ELSEVIER

Journal of Chromatography A, 846 (1999) 359–367

JOURNAL OF CHROMATOGRAPHY A

Separation of phenoxy acid herbicides and their enantiomers in the presence of selectively methylated cyclodextrin derivatives by capillary zone electrophoresis

Masaru Miura[a], Yuichi Terashita[a], Koichi Funazo[b], Minoru Tanaka[a,*]

[a]*Research Center for Environmental Preservation, Osaka University, 2–4, Yamada-oka, Suita, Osaka 565-0871, Japan*
[b]*Department of Chemistry, Osaka Prefectural College of Technology, Neyagawa, Osaka 572-8572, Japan*

Abstract

The chiral and mutual separation of nine phenoxy acid herbicides including seven pairs of phenoxy acid enantiomers was examined by capillary zone electrophoresis using unmodified and selectively methylated α-cyclodextrin (α-CD), β-CD and γ-CD derivatives as separation additives. The selective methylation of the secondary hydroxyl groups on the rim of the CD cavities produced remarkable selectivity changes for the phenoxy acid herbicides. For the chiral separation of seven racemic phenoxy acid herbicides, unmodified and methylated α-CDs exhibited higher enantioselectivities than the β-CD additives and also much higher enantioselectivities than the γ-CD additives. Among the α-CD chiral selectors, hexakis(2,3-di-*O*-methyl)-α-CD (2,3-DM-α-CD) especially exhibited quite high enantioselectivity at 10 m*M* for all seven phenoxy acid herbicides. For the nine phenoxy acid herbicides, unmodified α-CD at 5 m*M* produced their complete mutual separation within 14 min, but could not resolve all (seven) pairs of their enantiomers. The simultaneous (both chiral and mutual) separation of 2-(3-chlorophenoxy)propionic acid, 2-(2,4,5-trichlorophenoxy)propionic acid, (2,4,5-trichlorophenoxy)acetic acid, 2-(2-chlorophenoxy)propionic acid and 2-phenoxypropionic acid could be readily attained, but 2-(2,4-dichlorophenoxy)propionic acid, 2-(4-chloro-2-methylphenoxy)propionic acid, (2,4-dichlorophenoxy)acetic acid (2,4-D) and 2-(4-chlorophenoxy)propionic acid (2,4-CPPA) could not be mutually separated by mixing 5 m*M* α-CD and 10 m*M* 2,3-DM-α-CD. However, 2,4-D and 2,4-CPPA could be completely separated with α-CD and 2,3-DM-α-CD at 2.5 m*M* each, though neither 2-(2,4-dichlorophenoxy)propionic acid nor 2-(4-chloro-2-methylphenoxy)propionic acid could be separated at all.

Keywords: Enantiomer separation; Pesticides; Phenoxy acids; Cyclodextrins

1. Introduction

Capillary electrophoresis is well established as one of the most efficient methods for separation of analytes in mixtures, and the number of publications has exponentially increased in recent years, due to its rapid run-times, extremely high separation efficiencies, low sample requirements, etc. [1,2]. Capillary zone electrophoresis (CZE) has become one of the most popular modes used in capillary electrophoresis.

Recently, cyclodextrins (CDs) have been most frequently used to separate a variety of enantiomers as chiral selectors in CZE [3]. CDs are able to encapsulate a variety of guest molecules into their hydrophobic cavities and to form inclusion complex-

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00285-X

es. Both primary and secondary hydroxyl groups located at the external rims of CDs are considered to play an important role in the formation and stabilization of their inclusion complexes. It is well-known that the chemical modifications of CDs bring about changes in the shapes and sizes of their cavities, in hydrogen-bonding abilities and in other physical properties. Lately, various modified CDs have been introduced into CZE separations to obtain different selectivities from those of the original CDs [4–23]. In previous papers [24–26], the chiral separations of dansylamino and α- and β-naphthalenesulfonylamino acids by CZE with selectively methylated β- and γ-CD derivatives were first reported. We found that the differences in the positions of selectively methylated hydroxyl groups of CDs and in the sizes of their cavities produced remarkable enantioselectivity changes for dansylamino and α- and β-naphthalenesulfonylamino acid enantiomers.

There have been an increasing number of applications of CZE to the analysis of pollutants in environmental samples. Pesticides, a case in point, are of great environmental concerns because of their high toxicities even at low concentrations and in common use. Among them, the separation of phenoxy acid herbicides and their enantiomers using unmodified CDs and 2,6-dimethyl, 2,3,6-trimethyl and sulfobutyl ether β-CDs as separation additives has been reported [27–30].

In this paper, the separation of phenoxy acid herbicides and their enantiomers by CZE with selectively 2- and 3-monomethylated and 2,3-dimethylated α-, β- and γ-CD derivatives is described.

2. Experimental

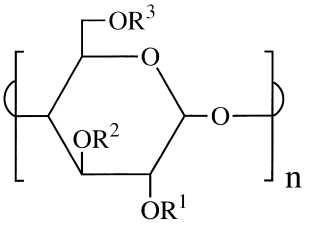
2.1. Apparatus

CZE measurements were carried out on a Model 270A automatic capillary electrophoresis system (Applied Biosystems, CA, USA) equipped with a variable-wavelength detector operated at 230 nm. An uncoated fused-silica capillary (Applied Biosystems; 72 cm total length, 50 cm effective length, 50 μm I.D.) was held at a constant temperature of 30°C. The system was operated at a constant voltage of 20 kV. Sample solutions (1.0 m*M* in water–acetonitrile, 50:50, v/v) were injected by a vacuum technique (12.7 cm Hg 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 separation buffer. Electropherograms were recorded with a D-2500 data processor (Hitachi, Tokyo, Japan). In order to minimize the impact of adsorbed CDs on the uncoated capillary, the capillary was sufficiently rinsed with 0.1 *M* NaOH and distilled water both before and after the experiments on that day. 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), hexakis(2- and 3-mono-*O*-methyl)-α-CDs, heptakis(2-mono-*O*-methyl)-β-CD and octakis(2- and 3-mono-*O*-methyl)-γ-CDs were prepared by modifying the method described in earlier papers [31–33]. Hexakis(2,3-di-*O*-methyl)-α-CD [31,32], heptakis(3-mono- and 2,3-di-*O*-methyl)-β-CDs [31,32] and octakis(2,3-di-*O*-methyl)-γ-CD [31] were also synthesized. 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 obtained mono- and di-methylated CDs are denoted by prefixing the unmodified CDs with MM- and DM-, respectively (Fig. 1).

Phenoxy acid herbicides including (2,4-dichlorophenoxy)acetic acid (2,4-D), (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), 2-(2,4-dichlorophenoxy)propionic acid (Dichlorprop), 2-(4-chloro-2-methylphenoxy)propionic acid (Mecoprop), 2-(2-chlorophenoxy)propionic acid (2,2-CPPA), 2-(3-chlorophenoxy)propionic acid (2,3-CPPA), 2-(4-chlorophenoxy)propionic acid (2,4-CPPA), 2-(2,4,5-trichlorophenoxy)propionic acid (Silvex) and 2-phenoxypropionic acid (2-PPA) were purchased from Aldrich (Milwaukee, WI, USA) and from Wako (Osaka, Japan), and others were obtained from Wako and Tokyo Kasei (Tokyo, Japan). The structures of



R^1	R^2	R^3	Abbreviation		
			n=6	n=7	n=8
H	H	H	α-CD	β-CD	γ-CD
Me	H	H	2-MM-α-CD	2-MM-β-CD	2-MM-γ-CD
H	Me	H	3-MM-α-CD	3-MM-β-CD	3-MM-γ-CD
Me	Me	H	2,3-DM-α-CD	2,3-DM-β-CD	2,3-DM-γ-CD

※ Me = CH_3

Fig. 1. Structures of methylated CD derivatives used as separation additives.

the phenoxy acid herbicides used in this study are given in Fig. 2.

Separation buffers for CZE measurements were prepared by dissolving each methylated CD in 0.1 *M* sodium borate–0.05 *M* sodium phosphate buffer (pH 9.0). They were filtered through a membrane filter (0.45 μm) after ultrasonication for 10 min prior to use.

3. Results and discussion

3.1. Characterization of methylated CD derivatives

Even after careful fractionation, each α-, β- or γ-CD derivative obtained was a mixture of hexakis(methyl)-α-, heptakis(methyl)-β- or octakis-(methyl)-γ-CD and its under- and/or over-

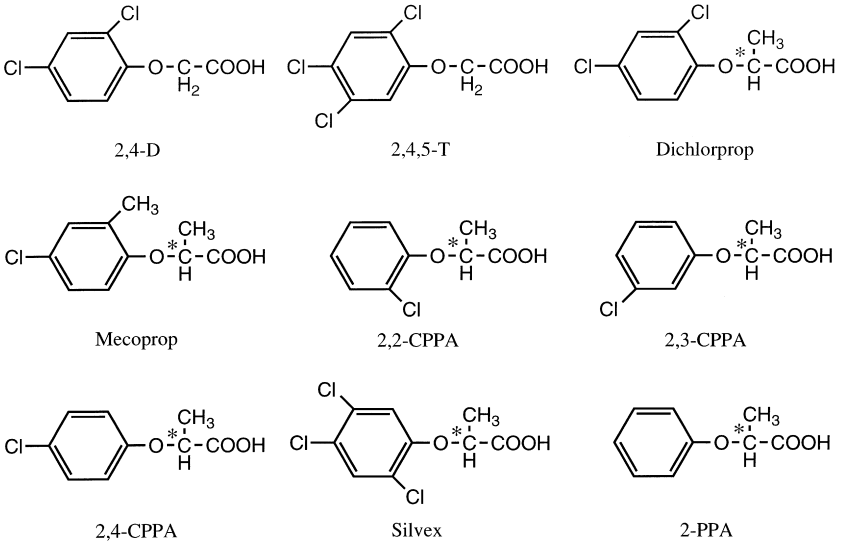


Fig. 2. Structures of the phenoxy acid herbicides.

methylated product(s). Ion peaks corresponding to the methyl–oxygen bond scission products (i.e., loss of methyl group(s)) were not observed in tandem FAB–MS spectra. Therefore, the composition of each methylated α-, β- or γ-CD derivative could be estimated from the relative intensities of the $(M+Na)^+$ ions (the samples were adulterated with NaI). Table 1 shows the composition of the methylated α-, β- and γ-CD derivatives used here for CZE. The content of the desired heptakis(2-mono-*O*-methyl)-β-CD in 2-MM-β-CD was only 70.3%. Compared with this, the other methylated CD derivatives were much more enriched in the desired methylated CD derivatives. Especially, the contents of heptakis(3-mono-*O*-methyl)-β-CD in 3-MM-β-CD and octakis(2,3-di-*O*-methyl)-γ-CD in 2,3-DM-γ-CD were 100%.

3.2. Chiral separation of phenoxy acid enantiomers in the presence of methylated CD derivatives

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.

Because the best chiral separation of seven pairs of phenoxy acid enantiomers was attained at pH 9.0 in the preliminary experiments run at pH 5.0, 7.0 and 9.0 with unmodified α-, β- and γ-CDs as chiral selectors (results not shown), the subsequent measurements were carried out at pH 9.0. Under these CZE conditions, the CD chiral selectors having no charge are transported toward the cathode by electroosmotic flow (V_{eo}). In the absence of the CDs, each negatively charged phenoxy acid migrates toward the cathode according to the difference between V_{eo} and its electrophoretic velocity (V_{ep}) due to $V_{eo}>V_{ep}$. When included into a CD cavity, the analyte is transported toward the cathode faster, because of the decrease in V_{ep}. This indicates that a faster migrating enantiomer interacts more strongly with the CD cavity.

Some of the phenoxy acid herbicides are racemic mixtures and only the D-isomers are the active ingredients. Chiral separations of these herbicides are required in order to assess the enantiopurities of formations and to optimize enantioselective production processes. Table 2 shows the R' values for the seven pairs of phenoxy acid enantiomers in the presence of unmodified and selectively methylated α-, β- and γ-CDs. The CD concentrations were 10 m*M*, except for 3-MM-α-CD (2 m*M*) whose solubility was much lower. The various CDs showed different enantioselectivities for these analytes. On the whole, it is apparent that the unmodified and methylated α-CDs exhibit higher enantioselectivities than the β-CDs and also much higher enantioselectivities than the γ-CDs. Judging from their migration times (data not shown), the α-CDs more strongly interact with the phenoxy acid herbicides than the β- or γ-CDs. These results are ascribed to the larger cavities of the β- and γ-CDs to ensure the chiral recognitions, compared with the α-CD cavities.

Among the α-CD chiral selectors, 2,3-DM-α-CD especially exhibited quite high enantioselectivity for all phenoxy acid herbicides ($R'=96.8$–100). 2- or

Table 1
Composition of methylated CD derivatives

	Composition (%)[a]								
	α-CD derivative			β-CD derivative			γ-CD derivative		
	$-1CH_3$	0	$+1CH_3$	$-1CH_3$	0	$+1CH_3$	$-1CH_3$	0	$+1CH_3$
2-MM	–	92.1	7.9	19.6	70.3	10.1	–	88.0	12.0
3-MM	2.3	97.7	–	–	100	–	11.8	88.2	–
2,3-DM	6.1	93.9	–	–	88.3	11.7	–	100	–

[a] 0=Desired methylated CD derivative; −=under-methylated CD derivative; +=over-methylated CD derivative.

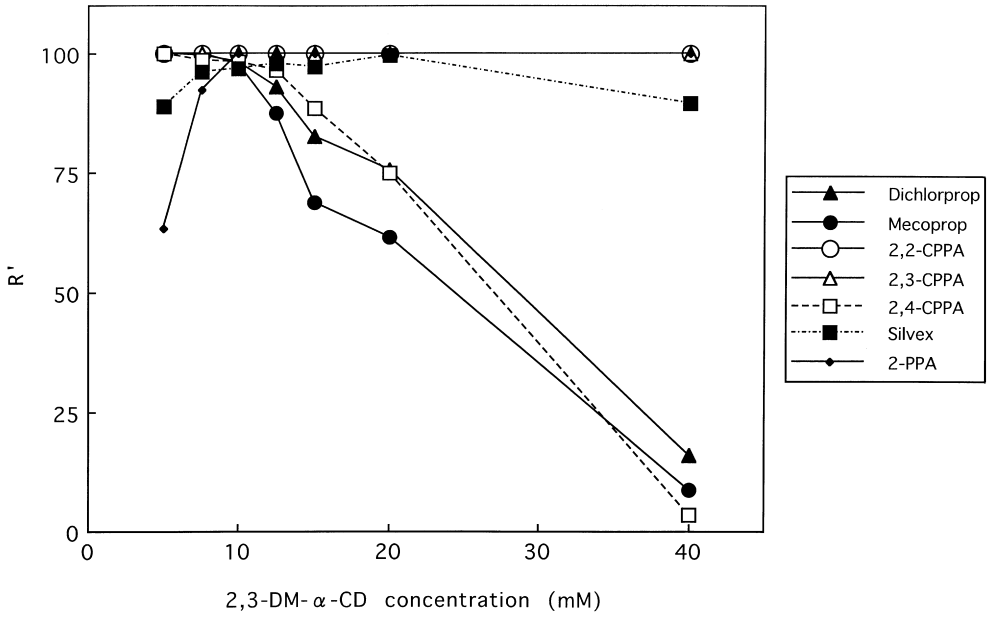


Fig. 3. Effect of 2,3-DM-α-CD concentration on the chiral separation of phenoxy acid herbicides.

3-Monomethylation of unmodified α-CD (2- or 3-MM-α-CD) was not as effective as the corresponding 2,3-dimethylation (2,3-DM-α-CD) in bringing about the chiral separations. For these analytes, it was confirmed that 2,3-DM-α-CD was the best chiral selector. Therefore, optimization of the 2,3-DM-α-CD concentration was conducted. Fig. 3 shows the effect of 2,3-DM-α-CD concentration in the range of 5–40 m*M* on the chiral separation of seven phenoxy acid enantiomers. For 2,2-CPPA and 2,3-CPPA (superimposed in the figure), the baseline separation was obtained in the concentration range

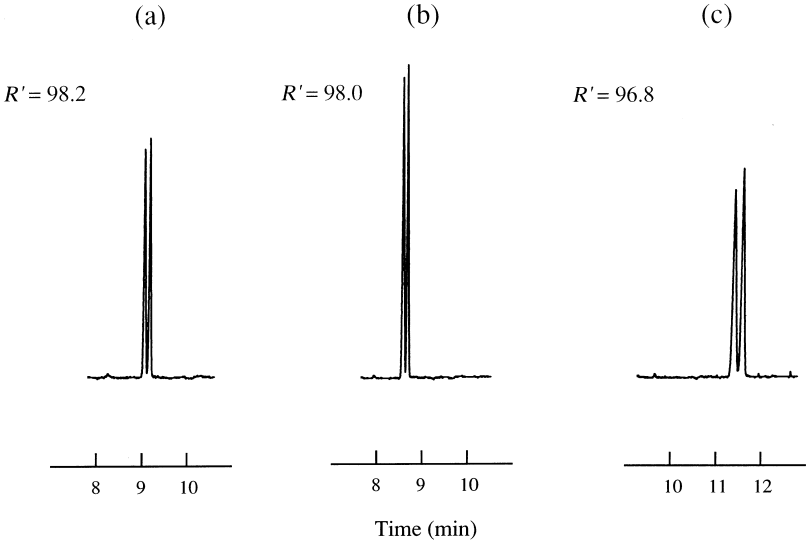


Fig. 4. Chiral separation of (a) Dichlorprop, (b) Mecoprop and (c) Silvex with 10 m*M* 2,3-DM-α-CD.

tested. The chiral separation of Dichlorprop, Mecoprop or 2,4-CPPA decreased with increasing 2,3-DM-α-CD concentration. Contrary to these analytes, 2-PPA was resolved better with an increase in the CD concentration. On the other hand, the chiral separation of Silvex increased with increasing 2,3-DM-α-CD concentration up to 20 m*M* and then decreased. Consequently, the optimum concentration of 2,3-DM-α-CD for resolving all seven phenoxy acid herbicides was fixed at 10 mM. Fig. 4 shows typical electropherograms of Dichlorprop, Mecoprop and Silvex in the presence of 10 m*M* 2,3-DM-α-CD.

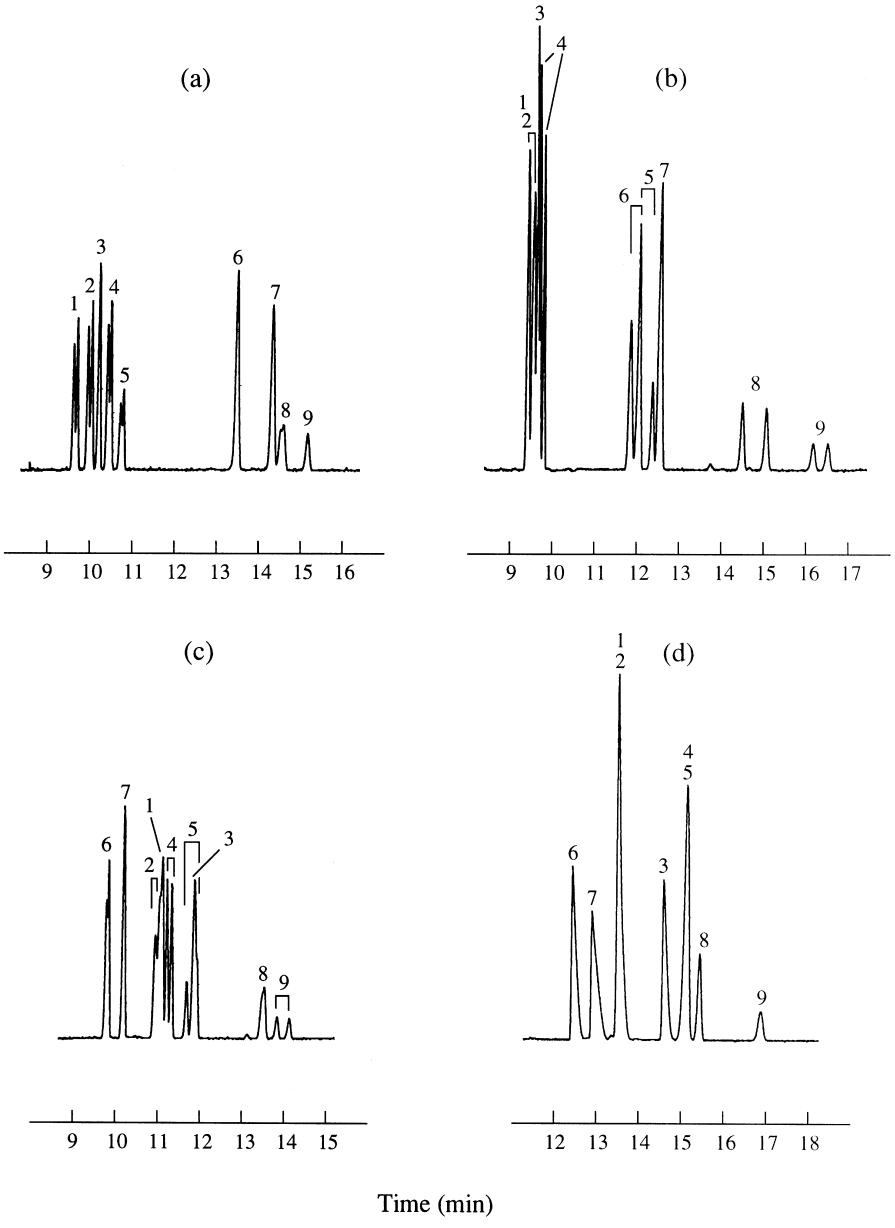


Fig. 5. Electropherograms of phenoxy acid herbicides with (a) α-CD, (b) 2,3-DM-α-CD, (c) β-CD and (d) γ-CD. Peaks: (1) Dichlorprop, (2) Mecoprop, (3) 2,4-D, (4) 2,4-CPPA, (5) 2,3-CPPA, (6) Silvex, (7) 2,4,5-T, (8) 2,2-CPPA, (9) 2-PPA.

3.3. Chiral and mutual separation of phenoxy acid herbicides with one of the CDs

The separation of nine phenoxy acid herbicides (the above-mentioned seven racemic ones, and non-racemic 2,4-D and 2,4,5-T) was investigated in the presence of unmodified and selectively methylated α-, β- and γ-CD derivatives (10 m*M* except for 2 m*M* 3-MM-α-CD at pH 9.0). Fig. 5 shows typical electropherograms of nine phenoxy acid herbicides with α-, 2,3-DM-α-, β- and γ-CDs. In the absence of CDs, the migration order of the phenoxy acid herbicides was Silvex<Mecoprop<Dichlorprop (Mecoprop and Dichlorprop were partially separated)<2,4,5-T<2,3-CPPA=2,4-CPPA (comigrated)<2,2-CPPA<2,4-D<2-PPA. The addition of each of the CDs used here produced a different migration order. Especially, the unmodified and selectively methylated α-CDs exhibited remarkably different selectivities from the β- or γ-CDs, except for 2,2-CPPA and 2-PPA. In the presence of the β- or γ-CDs, Silvex migrated fastest and secondly 2,4,5-T. These results are interpreted as follows. For these two analytes bearing three chlorine atoms on their phenyl rings, it is considered that these analytes are sterically difficult to penetrate into the α-CD cavities. On the other hand, these bulky analytes more tightly fit the β- or γ-CD cavities.

Among the CDs used in this study, unmodified α-CD afforded the best mutual separation (Fig. 5a). Though not with 10 m*M* α-CD, 2,4,5-T and 2,2-CPPA could be readily baseline-separated with 5 m*M* α-CD within 14 min (Fig. 6). In the presence of 10 m*M* 2,3-DM-α-CD that produced the best chiral separation of the optically active phenoxy acid herbicides, the mutual separation of all the herbicides could not be obtained as shown in Fig. 5b.

3.4. Chiral and mutual separation of phenoxy acid herbicides with mixed CDs

As described above, unmodified α-CD could mutually separate the nine phenoxy acid herbicides, but could not resolve all (seven) pairs of their enantiomers (Fig. 6). On the other hand, 2,3-DM-α-CD could completely or nearly baseline-separate them (Table 2). It is of great interest to investigate the simultaneous (both chiral and mutual) separation

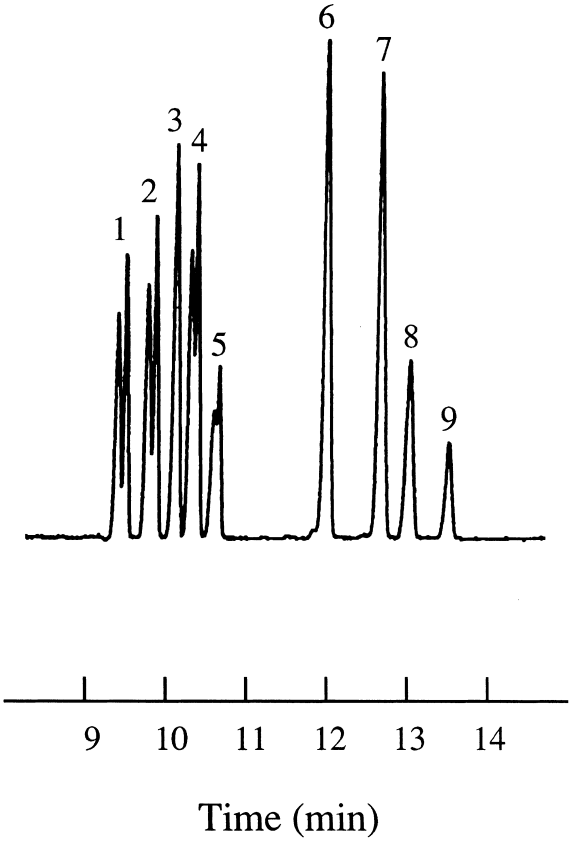


Fig. 6. Electropherogram of phenoxy acid herbicides with 5m*M* α-CD. Peaks as in Fig. 5.

of the herbicides with a mixture of unmodified α-CD and 2,3-DM-α-CD as the separation additives.

The separation was conducted with 2.5, 5.0 or 10 m*M* 2,3-DM-α-CD by changing the α-CD concentration from 2.5 to 10 mM. Fig. 7a shows the result for the simultaneous separation obtained with 5 m*M* α-CD and 10 m*M* 2,3-DM-α-CD. By mixing 5 m*M* α-CD with 10 m*M* 2,3-DM-α-CD, the separation of 2,3-CPPA, Silvex and 2,4,5-T was enhanced, and the simultaneous separation of 2,3-CPPA, Silvex, 2,4,5-T, 2,2-CPPA and 2-PPA could be readily attained. However, Dichlorprop, Mecoprop, 2,4-D and 2,4-CPPA could not be mutually separated. The separation of these four analytes was improved by decreasing the concentration of both α-CD and 2,3-DM-α-CD. Fig. 7b shows the separation with α-CD and 2,3-DM-α-CD at 2.5 m*M* each. The reduction and disappearance of the chiral separation have

Table 2
Chiral separation (R' values) of phenoxy acid herbicides in the presence of unmodified and selectively methylated CDs[a]

	R' value						
	Dichlorprop	Mecoprop	2,2-CPPA	2,3-CPPA	2,4-CPPA	Silvex	2-PPA
α-CD	45.9	57.9	0	12.9	29.9	0	0
2-MM-α-CD	50.2	0	0	6.0	14.4	0	0
3-MM-α-CD	100	100	40.8	0	100	0	0
2,3-DM-α-CD	98.2	98.0	100	100	98.2	96.8	100
β-CD	0	27.5	0	100	78.9	12.7	98.9
2-MM-β-CD	0	0	0	91.0	0	56.3	0
3-MM-β-CD	0	17.3	72.1	0	50.3	100	0
2,3-DM-β-CD	0	82.5	0	0	0	100	0
γ-CD	0	0	0	5.2	0	0	0
2-MM-γ-CD	30.0	20.8	0	0	0	4.8	0
3-MM-γ-CD	87.7	59.4	97.5	0	0	28.1	0
2,3-DM-γ-CD	0	0	0	0	0	0	0

[a] CD concentration; 10 m*M* (3-MM-α-CD; 2 m*M*).

resulted for Silvex and 2,2-CPPA and for 2-PPA, respectively. Dichlorprop and Mecoprop could not be mutually separated with the mixture, where the latter has a methyl group at the 2-position of the phenyl ring in place of a chlorine atom at the same position of the former.

4. Conclusions

The selective methylation of the secondary hydroxyl groups of the CD cavities produced remarkable selectivity changes for the phenoxy acid herbicides. 2,3-DM-α-CD especially exhibited quite

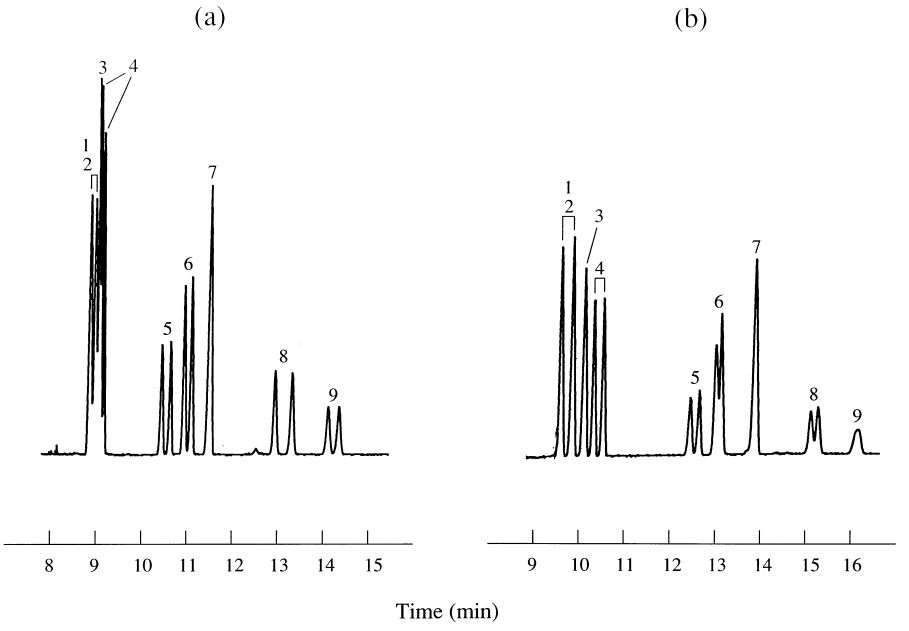


Fig. 7. Electropherograms of phenoxy acid herbicides with (a) 5 m*M* α-CD and 10 m*M* 2,3-DM-α-CD and (b) 2.5 m*M* of each α-CD and 2,3-DM-α-CD. Peaks as in Fig. 5.

high enantioselectivity at 10 m*M* for all seven phenoxy acids. For the nine phenoxy acids, α-CD at 5 m*M* produced their complete mutual separation within 14 min, but could not resolve all (seven) pairs of their enantiomers. The simultaneous (both chiral and mutual) separation of all analytes could not be achieved with the CD derivative alone, but was achieved by mixing α-CD with 2,3-DM-α-CD except for Dichlorprop and Mecoprop. The introduction of negatively charged groups into the CD derivatives used here may produce the complete simultaneous separation and is now in progress.

Acknowledgements

Partial support of this work by a Grant-in Aid for Scientific Research (A) from the Ministry of Education, Science, Sports and Culture, Japan, is gratefully acknowledged.

References

[1] K.D. Altria, Capillary Electrophoresis Guidebook, Humana, Totowa, 1996.
[2] H. Shintani, J. Polonsky, Handbook of Capillary Electrophoresis Applications, Blackie Academic and Professional, London, 1997.
[3] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[4] S. Fanali, J. Chromatogr. 474 (1989) 441.
[5] J. Snopek, H. Soini, M. Novotny, E. Smollova-Keulemansova, I. Jelinek, J. Chromatogr. 559 (1991) 215.
[6] M.J. Sepaniak, R.O. Cole, B.K. Clark, J. Liq. Chromatogr. 15 (1992) 1023.
[7] W. Schutzner, S. Fanali, Electrophoresis 13 (1992) 687.
[8] A. Nardi, A. Eliseev, P. Bocek, S. Fanali, J. Chromatogr. 638 (1993) 247.
[9] M.W.F. Nielen, Anal. Chem. 65 (1993) 885.
[10] M. Heuermann, G. Blaschke, J. Chromatogr. 648 (1993) 267.
[11] T. Schmit, H. Engelhardt, Chromatographia 37 (1993) 475.
[12] S.K. Branch, U. Holzgrabe, T.M. Jefferies, H. Mallwitz, M.W. Matchett, J. Pharm. Biomed. Anal. 12 (1994) 1507.
[13] R.J. Tait, D.O. Thompson, V.J. Stella, J.F. Stobaugh, Anal. Chem. 66 (1994) 4013.
[14] J. Szemán, K. Ganzler, J. Chromatogr. A 668 (1994) 509.
[15] K.-H. Gahm, A.M. Stalcup, Anal. Chem. 67 (1995) 19.
[16] W. Wu, A.M. Stalcup, J. Liq. Chromatogr. 18 (1995) 1289.
[17] S. Fanali, E. Camera, J. Chromatogr. A 745 (1996) 17.
[18] B. Chankvetadze, G. Endresz, G. Blaschke, Chem. Soc. Rev. 25 (1996) 141.
[19] J.B. Vincent, A.D. Sokolowski, T.V. Nguyen, G. Vigh, Anal. Chem. 69 (1997) 4226.
[20] Z. Juvancz, L. Jicsinszky, K.E. Markides, J. Microcol. Sep. 9 (1997) 581.
[21] F. Lelievre, C. Gueit, P. Gareil, Y. Bahaddi, H. Galons, Electrophoresis 18 (1997) 891.
[22] G. Galaverna, R. Corradini, A. Dossena, R. Marchelli, G. Vecchio, Electrophoresis 18 (1997) 905.
[23] K. Ichibuchi, S. Izumoto, H. Nishi, T. Sato, Electrophoresis 18 (1997) 1007.
[24] M. Yoshinaga, M. Tanaka, J. Chromatogr. A 679 (1994) 359.
[25] M. Yoshinaga, M. Tanaka, Anal. Chim. Acta 316 (1995) 121.
[26] M. Miura, K. Funazo, M. Tanaka, Anal. Chim. Acta 357 (1997) 177.
[27] M.W.F. Nielen, J. Chromatogr. 637 (1993) 81.
[28] A.W. Garrison, P. Schmitt, A. Kettrup, J. Chromatogr. A 688 (1994) 317.
[29] Y. Mechref, Z.E. Rassi, Anal. Chem. 68 (1996) 1771.
[30] C. Desiderio, C.M. Polcaro, S. Fanali, Electrophoresis 18 (1997) 227.
[31] K. Takeo, H. Mitoh, K. Uemura, Carbohydr. Res. 187 (1989) 203.
[32] J. Canceill, L. Jullien, L. Lacombe, J.-M. Lehn, Helv. Chim. Acta 75 (1992) 791.
[33] D. Ichen, B. Gehrcke, Y. Piprek, P. Mischnick, W.A. König, M.A. Dessoy, A.F. Morel, Carbohydr. Res. 280 (1996) 237.