 30 μA at 20 kV (about half of that observed in the absence of urea) and to ca. 56 μA even at 30 kV, as expected. Table 3 gives the effect of the applied voltage (15–30 kV) on the chiral separation of dansylamino acids in the presence of both 10 m*M* β-CD and 7 *M* urea.

Table 3
Effect of the applied voltage on the chiral separation (*R'* values) of dansylamino acids in the presence of 10 m*M* β-CD and 7 *M* urea (pH 9.0)

Dansylamino acid	Applied voltage (kV)			
	15	20	25	30
α-Amino-*n*-butyric acid	100	98.6	98.5	93.5
Aspartic acid	100	100	100	100
Glutamic acid	100	100	100	100
Leucine	91.3	97.8	99.0	70.9
Methionine	89.3	92.6	94.8	75.6
Norleucine	87.3	93.8	95.0	83.6
Norvaline	90.7	96.8	97.5	79.1
Phenylalanine	85.3	84.8	90.6	50.0
Serine	97.0	98.1	99.2	93.9
Threonine	98.6	100	100	96.5
Tryptophan	43.7	10.5	10.7	27.3
Valine	100	100	100	98.2

Except for dansyl-D,L-α-amino-*n*-butyric acid and -tryptophan, an applied voltage of 20 or 25 kV produced slightly larger *R'* values than those at 15 or 30 kV. Compared with the *R'* values for the buffer containing 10 m*M* β-CD in the absence of urea (Table 2), the addition of 7 *M* urea dramatically enhanced the enantioselectivity of β-CD for the dansylamino acids. Namely, their enantiomers could be completely or nearly

Table 2
Effect of β-CD concentration on the chiral separation (*R'* values) of dansylamino acids (pH 9.0)

Dansylamino acid	Concentration of β-CD (m*M*)						
	0	5	10	15	20	30	40
α-Amino-*n*-butyric acid	0	26.7	9.2	0	0	0	0
Aspartic acid	0	100	100	100	100	100	100
Glutamic acid	0	100	100	97.7	96.6	75.9	26.4
Leucine	0	41.8	52.7	79.3	66.2	53.0	48.7
Methionine	0	12.2	28.0	22.1	2.5	0	0
Norleucine	0	16.3	29.3	59.8	35.8	12.5	2.7
Norvaline	0	28.1	36.7	40.5	30.8	10.5	0
Phenylalanine	0	0	0	0	11.6	32.8	47.1
Serine	0	19.6	28.8	31.6	0	0	0
Threonine	0	80.2	88.1	86.6	79.4	61.6	26.0
Tryptophan	0	0	0	0	0	0	0
Valine	0	68.3	70.8	75.7	53.7	3.1	0

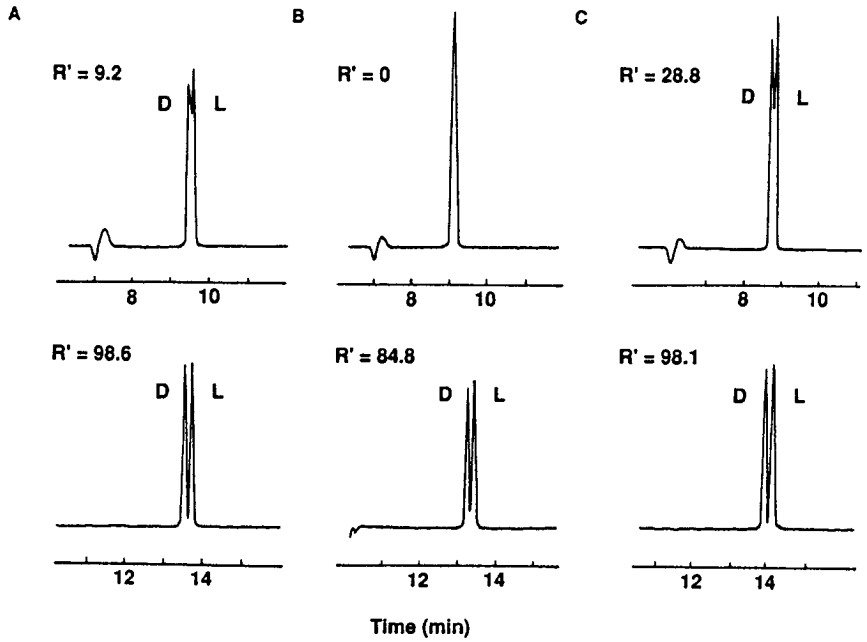

Fig. 1. Chiral separations of (A) dansyl-D,L-α-amino-*n*-butyric acid, (B) dansyl-D,L-phenylalanine and (C) dansyl-D,L-serine with 10 m*M* β-CD before (upper traces) and after (lower traces) the addition of 7 *M* urea.

baseline separated except for dansyl-D,L-tryptophan.

Fig. 1 shows typical electropherograms displaying the dramatic enhancement in the enantioselectivity of β-CD for dansyl-D,L-α-amino-*n*-butyric acid, -phenylalanine and -serine after the addition of 7 *M* urea. In the presence of unmodified β-CD, the D-enantiomer migrated faster than the corresponding L-enantiomer, regardless of the presence of urea. Under the CZE conditions described in the Experimental section, β-CD having no charge is transported toward the negative electrode by electroosmotic flow (V_{eo}). In the absence of β-CD, each negatively-charged dansylamino acid migrates toward the negative electrode with the difference between V_{eo} and its electrophoretic velocity (V_{ep}) being $V_{eo} > V_{ep}$. When included into a β-CD cavity, the solute is transported toward the negative electrode faster, because of the decrease in V_{ep}. This, therefore, indicates that a faster migrating enantiomer interacts more strongly with the β-CD cavity than a slower migrating one.

3.2. Effect of urea on the enantioselectivities of CDs other than β-CD

It is of great interest to investigate the effect of urea addition on the enantioselectivities of the CD derivatives other than the above-mentioned unmodified β-CD. The enhanced effect in the chiral separation with the already mentioned urea increased with an increase in the urea concentration (up to 7 *M* examined). Therefore, it was fixed at 7 *M*, while the CD concentration was fixed at 10 m*M* in further experiments. Table 4 gives the results for unmodified γ-CD, DM-γ-CD, TM-α-CD and TM-β-CD before and after the addition of 7 *M* urea, together with the results for unmodified β-CD already given. Except for dansyl-D,L-phenylalanine, the addition of urea reduced their enantioselectivities in the case of these CDs other than unmodified β-CD, in particular the methylated CD derivatives. The chiral separation of dansyl-D,L-phenylalanine was enormously enhanced by the addition of urea to the buffer solution containing γ-CD (*R'*, from 0 to 100) or DM-γ-CD (*R'*, from 37.3 to 98.0).

Table 4
Effect of urea addition on the chiral separation (R' values) of dansylamino acids (pH 9.0)

Dansylamino acid	Urea (M)									
	β-CD		γ-CD		DM-γ-CD[a]		TM-α-CD		TM-β-CD[a]	
	0	7	0	7	0	7	0	7	0	7
α-Amino-*n*-butyric acid	9.2	98.6	100	96.5	3.7	0	64.2	0	0	0
Aspartic acid	100	100	87.8	0	0	0	0	0	0	0
Glutamic acid	100	100	100	96.1	98.9	0	0	0	0	0
Leucine	52.7	97.8	100	100	0	0	99.2[a]	34.1[a]	100	90.7
Methionine	28.0	92.6	99.2	91.6	76.1	5.7	5.8	0	0	0
Norleucine	29.3	93.8	99.0	100	93.9	68.7	88.1	0	92.7	90.9
Norvaline	36.7	96.8	100	100	7.6	0	0	0	48.0	18.7
Phenylalanine	0	84.8	0	100	37.3	98.0	99.1[a]	20.6[a]	92.1	95.7
Serine	28.8	98.1	0	0	0	0	0	0	0	0
Threonine	88.1	100	100	76.1	16.1	0	0	0	0	0
Tryptophan	0	10.5	0	0	100	100	28.4[a]	0	76.6	0
Valine	70.8	100	99.4	98.6	0	0	43.9	0	38.7	0
Migration time of methanol (min)	7.05	9.79	7.63	10.22	7.35	10.00	7.88	10.67	7.67	10.61

[a] The L-enantiomer is the fast migrating enantiomer.

The dramatic enhancement in the enantioselectivity of unmodified β-CD induced by the addition of urea seems to be a unique behaviour. The reason for this enhanced effect in the chiral separation with urea is not clear at present. The resolution for a pair of enantiomers in CD-modified CZE varies with various parameters, such as the difference in apparent mobility between two enantiomers, their mobilities in the free and complexed forms and the electroosmotic flow. The electroosmotic flow is decreased by the addition of urea, as can be estimated from the migration times of methanol in Table 4. This decreased electroosmotic flow may result in the enhanced enantioselectivity. In the case of unmodified β-CD, the migration-time difference between the D- and L-enantiomers of each solute is larger after the addition of urea. However, this is not necessarily true for the other CDs listed in Table 4. Moreover, the larger time difference does not always bring about a higher enantioselectivity. The R' value for dansyl-D,L-phenylalanine increased from 0 to 84.8 by the addition of urea to the β-CD-containing buffer and also from 0 to 100 by the addition of urea to the γ-CD-containing buffer. It seems to be unlikely that these abrupt increases can be ascribed to only the decreased electroosmotic flow. The urea addition is also considered to affect the complex formation of the enantiomers with CDs. Consequently, in order to convincingly explain the enhanced effect in the chiral separation with urea, further quantitative work is needed.

Neither α-CD nor TM-γ-CD exhibited any enantioselectivity for the dansylamino acids, regardless of the presence of urea. This is ascribed to the weak and/or scarce interaction between the dansylamino acids and α-CD or TM-γ-CD. Both DM-α- and -β-CDs could separate only two dansylamino acid enantiomers.

3.3. Addition of substituted ureas to β-CD

The additional effect of three alkylated ureas (methyl-, ethyl- and 1,3-dimethylurea) on the enantioselectivity of β-CD was also examined. Methylurea at 1, 4 or 7 M was added to the running buffer containing 10 mM β-CD in order to investigate the effect of the methylurea concentration on the enantioselectivity. Fig. 2 shows

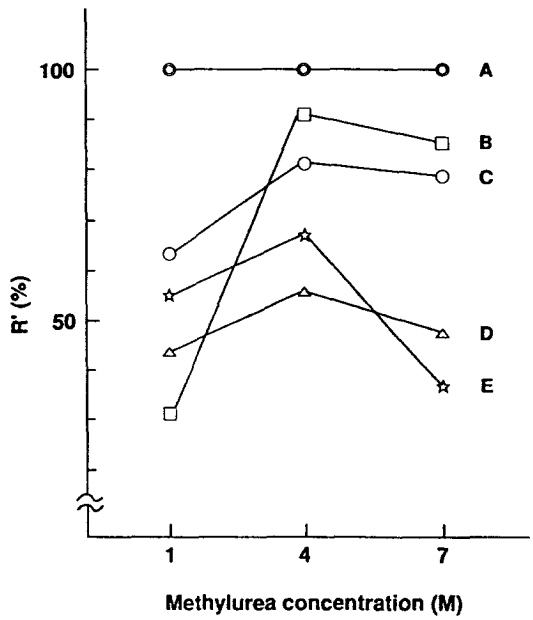

Fig. 2. Effect of methylurea concentration on enantioselectivity of β-CD (10 m*M*). Solutes: A = dansyl-D,L-aspartic acid, B = dansyl-D,L-leucine, C = dansyl-D,L-α-amino-*n*-butyric acid, D = dansyl-D,L-norleucine, E = dansyl-D,L-phenylalanine.

the results for five pairs of the dansylamino acid enantiomers. In the presence of 7 *M* methylurea, the migration time of each solute was more than 40 min and those of dansyl-D,L-aspartic and -glutamic acids exceeded 2 h. Therefore, the concentration of methylurea was fixed at 4 *M*. Table 5 gives the *R'* values for the dansylamino acids after the addition of 4 *M* methyl-, ethyl- or 1,3-dimethylurea to the 10 m*M* β-CD buffer solution. Compared with unsubstituted urea, these alkylated ones produced larger migration times for methanol and the solutes, which means smaller electroosmotic flows. Dansyl-D,L-aspartic and -glutamic acids were not eluted in the presence of 4 *M* ethyl- or 1,3-dimethylurea. Apparently, the addition of these alkylated ureas also enhanced the enantioselectivity of β-CD, as well as the addition of unsubstituted urea.

Fig. 3 shows typical electropherograms for the separations of the dansyl-D,L-leucine and -D,L-threonine enantiomers with 10 m*M* β-CD before and after the addition of 4 *M* 1,3-dimethylurea.

In conclusion, the addition of urea or the alkylated ureas produced a dramatic enhancement in the enantioselectivity of β-CD for dansylamino acid enantiomers. Further detailed work is needed to convincingly explain this increased enantioselectivity of β-CD and the decreased enantioselectivities of the other CDs. The proposed method can separate the dansylamino acid enantiomers but not the racemic dansylamino acids from each other.

Table 5
Effect of the addition of substituted ureas (4 *M*) on the chiral separation (*R'* values) of dansylamino acids in the presence of 10 m*M* β-CD (pH 9.0)

Dansylamino acid	Methylurea	Ethylurea	1,3-Dimethylurea[a]
α-Amino-*n*-butyric acid	81.6	78.0	94.7
Aspartic acid	100	–[b]	–[b]
Glutamic acid	100	–[b]	–[b]
Leucine	91.1	94.1	100
Methionine	45.6	31.0	80.4
Norleucine	56.7	37.1	91.2
Norvaline	59.7	56.1	85.0
Phenylalanine	68.5	55.7	85.3
Serine	74.0	70.2	93.1
Threonine	95.1	94.6	100
Tryptophan	0	0	0
Valine	93.3	94.7	98.3

[a] Applied voltage = 30 kV.
[b] Not eluted.

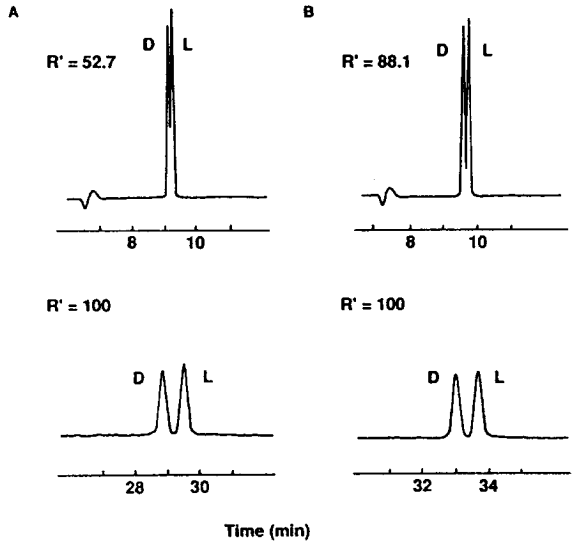

Fig. 3. Chiral separations of (A) dansyl-D,L-leucine and (B) dansyl-D,L-threonine with 10 m*M* β-CD before (upper traces) and after (lower traces) the addition of 4 *M* 1,3-dimethylurea.

More than twenty racemic dansylamino acids have been successfully separated by micellar electrokinetic chromatography using sodium dodecyl sulphate [25,26]. Thus the introduction of sodium dodecyl sulphate to the described β-CD method is of great interest in chiral separation of racemic dansylamino acid mixtures.

References

[1] S.F.Y. Li, Capillary Electrophoresis, Elsevier, Amsterdam, 1992.
[2] P.D. Grossman and J.C. Colburn, Capillary Electrophoresis, Academic, San Diego, CA, 1992.
[3] S. Fanali, J. Chromatogr., 474 (1989) 441–446.
[4] J. Snopek, H. Soini, M. Novotny, E. Smolkova-Keulemansova and I. Jelinek, J. Chromatogr., 559 (1991) 215–222.
[5] T.E. Peterson and D. Trowbridge, J. Chromatogr., 603 (1992) 298–301.
[6] S. Fanali, M. Flieger, N. Steinerova and A. Nardi, Electrophoresis, 13 (1992) 39–43.
[7] P. Gareil, J.P. Gramond and F. Guyon, J. Chromatogr., 615 (1993) 317–325.
[8] T.E. Peterson, J. Chromatogr., 630 (1993) 353–361.
[9] M.W.F. Nielen, Anal. Chem., 65 (1993) 885–893.
[10] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 635 (1993) 113–118.
[11] M. Heuermann and G. Blaschke, J. Chromatogr., 648 (1993) 267–274.
[12] C. Quang and M.G. Khaledi, J. High Resolut. Chromatogr., 17 (1994) 99–101.
[13] S.G. Penn, E.T. Bergström, D.M. Goodall and J.S. Loran, Anal. Chem., 66 (1994) 2866–2873.
[14] I.S. Lurie, R.F.X. Klein, T.A.D. Cason, M.J. LeBelle, R. Brenneisen and R.E. Weinberger, Anal. Chem., 66 (1994) 4019–4026.
[15] H. Nishi, Y. Kokusenya, T. Miyamoto and T. Sato, J. Chromatogr. A, 659 (1994) 449–457.
[16] M. Tanaka, M. Yoshinaga, S. Asano, Y. Yamashoji and Y. Kawaguchi, Fresenius J. Anal. Chem., 343 (1992) 896–900.
[17] M. Yoshinaga and M. Tanaka, J. Chromatogr. A, 679 (1994) 359–365.
[18] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235–241.
[19] D.Y. Pharr, Z.-S. Fu, T.K. Smith and W.L. Hinze, Anal. Chem., 61 (1989) 275–279.
[20] R.P. Frankewich, K.N. Thimmaiah and W.L. Hinze, Anal. Chem., 63 (1991) 2924–2933.
[21] J. Boger, R.J. Corcoran and J.-M. Lehn, Helv. Chim. Acta, 61 (1978) 2190–2218.
[22] J. Szejtli, A. Lipták, I. Jodá, P. Fügedi, P. Nanási and A. Neszmélyi, Starch/Stärke, 32 (1980) 165–169.
[23] D.W. Armstrong, T.J. Ward, R.D. Armstrong and T.E. Beesley, Science, 232 (1986) 1132–1135.
[24] S. Terabe, Y. Ishihama, H. Nishi, T. Fukuyama and K. Otsuka, J. Chromatogr., 545 (1991) 359–368.
[25] E. Skočir, J. Vindevogel and P. Sandra, Chromatographia, 39 (1994) 7–10.
[26] S. Michaelsen, P. Møller and H. Sørensen, J. Chromatogr. A, 680 (1994) 299–310.