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Three different approaches for the separation of MCPA and 2,4-D by capillary electrophoresis[1]

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Abstract

2,4-Dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) are phenoxyalkyl acid herbicides widely used in agriculture. In many cases, commercial formulations contain a mixture of both compounds so, it is important to develop analytical methods for their simultaneous determination. Phenoxyalkyl acid herbicides can be analysed by capillary electrophoresis (CE) by means of capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC). Good results have been obtained for the separation of some phenoxyalkyl acid herbicides, but the CE separation of MCPA and 2,4-D present some difficulties and only few works have addressed this problem. In this work, the CE separation of 2,4-D and MCPA has been studied via the addition of an organic modifier (acetonitrile), the use of a micellar system (sodium cholate) and the addition of different cyclodextrins. The use of acetonitrile resulted in the total separation of both herbicides in 15 min but with low efficiency and reproducibility values. On the other hand, the use of a micellar system solved the reproducibility problem but resulted in poor efficiency. Finally, when using α-, β- and γ-cyclodextrins selectivity differences were observed and the best results were obtained with the use of α-cyclodextrin. In this case, 2,4-D and MCPA have been completely resolved with a high efficiency in less than 5 min. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: 2,4-D; Pesticides; MCPA

1. Introduction

Phenoxy alkyl acid herbicides are widely used in agriculture as plant growth regulators. These compounds have been extensively analysed by chromatographic techniques. The analysis by GC requires an additional step in order to derivatize the carboxyl groups [1], whereas derivatization is not necessary for LC [2]. Capillary electrophoresis (CE) methods are also attractive for the analysis of phenoxyalkyl acid herbicides. In this sense capillary zone electrophoresis (CZE) [3], CZE with cyclodextrins [4,5], and micellar electrokinetic capillary chromatography (MECC) [6–8], have been applied to the separation of different mixtures of these herbicides, usually in samples also containing non-ionic pesticides.

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An interesting case is the analysis of 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA). These compounds are systemic hormone-type selective herbicides, readily adsorbed by leaves and roots. In many cases, commercial formulations contain a mixture of 2,4-D and MCPA [9]. So, the development of analytical methods for the simultaneous determination of that pesticide mixture is important.

CE separation of 2,4-D and MCPA presents some difficulties because they show very similar electrophoretic mobilities. Only few works have addressed this problem. Approaches to this problem include using CZE with low pH buffer systems [10], hexamethonium hydroxide as electroosmotic flow modifier [11], cyclodextrins [10,12], and sodium dodecylsulfate (SDS) with organic modifiers [13].

In this work the CE separation of MCPA and 2,4-D in a phosphate–borate buffer has been studied by using three different approaches: by the addition of an organic modifier, by using micellar systems and by the addition of different cyclodextrins.

2. Experimental

2.1. Chemicals

Phenoxyalkyl acid standards were obtained from Riedel-de Haën (Heelze-Hannover, Germany); α- and β-cyclodextrins were obtained from Sigma (St. Louis, MO, USA), and γ-cyclodextrin was purchased from Aldrich (Milwaukee, WI, USA).

Buffer salts, sodium borate and sodium phosphate, were purchased from Sigma. All other reagents were as described previously [14].

2.2. Apparatus

Columns used were untreated fused-silica capillaries (50 cm length, 40 cm to the detector, 50 μm I.D.) provided by Polymicro Technologies (Phoenix, AZ, USA).

A Hipotronics (Brewster, NY, USA) Model 340A high-voltage power supply with inlet and outlet reservoirs comprised of microcentrifuge tubes with platinum wire electrodes, was used.

Detection was carried out by on-column measurements at 200 nm on a Linear (Reno, NV, USA) Model 204 spectrophotometric detector.

2.3. Procedures and operating conditions

The capillaries were initially rinsed with 0.1 *M* NaOH. Stock solutions of 1 g l^{-1} for the phenoxyacid pesticides were prepared in MeOH. Working sample solutions were diluted in distilled water. All the runnings were accomplished at a +15 kV without temperature control. Hydrostatic injections were performed by a common siphoning procedure [15], using an inlet elevation of 10 cm and an injection time of 10 s. The injected volume was 10 nl. Number of theoretical plates (N) and resolution (R_s) were determined as $16t_r^2w^{-2}$ and $2(t_{r2}-t_{r1})$. $(w_1+w_2)^{-1}$, respectively, as in liquid chromatography, where t_r is the migration time of the analyte and w is the peak width. Migration time corresponding to the electroosmotic flow (t_o) was determined by the disturbance signal shown by the methanol present in the injected sample.

3. Results and discussion

2,4-D and MCPA differ only by one substituent in the aromatic ring, so it is not surprising that they present a separation challenge. Previous studies [7,15] developed different methods for the CE analysis of phenoxyacid, phenylureic, triazines and organophosphorous pesticides. It was observed that a 10 m*M* sodium phosphate, 6 m*M* sodium borate buffer, pH 9.3, gave good results, and so it was evaluated for its capacity to separate 2,4-D and MCPA. Because their electrophoretic mobilities are very close and much lower than the electroosmotic flow, which moves in the opposite direction, both analytes could not be separated under the used experimental conditions (Fig. 1a). In order to achieve the baseline separation of both herbicides three different ways based on three different approaches were studied: (i) the use of organic modifiers to decrease the electroosmotic flow in a CZE system; (ii) the use of a MECC system; and (iii) the use of different cyclodextrins to form inclusion complexes.

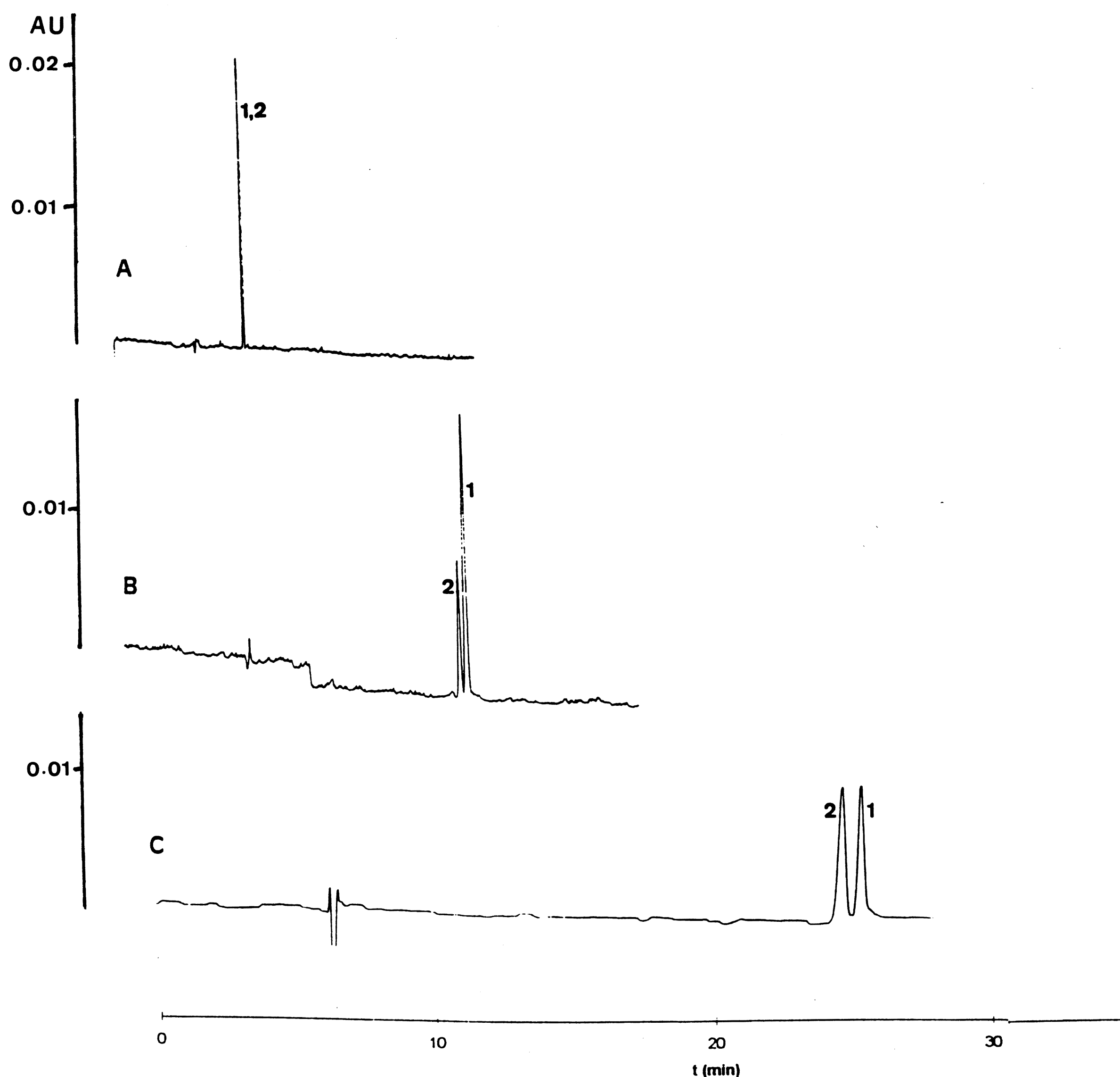

Fig. 1. Electropherograms for a mixture of 5 mg l^{-1} of 2,4-D (1) and MCPA (2) in: (A) 0.01 *M* Na_2HPO_4, 0.006 *M* $Na_2B_4O_7$ buffer (P/B buffer); (B) P/B buffer +50% ACN; (C) P/B buffer +0.05 *M* NaCh +35% ACN.

3.1. Organic modifiers

The theoretical expression for resolution in CZE is given by $R_s = N^{1/2}(\mu_2 - \mu_1)/4(\bar{\mu} + \mu_{eo})$ where μ_1, μ_2, $\bar{\mu}$, and μ_{eo} are the electrophoretic mobilities of solute 1, solute 2, average of the solutes, and electroosmotic flow, respectively. One way to improve the separation of 2,4-D and MCPA is to work with a system where $\bar{\mu}$ and μ_{eo} have more similar absolute values. This can be achieved by decreasing the electroosmotic flow by means of the addition of organic modifiers to the buffer [16]. Two organic solvents were tested: methanol and acetonitrile. It was observed that the decrease in electroosmotic flow was more acute when methanol was used. This fact can be attributed to changes in the viscosity, and

Table 1
Migration times (t), resolution (R_s) and number of theoretical plates (N) as a function of the percentage of acetonitrile (%ACN) added to a 0.01 M Na_2HPO_4, 0.006 M $Na_2B_4O_7$ (pH 9.3) buffer (P/B)

Buffer	t_o	t_{MCPA}	$t_{2,4\text{-D}}$	R_s	N_{MCPA}	$N_{2,4\text{-D}}$
P/B	3.4	5.4	5.4	–	–	–
P/B+10%ACN	3.7	6.1	6.1	–	–	–
P/B+20%ACN	4.0	7.0	7.0	–	–	–
P/B+30%ACN	4.4	8.3	8.4	0.66	49 000	50 000
P/B+40%ACN	4.9	10.7	10.9	0.87	35 000	36 000
P/B+50%ACN	5.3	13.9	14.2	1	34 500	36 000
P/B+55%ACN	5.7	19	19.5	1.05	36 000	16 500
P/B+60%ACN	6.4	31.6	33.5	1.81	20 000	12 500

to a modification of the capillary wall, thus affecting the zeta potential. The best results, a baseline separation in a lower time, were obtained by using acetonitrile.

Table 1 shows the effect of acetonitrile on the separation of MCPA and 2,4-D. As can be observed, a minimum addition of 50% acetonitrile was required in order to achieve a good resolution (Fig. 1B). Nevertheless, it has a cost on analysis and on efficiency if compared with the CZE analysis of phenoxyacids [3]. Moreover, reproducibility of the migration times at high acetonitrile rates (50%) was very low, with R.S.D. values of 10.5 and 11% for MCPA and 2,4-D, respectively. This fact is probably due to run to run changes in μ_{eo} which produces greater relative standard deviation (R.S.D.) when $\overline{\mu}$ and μ_{eo} are in the opposite direction and have similar values.

3.2. Micellar systems

In order to obtain better results, new separation conditions were tested. As is known, when working in a MECC system obtained by the addition of an anionic surfactant above its critical micelle concentration (cmc) to the running buffer, the analytes may differentially interact with the electrophoretic-retarded micelle. In those systems, resolution often improves with the addition of organic modifiers due to an increase in the elution window [14].

Two bile salts, sodium cholate (NaCh) and sodium deoxycholate (NaDCh), were tested as anionic surfactants. They were selected because of their larger tolerance to organic solvents than the widely used surfactant in MECC, SDS. In a previous work [15] it was observed that, for the same amount of organic solvent, analysis times were shorter for NaCh than for NaDCh micelles due to its higher polarity. This fact, together with the greater sensitivity of the NaDCh cmc to organic solvents, yielded better results when working with NaCh micelles.

Table 2 summarises the results obtained for the separation of MCPA and 2,4-D by the addition of acetonitrile to a phosphate–borate buffer containing NaCh.

When comparing these results with those presented in Table 1, it can be observed that the percentage of acetonitrile required to allow the

Table 2
Migration times (t), resolution (R_s) and number of theoretical plates (N) as a function of the percentage of acetonitrile (%ACN) added to a 0.05 M NaCh, 0.01 M Na_2HPO_4, 0.006 M $Na_2B_4O_7$ (pH 9.3) buffer (P/B)

Buffer	t_o	t_{MCPA}	$t_{2,4\text{-D}}$	R_s	N_{MCPA}	$N_{2,4\text{-D}}$
P/B, NaCh	3.5	6.4	6.4	–	–	–
P/B, NaCh+25%ACN	5.4	12.7	12.7	–	–	–
P/B, NaCh+30%ACN	6.1	18.6	19.0	0.55	11 000	10 000
P/B, NaCh+35%ACN	6.5	24.7	25.4	1.12	27 000	24 500
P/B, NaCh+40%ACN	6.6	30.0	31.5	1.87	25 500	22 000
P/B, NaCh+50%ACN	7.2	>50	>50	–	–	–

baseline separation of MCPA and 2,4-D decreases from 50 to 35%. Under these conditions R.S.D. values of 6.2 and 5.7% for MCPA and 2,4-D, respectively, were obtained so migration time reproducibility was improved. Nevertheless, for the same resolution value the migration times were higher for MECC than for CZE (Fig. 1C). On the other hand, the use of a micellar system also degrades on efficiency. So, reproducibility problem was solved but analysis times and efficiencies are still not good.

3.2.1. Cyclodextrins

Cyclodextrins (CDs), sometimes called Schardinger dextrins, cycloamyloses or cycloglucans, are a series of oligosaccharides composed of α-linkages of a number of D(+)-glucopyranose units. Native CDs are neutral, cylindrically shaped molecules consisting of an apolar hydrophobic cavity and a hydrophilic exterior [17,18]. Cavity diameters have been reported to be about 5.0, 6.3 and 8.0 Å for α-, β- and γ-cyclodextrins, respectively [19]. As a consequence of this structure, cyclodextrins can include apolar molecules of appropriate dimensions [17].

Since the first applications of CDs to CE by Terabe et al. [20], they have been used extensively in CZE or MECC [21–24].

In the present study, the addition of 0.005 *M* α-, β- and γ-cyclodextrins to the phosphate–borate buffer was tested and the obtained results are summarised in Fig. 2. Comparing Fig. 1A and Fig. 2 indicates that migration times decreased with the addition of cyclodextrins, because the CD–analyte

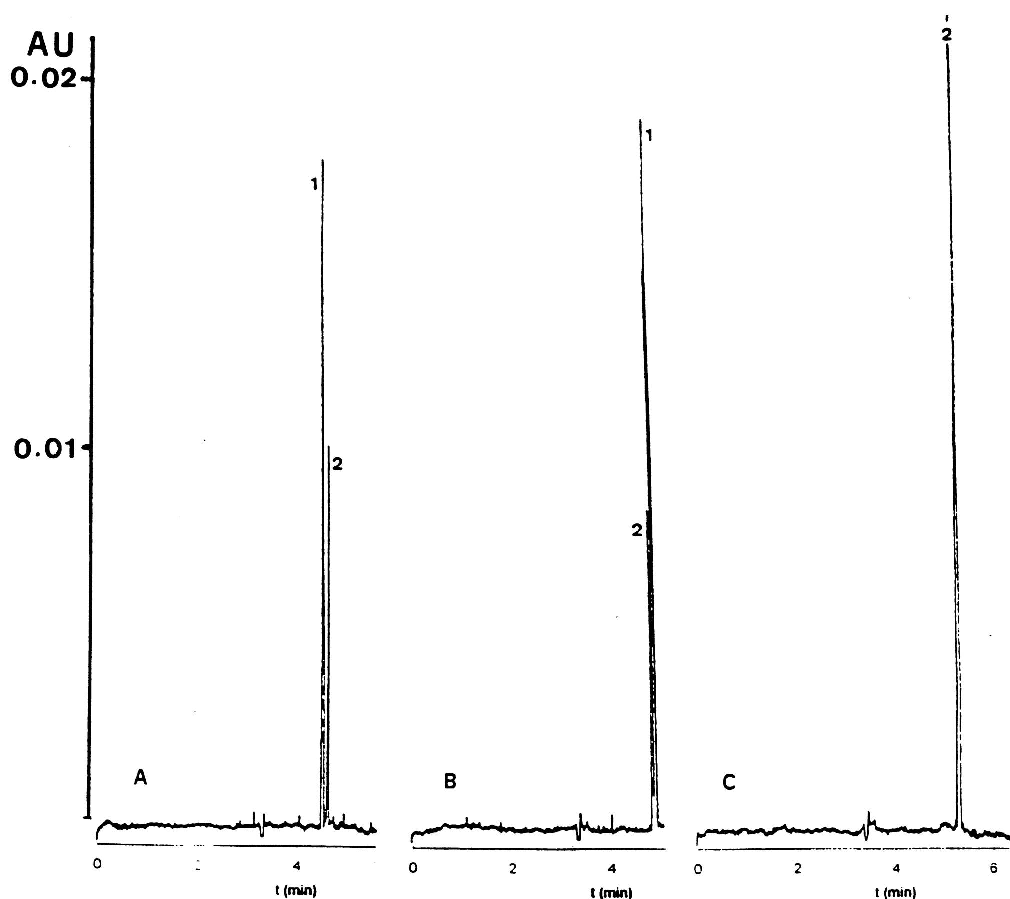

Fig. 2. Electropherograms for a mixture of 5 mg l^{-1} of 2,4-D (1) and MCPA (2) in a 0.01 *M* Na_2HPO_4, 0.006 *M* $Na_2B_4O_7$ buffer containing: (A) 0.005 *M* α-CD; (B) 0.005 *M* β-CD; (C) 0.005 *M* γ-CD.

Table 3
Migration times (t), resolution (R_s) and number of theoretical plates (N) when 0.005 M of α-, β- and γ-cyclodextrins was added to a 0.01 M Na_2HPO_4, 0.006 M $Na_2B_4O_7$ (pH 9.3) buffer (P/B)

Buffer	t_o	$t_{2,4-D}$	t_{MCPA}	R_s	$N_{2,4-D}$	N_{MCPA}
P/B+α-CD	3.3	4.55	4.65	1.6	132 500	61 500
P/B+β-CD	3.3	4.90	4.85	0.8	153 500	67 000
P/B+γ-CD	4.4	5.30	5.3	–	–	–

complex has a less negative electrophoretic mobility than the uncomplexed compound. This phenomenon indicates that both herbicides could form inclusion complexes with the three studied cyclodextrins. Therefore, selectivity differences have been observed. 2,4-D showed a stronger interaction with α-CD, eluting earlier than MCPA, while when β-CD was used, opposite results were observed. For γ-CD, 2,4-D and MCPA coeluted. Such results could be accounted for the difference of the stability constants of the inclusion complex. As has been already suggested [12], it seems that when the molecular size of the herbicide approach the interior size of the cyclodextrin, the stability of the inclusion complex increases. MCPA is a larger molecule than 2,4-D. Therefore, MCPA and 2,4-D form more stable inclusion complexes with β-CD and α-CD, respectively. In the case of γ-CD, the interior size is too high to differentiate between the herbicides.

As is shown in Table 3, the best separation was achieved with the use of α-CD. In this case, the two peaks were completely resolved in a short analysis time and with a high efficiency.

Comparing the obtained results for the three studied approaches (Tables 1–3), it can be concluded that the addition of α-CD to the running buffer is the best choice for the analysis of 2,4-D and MCPA because it allows the separation of the two phenoxy-acids with better efficiency and in shorter analysis time than with the use of acetonitrile and micellar systems. Therefore, the performance of the α-CD system in terms of linearity, reproducibility and detection limits was studied and the obtained results are presented in Table 4.

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References

[1] A.L.S. Pena, M.I.N. Silveira, J. Chromatogr. A 758 (1997) 324–331.
[2] D. Barcelo, Chromatographia 25 (1988) 928–936.
[3] M. Aguilar, A. Farran, V. Martí, Sci. Total Environ. 132 (1993) 133–140.
[4] S.K. Yeo, H.K. Lee, S.F.Y. Li, J. Chromatogr. 594 (1992) 335–340.
[5] A.W. Garrison, P. Schmitt, A. Kettrup, J. Chromatogr. A 688 (1994) 317–327.
[6] A. Farran, S. Ruiz, Anal. Chim. Acta 317 (1995) 181–188.
[7] A. Farran, S. Ruiz, C. Serra, M. Aguilar, J. Chromatogr. A 737 (1996) 109–116.
[8] Y. Mechref, Z. El Rassi, J. Chromatogr. A 757 (1997) 263–273.
[9] European Directory of Agrochemical Products, Part 2, Herbicides, Royal Society of Chemistry, 1984.
[10] M.W.F. Nielen, J. Chromatogr. 637 (1993) 81–90.
[11] G.M. McLaughlin, A. Weston, K.D. Hauffe, J. Chromatogr. A 744 (1996) 123–134.

Table 4
Linearity data, reproducibility and limits of detection for 2,4-D and MCPA

Component	Regression (r^2)	Slope	Intercept	R.S.D. (%)		LOD (mg l^{-1})
				Time	Area	
2,4-D	0.999	0.788	0.144	1.63	3.20	0.15
MCPA	0.999	0.776	−0.011	1.67	3.35	0.14

Buffer: 0.005 M α-CD+0.01 M Na_2HPO_4+0.006 M $Na_2B_4O_7$ (pH 9.3). Linearity range from 1 to 10 mg l^{-1}. Reproducibility determined as the relative standard deviations (%R.S.D.) of the mean values of six replicates. Limits of detection (LOD) as three times the standard deviation of 10 measures of a reagent blank.

[12] Y.-Z. Hsieh, H.-Y. Huang, J. Chromatogr. A 745 (1996) 217–223.
[13] M. Jung, W.C. Brumley, J. Chromatogr. A 717 (1995) 299–308.
[14] R.O. Cole, M.J. Sepaniak, W.L. Hinze, J. Gorse, K. Oldiges, J. Chromatogr. 557 (1991) 113–123.
[15] M. Aguilar, A. Farran, C. Serra, M.J. Sepaniak, K.W. Whitaker, J. Chromatogr. A 778 (1997) 201–205.
[16] M.J. Sepaniak, D.F. Swaile, A.C. Powell, J. Chromatogr. 480 (1989) 185–186.
[17] M.L. Berder, M. Komiyama, Cyclodextrin chemistry, in: Reactivity and Structure contents in Organic chemistry, vol 6, Springer–Verlag, Berlin, 1978.
[18] J. Szejtli, B. Zsadon, T. Cserhati, Cyclodextrin use in separations, in: W.L. Hinze, D.W. Armstrong (Eds.), Ordered Media in Chemical Separations, ACS Symposium Series 342, 1987.
[19] C.L. Copper, M.J. Sepaniak, Anal. Chem. 66 (1994) 147–154.
[20] S. Terabe, H. Ozaki, K. Otsuka, T. Ando, J. Chromatogr. 332 (1985) 211–217.
[21] H. Nishi, T. Fukuyama, S. Terabe, J. Chromatogr. 553 (1991) 503–516.
[22] T.L. Bereuter, LC·GC Int. 7(2) (1994) 78–93.
[23] V.C. Anigbogu, C.L. Copper, M.J. Sepaniak, J. Chromatogr. A 705 (1995) 343–349.
[24] M.J. Sepaniak, C.L. Copper, K.W. Whitaker, V.C. Anigbogu, Anal. Chem. 67 (1995) 2037–2041.