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Separation of phenoxy acid herbicides and their enantiomers by high-performance capillary electrophoresis

A.W. Garrison*, P. Schmitt, A. Kettrup

Institut für Ökologische Chemie, GSF-Forschungszentrum für Umwelt und Gesundheit, Neuherberg, D-85758 Oberschleissheim, Germany

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Abstract

Capillary electrophoresis conditions in the free solution mode (capillary zone electrophoresis) were established for the separation and detection of 2,4-dichlorophenoxyacetic acid and three optically active phenoxy acid herbicides (dichlorprop, mecoprop and fenoprop).

A 50 m*M* acetate buffer at pH 4.5 gave the best separation, using a 50 cm (to detector) × 75 μm I.D. fused-silica column; the column temperature was 30°C, separation voltage 20 kV and optimum detector wavelength 230 nm. Separation of the four herbicides required less than 15 min under these conditions. Baseline separation of the two enantiomers of each of the three optically active herbicides, separately and in mixtures of the three, was accomplished by the addition of 25 m*M* tri-O-methyl-β-cyclodextrin to the acetate separation buffer. Di-O-methyl-β-cyclodextrin or α-cyclodextrin (CD) separated enantiomers of dichlorprop and mecoprop, but not those of fenoprop; β-CD provided very little separation and γ-CD gave no separation. Addition of methanol to the separation buffer increased separation, but doubled migration times. Over a variety of sample concentrations and injection times, reproducibilities of migration times of racemates and enantiomers ranged from 1.3 to 4.6% R.S.D.; peak area and peak height reproducibilities ranged from 1.6 to 17.9% R.S.D.

1. Introduction

The recent advent of high-performance capillary electrophoresis (HPCE or CE) adds a separation tool of unprecedented efficiency to the more conventional chromatographic instrumentation, and CE has been applied to numerous pharmaceutical and biochemical separation problems [1], but not often to pesticide analysis or to other environmental problems [2–7].

Currently available chiral solid phases for gas chromatography (GC) and high-performance liquid chromatography (HPLC) columns and chiral reagents allow the chromatographic separation of optical isomers, and a fertile field of investigation has ensued, especially for pharmaceutical products and amino acids. Techniques for chiral separations by CE, usually involving addition of chiral reagents such as cyclodextrins (CDs) to the separation buffer [8,9], are now also available, and have been widely applied in

* Corresponding author. Visiting Scientist at GSF, now returned to the Athens Environmental Research Laboratory, US Environmental Protection Agency, Athens, GA 30605-2720, USA.

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the pharmaceutical and biomedical fields during the past few years [10–13].

Biological activity in soil or water environments may result in the preferential reactivity of one enantiomer or other optical isomer of a pesticide in terms of microbial degradation, biological uptake, metabolism or toxicity [14,15]. Investigations of this preferred reactivity phenomenon could produce important results; manufacturers, for example, may be able to tailor pesticide formulations that are more selective for target organisms and vegetation, thereby reducing total chemical application significantly. Improved targeting of pests also should result in a direct reduction in adverse environmental impacts [14].

Investigations of preferential reactivity require advanced separations technology, as do the analyses required for research and monitoring during development, testing and production of optical isomers of insecticides and herbicides. Analysis usually involves GC or HPLC with chiral solid phases [16,17].

Phenoxy acid herbicides, including the well known 2,4-dichlorophenoxyacetic acid (2,4-D), are important as selective pre- and post-emergence herbicides; their toxicity and herbicidal effects have been studied in detail, and a variety of methods have been developed for their analysis, most being based on HPLC or GC [18–20]. Their environmental persistence also has been studied; for example, the degradation kinetics of 2,4-D and dichlorprop (Fig. 1) in soils were measured in the early 1980s [21]. Several of these herbicides are optically active —those with the phenoxy substituent on the 2-position of propionic acid, for example. Enantiomers of dichlorprop and mecoprop [17] have been separated by HPLC and their biological properties studied; in each case, only the (+)-isomer is herbicidally active [22].

These phenoxy acids are excellent candidates for separation by CE. Their pK_a values are such as to allow separation by the simplest form of CE, free solution CE (FSCE), otherwise known as capillary zone electrophoresis (CZE). For example, Nielen [31] recently described the CZE separation of several phenoxy acid herbicides

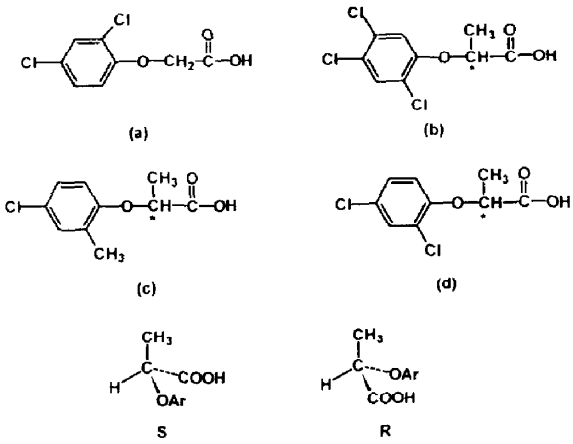

Fig. 1. Structures phenoxy acid herbicides. a = 2,4-Dichlorophenoxyacetic acid (2,4-D); pK_a = 3.3 (SPARC), 2.64 (literature). b = 2-(2,4,5-Trichlorophenoxy)-propionic acid (fenoprop); pK_a = 3.2 (SPARC), 2.84 (literature). c = 2-(4-Chloro-2-methylphenoxy)propionic acid (mecoprop); pK_a = 3.4 (SPARC); d = 2-(2,4-Dichlorophenoxy)propionic acid (dichlorprop); pK_a = 3.3 (SPARC). *S* and *R* = designation of absolute configuration of enantiomers of the optically active compounds; Ar is the substituted phenyl group. * = Optically active carbon.

and related impurities originating from production processes, as well as chiral separation of some phenoxypropionic acid herbicides using CD chiral selectors. Of special interest in our study was the optimization of separation of the enantiomers of the optically active herbicides by CZE with CD reagents. Our objective was to establish the optimum conditions necessary for CZE analysis of representative phenoxy acid herbicides and their enantiomers.

2. Experimental

Instrumentation consisted of a Beckman P/ACE 2100 series HPCE with Beckman System Gold chromatography software. The fused-silica CE column [65 cm (50 cm to the detector) × 300 μm O.D. × 75 μm I.D.] was obtained from Beckman and fitted into a 100 × 200 μm aperture cartridge. Usual CE conditions for separation of the phenoxy acid compounds were: temperature, 30°C; voltage, 20 kV; detector wavelength, 230 nm.

The three buffers were composed as follows: (a) acetate [50 m*M*, pH 4.45; 0.05 *M* glacial acetic acid–0.05 *M* sodium acetate (1:1, v:v)], (b) borate [100 m*M*, pH 7.0; 0.1 *M* boric acid–0.4 *M* sodium tetraborate (6.5:2.5, v:v)] and (c) phosphate [100 m*M*, pH 6.95; 0.1 *M* sodium dihydrogenphosphate–0.1 *M* disodium hydrogenphosphate (85:15, v:v)]. Buffer stock solutions were stored under refrigeration.

The phosphate buffers were apparently not stable for more than about 2 days, even when kept cold. Older solutions caused "spikes" on the electropherograms, perhaps because of bacterial growth. Filtration (through 0.45-μm syringe filters) followed by sonication for various times usually resulted in even more "spikes".

CD solutions were prepared in small volumes, as needed, in the acetate separation buffer. It was usually necessary to sonicate for 30 s or longer to achieve solution except for the methylated CDs, which were very soluble. β-CD was the least soluble; it was necessary to warm the solution to reach even 25 m*M* concentration. These CD solutions were not filtered. In the experiments where methanol was added in an attempt to enhance enantiomeric separation, solutions of α-CD and β-CD in buffer and methanol were filtered through 0.2-μm syringe filters.

Phenoxy acid analyte stock solutions were prepared as follows: 40 mg of each analyte were dissolved in 100 ml of pesticide-grade methanol; this was diluted 1:100 to give a final solution concentration of 4 μg/ml. This solution was used directly for CE analysis.

Typically, analyses were performed automatically by the Beckman P/ACE system and the System Gold software, using the software's sample table. One or two samples could be run to optimize conditions, or up to ten samples could be automatically analyzed. In any case, the run sequence always included the following steps: (1) 2-min rinse with the separation buffer, in a separate inlet vial; (2) hydrodynamic sample injection from the sample vial, for 5 to 15 s; (3) sample separation run for 15 to 20 min with separation buffer in inlet and outlet vials; and (4) 2-min rinse with 0.1 *M* sodium hydroxide. At the beginning of the day, the column was rinsed with 0.1 *M* sodium hydroxide for 20 min, followed by distilled water for 15 min, and finally by 0.1 *M* sodium hydroxide for 2 min just before beginning a run sequence. For step 3, separation, inlet and outlet buffers were usually renewed for each sample; however, there were many exceptions. If the buffer appeared to be stable (i.e., provided fairly reproducible migration times) the same solutions (in the same inlet and outlet vials) were often used for as many as four samples. This also held true for buffers containing CDs.

Chemical sources and purity: distilled/deionized water was obtained from a "Milli-Q plus" still (Millipore, Bedford, MA, USA). Dichlorprop, mecoprop, fenoprop and 2,4-D were obtained in greater than 99% purity from Dr. Ehrenstorfer GmbH, Augsburg, Germany. *R*-(+)-Mecoprop, *R*-(+)-dichlorprop, (designated "D" instead of "*R*" by the supplier) and methanol (Pestanal grade) were obtained from Riedel-de Haen, Munich, Germany. α-, β- and γ-CDs were from Serva, Heidelberg, Germany. Heptakis(2,6-di-O-methyl)-β-cyclodextrin and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin were from Sigma, Deisenhofen, Germany. Boric acid (electrophoresis grade) and sodium tetraborate (99% pure) were also from Sigma. Sodium dihydrogenphosphate, disodium hydrogenphosphate, sodium acetate and glacial acetic acid, all analytical-reagent grade, were from E. Merck, Darmstadt, Germany.

3. Results

3.1. Separation of the racemic herbicides

Fig. 1 shows the structures, and the caption the pK_a values, of the four phenoxy acid herbicides studied. The pK_a values of 2,4-D and fenoprop were available from the literature; values for all four compounds were also calculated using SPARC [23], a computer program developed for calculation of chemical and physical properties of chemicals strictly from their structures. Fig. 1 also shows the absolute con-

figuration of the three optically active herbicides. Only the *R*-isomers of dichlorprop and mecoprop are herbicidally active [22]; these are correctly named *R*-(+)-2-(2,4-dichlorophenoxy)-propionic acid and *R*-(+)-2-(4-chloro-2-methylphenoxy)propionic acid, respectively, since they each rotate the plane of polarized light in a clockwise direction (+).

Selection of the appropriate buffer (background electrolyte) was critical in separation of the closely related racemates. Buffer components, pH and ionic strength are all important variables [24,25]. Neither phosphate nor borate buffers provided adequate separation of dichlorprop, fenoprop and mecoprop (2,4-D was not included in these particular experiments) at pH levels within their optimum pH ranges (Fig. 2). A 50 m*M* acetate buffer of about pH 4.5 gave the best separation; higher pH acetate buffers were unsuccessful. Fig. 2 also shows the effect of changing ionic strength on resolution of these three compounds; increasing the buffer concentration from 20 to 50 m*M* at pH 4.47 increased resolution.

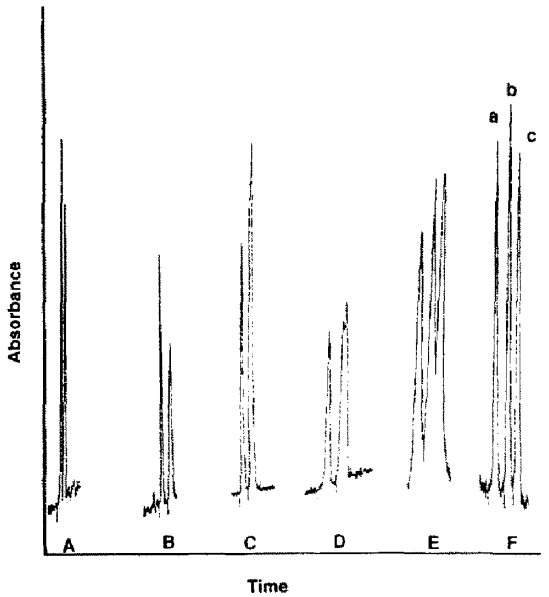

Fig. 2. Buffer trials for separation of fenoprop (a), mecoprop (b) and dichlorprop (c). (A) 100 m*M* borate, pH 8.35; (B) 100 m*M* phosphate, pH 6.40; (C) 50 m*M* acetate, pH 5.80; (D) 50 m*M* acetate, pH 5.20; (E) 20 m*M* acetate, pH 4.47; (F) 50 m*M* acetate, pH 4.47. Other separation conditions as in Fig. 3B. Concentration of each analyte in sample is 1.5 μg/ml.

Apparently the balance between the electroosmotic flow and the electrophoretic mobility of the analytes (corresponding to their pK_a values) is optimum at about pH 4.5 and 50 m*M* buffer concentration. At pH much below this value, a significant fraction of each analyte would exist in its neutral form, because their pK_a values range between 3.2 and 3.4, and the net charge on the analyte would decrease. In earlier trials with a different acetate buffer system, we found that resolution decreased when pH decreased from 4.8 to 4.0. On the other hand, the lack of complete separation with borate and phosphate buffers at relatively high pH values where the analytes are completely ionized suggests some sort of secondary buffer effect on mobilities.

Use of a 10 kV separation voltage instead of the usual 20 kV approximately doubled migration times, but did not significantly increase resolution. This was as expected, because efficiency is proportional to voltage [24]. Temperature of the column was maintained at 30°C for all analyses. This was a stable temperature for the Beckman CE system, which employs a liquid bath to control column temperature.

3.2. Detection and reproducibility of the racemates

Fig. 3 shows that 230 nm is the optimum wavelength for detection of the four herbicides with the Beckman standard UV detector. The herbicides do not absorb at 254 nm. Although the absorbance for fenoprop is about three times higher at 214 nm than at 230 nm, the latter wavelength gives only slightly poorer absorbances (about 25% less) for the other three compounds; in addition, the absorbances of all four compounds are more uniform at 230 nm. Finally, the acetate buffer gives a much smaller negative peak at 230 nm. Electropherogram peaks were identified by spiking with standards.

The detection limit for each herbicide is 0.05 μg/ml, or about $5 \cdot 10^{-7}$ *M*, at a signal-to-noise

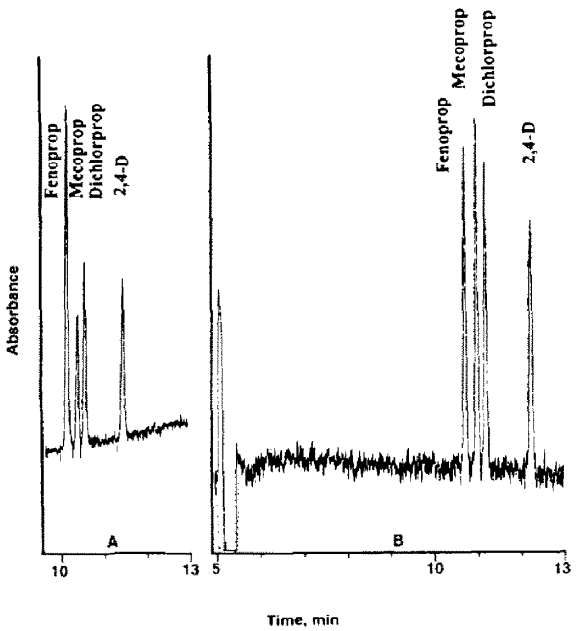

Fig. 3. Absorbances of phenoxy acid herbicides at (A) 214 and (B) 230 nm. Buffer: 50 m*M* acetate, pH 4.45. Capillary: 50 cm (to detector) × 0.075 mm I.D., fused silica. Separation voltage/current: 20 kV (400 V/cm)/41 μA. Hydrodynamic injection: 5 s. Temperature: 30°C. Concentration of each analyte in (A) and (B): 1 μg/ml. Signal at 5.07 min in (B) is the neutral peak.

ratio of 2. Table 1 gives reproducibility data for migration time, peak area and peak height for CE analysis of these herbicide racemates at 1 and 10 μg/ml and at two different injection times.

3.3. Separation of the herbicide enantiomers

Fig. 4 depicts the degree of enantiomeric separation of the three optically active herbicides (Fig. 1) achieved through addition of 10 m*M* α- or β-CD or di-O-methyl-β-cyclodextrin (DMBCD) to the acetate run buffer (50 m*M*, pH 4.45). γ-CD had no effect on the resulting electropherogram; no separation occurred. The addition of β-CD caused slight peak splitting of fenoprop and mecoprop, but no separation of dichlorprop enantiomers. DMBCD caused baseline separation of mecoprop and dichlorprop enantiomers, but had no effect on fenoprop. Finally, the addition of α-CD resulted in almost baseline separation of dichlorprop and mecoprop enantiomers, but, as with DMBCD, had no effect on fenoprop. Surprisingly, α-CD caused a major shift in migration times of the herbicides; fenoprop and dichlorprop changed relative migration times.

Higher concentrations of these CDs had little effect on separation results, although an increase of β-CD concentration from 10 to 25 m*M* decreased enantiomeric separation to almost zero. Higher concentrations sometimes caused very noisy baseline levels and spikes. The γ-CD background/baseline was very noisy, even at 10 m*M*. Table 2 summarizes the interactions among the five cyclodextrins and the three optically active herbicides. (2,4-D always existed as a single peak, of course, because it is not optically active).

The best enantiomeric separation was achieved by addition of tri-O-methyl-β-cyclodextrin (TMBCD) to the acetate run buffer. Fig. 5 shows two representative electropherograms of 2,4-D and the six enantiomers of fenoprop, mecoprop and dichlorprop. Resolution is better at 25 m*M* than at 12.5 m*M*, but the baseline is noisier, probably because of the lower concentration of analytes. Spiking the Fig. 5B sample solution with 1 μg/ml *R*-(+)-dichlorprop and *R*-(+)-mecoprop enantiomer standards showed the second peak of each pair of enantiomers to be the *R*-(+) isomer. No standards were commercially available for the enantiomers of fenoprop.

Experiments were conducted to determine the optimum level of TMBCD. Fig. 6 shows the relationship between the α value [26] for separation of each (+) and (−) pair of isomers and the concentration of TMBCD. $\alpha = t_{R2}/t_{R1}$, where t_{R2} is the migration time of the later-eluting peak (the + isomer) and t_{R1} is the migration time of the first peak. Based on these data, 25 m*M* was deemed to be the optimum concentration of TMBCD in the run buffer, even though 50 m*M* gives a slightly higher α value for dichlorprop.

Table 1
Reproducibility of CE data for herbicides and their enantiomers

Herbicide	Concentration (μg/ml)	Injection time (s)	Migration time (min)			Peak area		Peak height	
			n	Mean	R.S.D.	*n*	R.S.D.	*n*	R.S.D.
Fenoprop									
Racemate	1	15	6	13.13	3.91	6	6.84	6	13.08
	10	15	6	12.31	3.05	6	4.78	6	4.36
	10	5	7	14.77	1.30	7	13.05	7	11.55
(−)-Isomer	1	15	4	10.76	2.91	4	1.55	4	11.13
(+)-Isomer	1	15	4	11.20	3.05	4	4.97	4	6.80
Mecoprop									
Racemate	1	15	6	13.53	3.91	6	8.70	6	12.24
	10	15	6	12.63	3.12	6	5.31	6	5.31
	10	5	7	15.26	1.34	7	12.03	7	13.78
(−)-Isomer	1	15	4	12.69	3.42	4	5.57	4	6.37
(+)-Isomer	1	15	4	13.19	3.69	4	8.06	4	7.62
Dichlorprop									
Racemate	1	15	6	13.84	4.08	6	8.37	6	12.67
	10	15	6	12.97	3.11	6	5.75	6	5.51
	10	5	7	15.64	1.29	7	13.96	7	16.11
(−)-Isomer	1	15	4	13.87	4.14	4	2.46	4	9.78
(+)-Isomer	1	15	4	14.19	4.27	4	7.50	4	9.92
2,4-D	1	15	6	15.45	4.56	6	9.44	6	13.13
	10	15	6	14.32	3.25	6	6.32	6	8.25
	10	5	7	17.68	1.54	7	17.86	7	15.45

3.4. *Effects of methanol on enantiomeric separation*

Even though use of TMBCD under the above conditions gave good separations of all three enantiomer pairs, the effects of methanol addition were briefly investigated. Experiments were conducted with 0, 5, 10 or 20% methanol added to each run buffer, which also contained 12.5 m*M* of one of the five CD reagents. With one exception, qualitative results were the same with as without methanol: (1) α-CD with methanol separated the isomers of dichlorprop and mecoprop, and not those of fenoprop, while shifting the migration order of the herbicides in the same way as without methanol; (2) DMBCD separated the isomers of dichlorprop and mecoprop, and not those of fenoprop; (3) β-CD slightly separated the isomers of fenoprop but not those of the other two herbicides (without methanol, the mecoprop peak was also very slightly split); and (4) TMBCD still afforded the best separation, giving good baseline separation of all three pairs of isomers. A methanol content of 10% resulted in even better separation using the TMBCD reagent than that obtained with no methanol added. This was generally also true with the α-CD, DMBCD and β-CD reagents; i.e., whenever separation of isomers occurred upon the addition of a CD, the addition of 10% methanol increased the separation.

The one exception with methanol addition mentioned above was that γ-CD in the presence of methanol did cause some separation of the dichlorprop and mecoprop optical isomers, as opposed to the absence of methanol, where γ-CD caused no separation at all. Separation was maximum with 20% methanol, in which case the

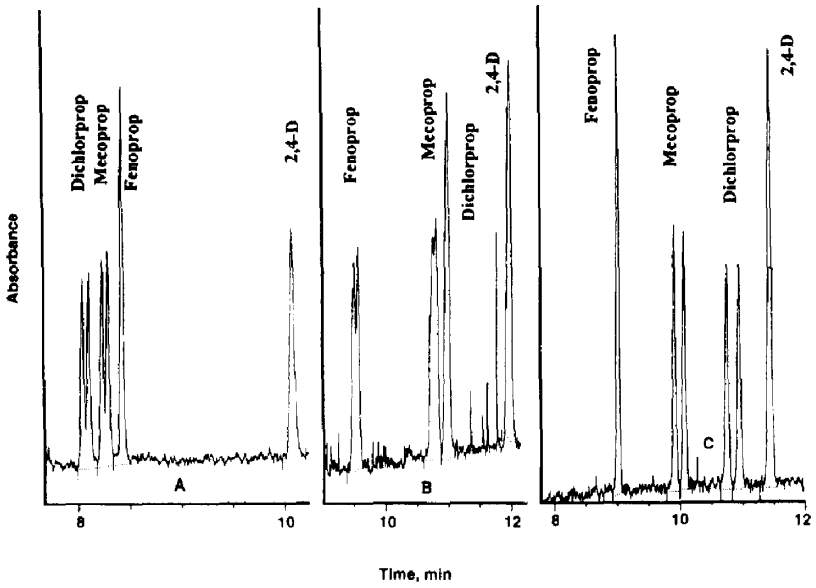

Fig. 4. Separation of (+) and (−) isomers of optically active herbicides by cyclodextrin reagents. (A) 10 m*M* α-CD; (B) 10 m*M* β-CD; (C) 10 m*M* DMBCD. Conditions as in Fig. 3B, except for addition of 10 m*M* CD to the acetate run buffer.

dichlorprop isomers had an α value of 1.01, showing peak splitting but not baseline separation.

One disadvantage of methanol addition was the increase in migration time for all optical isomers and for 2,4-D. This increase correlated with the amount of methanol added; for example, with TMBCD but without methanol, the latest migrating peak, 2,4-D, required 13.57 min for elution. With the addition of 10% methanol, migration time increased to 24.17 min, and with 20%, to 29.67 min. This degree of increase of migration time with addition of methanol was about the same when the other four CDs were used; i.e., addition of 20% methanol more than doubled the migration times for the optical isomers and for 2,4-D.

3.5. Reproducibility of enantiomer electropherograms

Table 1 presents data on the reproducibility of migration times, peak heights and peak areas of the (+) and (−) isomers of the three herbicides at a concentration of 1 μg/ml, separated by complexation with TMBCD at 25 m*M* as described above.

Table 2
Separation of herbicide enantiomers by cyclodextrins

	α-CD	β-CD	γ-CD	DMBCD	TMBCD
Fenoprop	−	+	−	−	+
Mecoprop	+	+	−	+	+
Dichlorprop	+	-	−	+	+

+ = Some degree of separation; − = no separation.

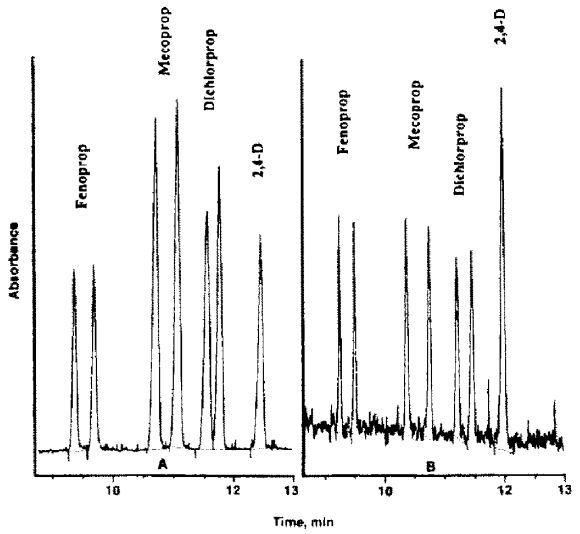

Fig. 5. Separation of (+) and (−) isomers of optically active herbicides by TMBCD. (A) 12.5 m*M* TMBCD, concentration of each herbicide is greater than 100 μg/ml; (B) 25 m*M* TMBCD, 1 μg/ml each herbicide. Other conditions as in Fig. 3B.

4. Discussion

TMBCD added to the acetate run buffer at 25 m*M* results in excellent separation of the optical isomers of the three herbicide racemates. None of the other four cyclodextrin derivatives used in this study separated all six optical isomers.

Both cavity size and hydrogen bonding and/or hydrophobic interactions at the larger lip of the truncated CD cone may play a role in its complexation with phenoxy acids [8,9,27]. Apparently, cavity size alone does not determine whether there is sufficient difference in the complexation constants of the (+) and (−) phenoxy acid isomers to cause separation. Racemic dichlorprop, the phenoxy acid with the smallest effective molecular diameter —corresponding to the molecular volume of the substituted phenoxy moiety (molecular volume of 2,4-dichlorophenol = 112 ml/mol)— is separated by the α-CD, which has the smallest cavity size, but not by the β-CD, which may simply be too large for differential complexation. This size restriction is logical, since the usual complexation mechanism involves insertion of the more hydrophobic (usually the aromatic) portion of the molecule into the hydrophobic CD cavity [8,9]. However, the fact that the dichlorprop racemate is separated by DMBCD and TMBCD, which have the same inner diameter as β-CD, implies an additional complexation mechanism.

Fenoprop, which has the largest effective molecular diameter (molecular volume of 2,4,5-trichlorophenol = 124 ml/mol) probably does not fit into the small α-CD cavity. Its isomers are separated by TMBCD and to a small extent by β-CD, but not at all by DMBCD. This apparent

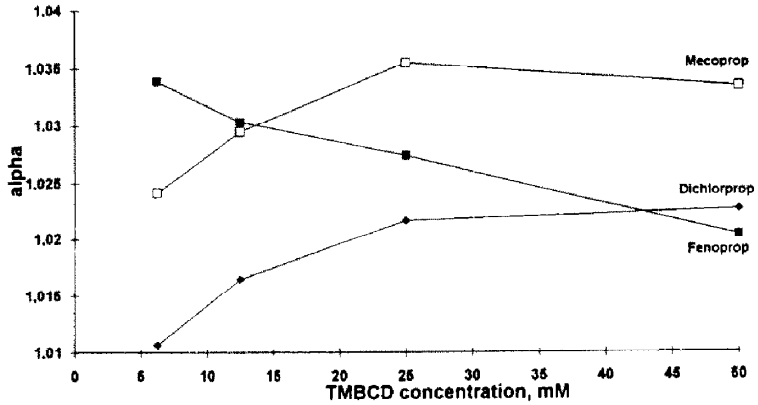

Fig. 6. Relative migration times (α) of (+) and (−) isomers of three herbicides at varying concentrations of TMBCD. Conditons as in Fig. 3B.

anomaly also implies an additional interaction mechanism. Mecoprop, the middle-sized (molecular volume of 2-methyl-4-chlorophenol = 118 ml/mol) of the racemic phenoxy acids, is separated to some extent into its optical isomers by all CDs used here except for γ-CD. γ-CD did not separate any of the racemates; its internal diameter is apparently too large.

The effects of methanol addition on these enantiomeric separations should provide clues to the CD–phenoxy acid complexation mechanisms [27]. For example, the partial separation of dichlorprop and mecoprop isomers with γ-CD in the presence of methanol may be caused by inclusion of a phenoxy acid–methanol adduct into the CD cavity, which may be too large to form effective complexes with the phenoxy acid alone. The general increase in optical isomer separation with addition of methanol, as well as the overall increase in migration times of all analytes, is likely caused by reduction of electroosmotic flow because of solvent interactions with the column wall [25]. Migration times in the presence of methanol could probably be reduced while retaining good separation by increasing the voltage from 20 to 30 kV.

Another puzzling phenomenon is the large shift in migration time observed with some of the optical isomers after CD complexation, relative to the migration time of the uncomplexed racemate. The complexes are new chemical species, of course, and would be expected to have different migration times from the uncomplexed racemates. An extreme case, however, is the large reduction in migration times of mecoprop and dichlorprop after the addition of α-CD; this large shift does not occur with other CDs. In this case, fenoprop's migration time changes very little; since its isomers are not resolved with α-CD, as mentioned above, and since fenoprop is the largest of the three herbicides, it is assumed that it does not form a complex. The greatly reduced migration time of mecoprop, which is even more reduced for dichlorprop, means that the α-CD complexes of these enantiomers have lower electrophoretic mobilities than do the uncomplexed racemates. Perhaps the pK_a values of the complexed analytes are increased relative to the uncomplexed forms, resulting in a net reduction of charge at the pH of the buffer.

Carbon-14 and proton nuclear magnetic resonance experiments are underway in an attempt to provide explanations for these complexation processes and mechanisms.

Changes in migration times upon CD complexation can cause confusion in identification of optical pairs, and it is necessary to spike samples with at least one of the optical isomers of each pair to assure positive identification. Sample spiking would be particularly important in the analysis of complex matrices, e.g., environmental samples. It should also be realized that migration time depends on the concentration of the CD used for separation, as well as on the CD structure.

Reproducibility of migration times of 2,4-D and the three optically active racemates and their (+) and (−) isomers is good; relative standard deviations (R.S.D.s) range from 1.3 to 4.6%. The R.S.D.s are considerably better for 5- than for 15-s injections. It is also noteworthy that the migration time R.S.D.s corresponding to 10 μg/ml sample concentrations are slightly better than those for 1 μg/ml levels at a 15-s injection time. On the other hand, reproducibility of quantitative parameters, i.e. peak areas and heights, varies from about 1.6 to 11.1% R.S.D. for the complexed optical isomers, and is even higher for most of the racemates, ranging from 4.4 to 17.9%. R.S.D.s for these parameters for the racemates are much better for 10 μg/ml than for 1 μg/ml concentrations at 15-s injection times. Area and peak-height R.S.D.s are much better for 15- than for 5-s injection times at the 10 μg/ml concentrations. For the optical isomers, peak area reproducibility was better than that for peak height.

In summary, the best quantitative precision occurs by using peak area measurements at the higher sample concentration and longer injection time. Under these conditions, migration time reproducibility is lower but still acceptable. For these optimal conditions, R.S.D.s for migration times range from 3.1 to 3.3%, and R.S.D.s for peak areas range from 4.8 to 6.3% for the

racemates. Similarly, migration time R.S.D.s range from 2.9 to 4.3% and peak area R.S.D.s range from 1.6 to 8.1% for the optical isomers, which were only run at the 1 μg/ml concentration.

5. Conclusions

A 50 m*M* acetate run buffer of pH 4.5 provides for baseline separation of 2,4-D, dichlorprop, mecoprop and fenoprop, using FSCE at 20 kV and 30°C. UV detection at 230 nm allows a lower detection limit of 0.05 μg/ml (about $5 \cdot 10^{-7}$ *M*).

TMBCD added to the acetate run buffer at 25 m*M* results in baseline separation of the (+) and (−) isomers of the three optically active herbicides; none of the other four CD derivatives used in this study separates all six isomers. NMR studies in progress should help explain the interactions between these phenoxy acids and the various CDs. The addition of methanol to the run buffer along with the CD reagent is not overall advantageous; separations are marginally improved, but migration times are generally doubled.

Reproducibility of migration times of 2,4-D and the three optically active racemates and their enantiomers is good. Reproducibility of peak areas and heights is acceptable; best results are obtained by using peak area instead of peak height measurements at higher concentrations and longer injection times.

5.1. Environmental applications

Possible applications of these results to environmental problems are obvious. HPCE has particular advantages over GC or HPLC: resolution is better; chiral separation is simpler; analysis is faster; and finally, less sample preparation is necessary, mostly because in the FSCE mode used here, only anions are detected. This latter advantage eliminates the need for derivatization, as well as screening out all cationic and neutral interferences.

CE also has disadvantages relative to GC or HPLC; for one, reproducibility is lower. In addition, CE does not have the apparent sensitivity of GC or HPLC separation/detection techniques; although the inherent sensitivity (mass sensitivity) is excellent in terms of the amount on column, the injection volume is very small. Detection levels are adequate, however, for many environmental analyses. Furthermore, laser-induced fluorescence detectors provide very high sensitivity for certain types of analytes. Another important current disadvantage of CE over the other techniques is that interfaces with mass spectrometers are still more or less in the experimental stage; positive identification of unknown peaks, therefore, is not the routine procedure possible with GC–MS or HPLC–MS. Current research is rapidly changing this situation, however [28,29].

The CE methods described here have recently been applied to determine the relative degradation of the two optical isomers of dichlorprop with time in soil samples from an agricultural site treated with a commercial herbicide formulation [30].

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