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On-line preconcentration and enantioselective separation of triadimenol by electrokinetic chromatography using cyclodextrins as chiral selectors

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

Enantioselective separation of triadimenol, a component of systemic agricultural fungicide, by electrokinetic chromatography (EKC) using cyclodextrins (CDs) as chiral selectors was investigated. Both a neutral CD derivative, hydroxypropyl-γ-CD (HP-γ-CD), and an ionic one, heptakis-6-sulfato-β-CD (HS-β-CD), were employed as an additive in cyclodextrin–modified micellar electrokinetic chromatography (CD–MEKC) and as a chiral pseudostationary phase in CDEKC, respectively. In each system, four stereoisomeric peaks were completely or partially separated from each other. To enhance the detectability or the concentration sensitivity, on-line preconcentration techniques were applied to both EKC systems. Sweeping was used in the CD–MEKC system under an acidic condition, whereas stacking with a reverse migrating pseudostationary phase (SRMP) in the CDEKC system. Around 10-fold increase in the detection sensitivity for each peak was attained with both sweeping and SRMP systems. Good repeatabilities in the migration time, corrected peak area, and peak height were recognized in terms of the relative standard deviation. The limit of detection for each peak in the SRMP-CDEKC system, calculated from the calibration curve, was found to be 0.8–3.8 ppm.

Keywords: Capillary electrophoresis; Electrokinetic chromatography; Cyclodextrins; CD–MEKC; CDEKC; Enantiomer separation; Stacking; Sweeping

1. Introduction

Capillary electrophoresis (CE) has been recognized as one of highly efficient separation techniques in various analytical fields owing to its high resolving power. In the area of pharmaceutical and

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agricultural sciences, CE has become popular in terms of the micro scale and fast analysis, including chiral separations.

Separation of enantiomers is one of major objectives in the chromatographic area as well as in CE, especially in the pharmaceutical and biochemical fields. Among several methods used for enantiomeric separations by CE, the use of cyclodextrins (CDs) as chiral selectors in capillary zone electrophoresis (CZE) is one of the most popular techniques [1], which is recognized as CD modified or CD mediated CZE (CD-CZE). In CD-CZE, various types of CDs are employed to achieve chiral recognition [2], including underivatized or natural nonionic CDs, e.g., α-, β-, and γ-CDs, or derivatized neutral CDs, e.g., dimethyl- and trimethyl- and hydroxypropyl (HP)-CDs. At the present stage, several derivatized ionic CDs, e.g., carboxymethylated- and sulfated-CDs, are available, and they are used as pseudostationary phases in electrokinetic chromatography (EKC) or CDEKC, where enantiomer separation is also carried out [2]. In micellar electrokinetic chromatography (MEKC), the addition of CD to the micellar solution as a chiral selector has also been employed for chiral separation [3], namely the cyclodextrin-modified micellar electrokinetic chromatography (CD–MEKC) technique.

There are several pesticide components that are optically active. Triadimenol, a triazole fungicide, which is usually used as a systemic agricultural fungicide effective to powdery mildew and grain rust, is a chiral compound that has two enantiomeric pairs or four individual stereoisomers, i.e., (−)-threo-1S,2R−, (+)-threo-1R,2S−, (−)-erythro-1S,2S−, and (+)-erythro-1R,2R−, due to two chiral centres, as shown in Fig. 1. Generally

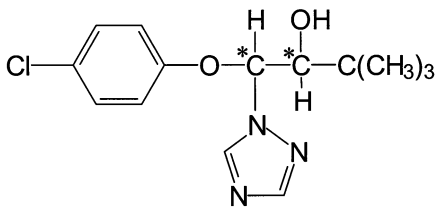

Fig. 1. Chemical structure of triadimenol.

the biochemical activity of each enantiomer of such pesticide components is different from each other, and sometimes it is necessary to know the exact content of each enantiomer in the environmental or actual samples. Differences in biological activities among the individual stereoisomers of triadimenol have been investigated. For example, the (−)-threo-1S,2R enantiomer showed the highest fungitoxicity against the rhizoctonia solani among the four enantiomers [4]: the ED_{50} (50% effective dose) values for (−)-threo-1S,2R−, (+)-threo-1R,2S−, (−)-erythro-1S,2S−, and (+)-erythro-1R,2R−enantiomers are 1, 50, 100, and 50 ppm, respectively. To determine the quantity of each enantiomer, resolution of stereoisomers is then required.

One of general problems in CE is relatively poor detectability in terms of the concentration sensitivity as long as spectrophotometric detection is employed. To overcome this or to enhance the detectability, several on-line sample preconcentration techniques have been developed and applied to CE systems including EKC. Both stacking [5] and sweeping [6] are sometimes effective to improve the detection sensitivity significantly in EKC, especially under acidic conditions.

In this study, enantiomer separations of triadimenol by CD–MEKC, where HP-γ-CD is used as a chiral selector and sodium dodecyl sulfate (SDS) as an achiral micelle, and by CDEKC, where hepta-6-sulfato-β-cyclodextrin (HS-β-CD) as a chiral pseudostationary phase, were investigated to compare the resolution performance in each system. To investigate the possibility of the enhancement of the detection sensitivity by using on-line sample preconcentration techniques for the enantioseparation of triadimenol by EKC, sweeping was applied to the HP-γ-CD–MEKC system, while stacking with a reverse migrating pseudostationary phase (SRMP), which is one of stacking methods available in EKC, was applied to the CDEKC system, respectively. Repeatabilities in both systems using on-line preconcentrations in terms of the migration time, peak area, and peak height were briefly examined and the limits of detection (LOD) were also estimated with the calibration curve.

2. Experimental

2.1. Chemicals and reagents

HS-β-CD was a gift from Prof. G. Vigh (Texas A&M Univ.), whereas HP-γ-CD was purchased from Aldrich (Milwaukee, WI), SDS from Nacalai Tesque (Kyoto Japan), and triadimenol from Wako (Osaka, Japan), respectively. A sample stock solution was prepared by dissolving triadimenol in methanol at the concentration of 2000 ppm. Sample solutions were prepared by diluting the stock solution with separation solutions for conventional CD–MEKC, with a buffer solution without pseudostationary phases for sweeping, and with pure water for SRMP. All other reagents used were of analytical grade. Water was purified with a Milli-Q Labo system (Nihon Millipore, Yonezawa, Japan).

Separation solutions were prepared by dissolving CDs at an adequate concentration in phosphate buffers (pH 2.2 or 7.0) and filtered through a 0.2 μm membrane filter prior to use.

2.2. Instrumentation

CE was performed by a Hewlett-Packard ^{3D}CE System (Waldbronn, Germany) equipped with a photodiode array detector. The operation of the CE instrument and data processing was carried out by the HP ChemStation software running on Windows NT 4.0. The separation capillary used was an untreated fused silica capillary of 50 μm i.d. × 50 cm (41.5 cm effective) obtained from Polymicro Technologies (Phoenix, AZ).

In the CD–MEKC system, the voltage of +16 kV was applied for pH 7.0, whereas −16 kV for pH 2.2. While in the CDEKC system, −16 kV was applied. The detection wavelength used was 200 nm and temperature was maintained at 25 °C.

2.3. Procedure

Sweeping in CD–MEKC was carried out under the acidic condition (pH 2.2) according to the procedure previously reported [7]: The sample solution, of which conductivity was adjusted as almost the same as that of the separation solution, was injected hydrodynamically with an air pressure of 50 mbar for 20 s following the rinse of the capillary with the separation or micellar solution.

SRMP in CDEKC was performed also under the acidic condition (pH 2.2). The procedure was similar to that in the previous paper [8]: The sample solution, prepared by diluting the stock solution with pure water, was injected hydrodynamically at 50 mbar for 15 s after filling the separation solution into the capillary.

3. Results and discussion

3.1. Chiral separation by CD–MEKC with HP-γ-CD

According to previous experiments concerning chiral separations of several stereoisomeric pesticides [9], a CD–MEKC mode was first investigated using HP-γ-CD as a chiral selector and SDS as an achiral pseudostationary phase under both neutral and acidic conditions.

At pH 7.0, an almost complete resolution of four stereoisomers was attained, as shown in Fig. 2, where detection was carried out at the cathodic

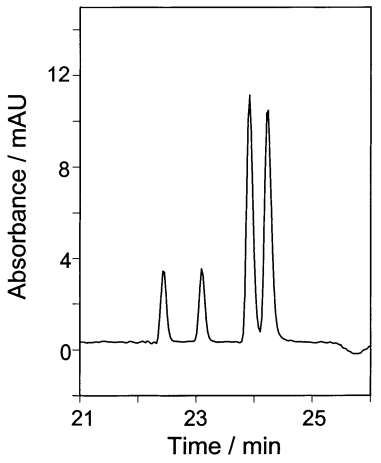

Fig. 2. Enantiomeric separation of triadimenol by conventional CD–MEKC with HP-γ-CD. Sample, 200 ppm triadimenol injected for 1 s at 50 mbar; separation solution, 25 mM HP-γ-CD—50 mM SDS in 50 mM phosphate buffer (pH 7.0) containing 15% (v/v) methanol; capillary, 50 μm i.d. × 50 cm (effective length, 41.5 cm); applied voltage, 16 kV; detection wavelength, 200 nm; temperature, 25 °C.

end of the capillary or all isomers migrated toward the negative electrode due to the strong electroosmotic flow (EOF). Although detailed examination was not carried out, the migration times of triadimenol isomers were decreased with the decrease in the SDS concentration. However, no peak was detected without an SDS addition. The addition of methanol affected resolution: the third and fourth peaks were completely resolved from each other with the addition of 15% (v/v) methanol, whereas less than 10% (v/v) addition brought incomplete resolution.

To apply on-line sample preconcentration techniques, acidic conditions are generally more preferable than neutral ones in terms of the achievable concentration efficiency when sweeping or stacking methods are employed in EKC [10]. Then the acidic condition or pH 2.2 was applied for the enantioseparation. Effects of the concentrations of HP-γ-CD and SDS as well as the content of methanol were briefly examined to obtain an optimal resolution. As a result, a 30 mM HP-γ-CD—50 mM SDS—20% (v/v) methanol solution gave the best separation among the conditions examined (data not shown). In this instance, the detection point was placed at the anodic end or all isomers migrated toward the positive electrode due to the suppressed EOF. However, a complete separation of individual four peaks could not be attained.

3.2. Sweeping in CD–MEKC

After obtaining the optimal condition, sweeping was applied to the system to improve the concentration detection sensitivity. A typical electrokinetic chromatogram of sweeping–CD–MEKC detected at the anodic end of the capillary is shown in Fig. 3, where a 20 s duration for the injection at 50 mbar was carried out. Although, as mentioned before, the complete resolution for each peak pair was not fully attained, the detectability was found to be approximately 10-fold increase in terms of the sensitivity enhancement factor obtained with the peak height (SEF_{height}) for each enantiomer peak. Longer injection lengths or longer injection times of the sample solution were examined, but no better sensitivity enhancement was obtained. Although the authentic was not available, the first two peaks or larger peak pair should be an enantiomeric pair and the latter two peaks or smaller peak pair be another enantiomeric pair. From this point of view, the migration order in Fig. 3 was changed from that in Fig. 2, simply because of the reversal of the migration direction. The repeatability in the sweeping–CD–MEKC system concerning the migration time, peak area, and peak height for each enantiomeric peak was briefly examined ($n = 3$) for the injection of the 5 ppm sample and the results are summarized in Table 1 as the relative standard deviation (RSD%). The repeatability in the migration time of each peak was acceptable value ranging 0.36–0.47%. As for the corrected peak area and peak height, slightly poor repeatabilities were observed, mainly due to the incomplete separation between each adjacent peak pair and/or tailed peak shape. The concentration sensitivity for each peak was about 10-fold increased in the sweeping–CD–MEKC system compared to the conventional CD–MEKC system. Although these values are not fully satisfactory for the analysis of real samples, the result clearly shows the possibility of improving the detectability by using sweeping in CD–MEKC.

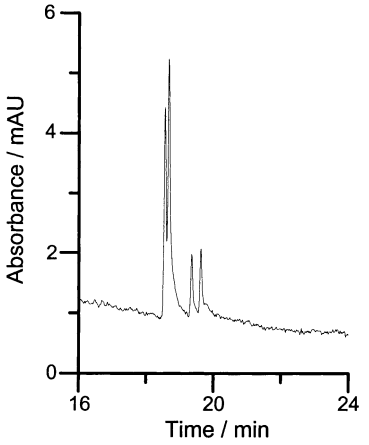

Fig. 3. On-line preconcentration and enantiomeric separation of triadimenol by sweeping–CD–MEKC with HP-γ-CD. Sample, 20 ppm triadimenol injected for 15 s at 50 mbar; separation solution, 30 mM HP-γ-CD—50 mM SDS in 50 mM phosphate buffer (pH 2.2) containing 20% (v/v) methanol; applied voltage, −16 kV. Other conditions as in Fig. 2.

Table 1
RSDs and SEF_{height} for triadimenol in sweeping–CD–MEKC

	Peak 1[a]	Peak 2[a]	Peak 3[a]	Peak 4[a]
Calibration line [b]	$y = 0.7140x + 0.0591$	$y = 0.9265x - 0.2862$	$y = 0.2272x - 0.0316$	$y = 0.2348x - 0.0599$
Correlation coefficient (r)	0.9959	0.9980	0.9973	0.9985
RSD (%, $n = 3$)				
(a) Migration time	0.47	0.47	0.41	0.36
(b) Corrected peak area	3.1	3.8	2.0	9.2
(c) Peak height	3.8	3.4	5.3	2.9
SEF_{height} [c]	12	10	11	9

Conditions as in Fig. 3.
[a] Peaks 1–4 are the first, second, third, and fourth migrated (detected) peaks, respectively.
[b] Calibration line: concentration (ppm) = slope × peak height (mAU) + y-intercept.
[c] SEF_{height} = (peak height obtained with sweeping/peak height obtained with usual CD–MEKC injection) × dilution factor.

3.3. Chiral separation by CDEKC with HS-β-CD

As an alternative method to CD–MEKC, CDEKC can be employed if an adequate ionic CD is available. The single isomeric ionic CD, HS-β-CD, was introduced [11] and has been found to be effective as a pseudostationary phase in CDEKC for enantioseparations [12]. Recently, Wu et al. reported chiral separation of fourteen triazole fungicides including triadimenol by CDEKC under acidic conditions (pH 3.0), where commercially available sulfated β-CD (S-β-CD) was employed as a chiral pseudostationary phase [13]. Note that this S-β-CD is not the single isomeric compound, but rather a mixture of sulfated CDs whose substitution degrees ranging between seven and eleven [13]. In their work, enantioseparations of most enantiomers were successfully achieved. However, the sample concentrations were not carefully concerned or typically the concentrations were around 50 ppm and the LOD was not discussed.

In the present study, SRMP was applied to the CDEKC system to enhance the detectability. After brief investigations on separation conditions, 20 mM HS-β-CD in 20 mM phosphate buffer (pH 2.2) was found to bring the best enantiomeric resolution, as shown in Fig. 4(A). Almost complete resolution was achieved for each peak pair.

To apply the SRMP method to the CDEKC system, the conductivity of the sample solution should be lower than that of the separation solution or the HS-β-CD solution. The sample stock solution was then diluted with pure water to give a 20, 10, 5, and 2.5 ppm solutions. A typical example of the SRMP–CDEKC separation is shown in Fig. 4 (B), where the 20 ppm sample solution was injected for 15 s at 50 mbar pressure. Although resolution for each peak pair, especially between the first and second migrated peaks, was decreased compared to that in conventional separation (Fig. 4A), better enantiomer separation compared to the CD–MEKC system was achieved, where on-line sample preconcentration techniques were applied.

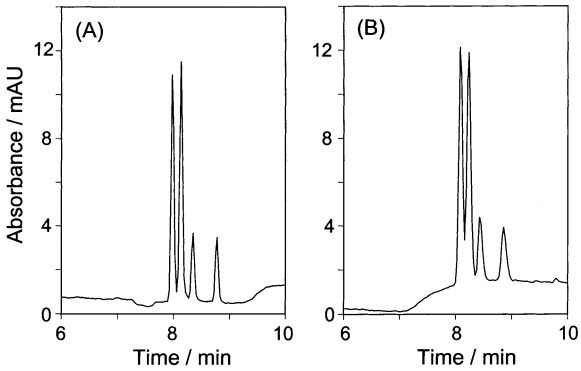

Fig. 4. Enantioseparations of triadimenol by (A) conventional CDEKC and (B) SRMP–CDEKC. Separation solution, 20 mM HS-β-CD in 20 mM phosphate buffer (pH 2.2); sample, (A) 200 ppm triadimenol injected for 1 s at 50 mbar, (B) 20 ppm triadimenol injected for 15 s at 50 mbar. Other conditions as in Fig. 3.

The performance of the system was examined in terms of the LOD, repeatability, and increase in

Table 2
LODs, RSDs, and SEF_{height} for triadimenol in SRMP-CDEKC

	Peak 1[a]	Peak 2[a]	Peak 3[a]	Peak 4[a]
Calibration line	$y = 0.5676x - 0.3660$	$y = 0.5449x - 0.4075$	$y = 0.1497x - 0.1068$	$y = 0.1230x - 0.0803$
Correlation coefficient (r)	0.9997	0.9997	0.9996	0.9994
LOD (S/N = 3)				
(a) ppm	0.82	0.86	3.1	3.8
(b) $\times 10^{-6}$ M	2.78	2.90	10.6	12.8
RSD (%, n = 3)				
(a) Migration time	0.30	0.26	0.24	0.24
(b) Corrected peak area	4.7	4.6	6.0	5.8
(c) Peak height	5.2	5.4	6.9	5.5
SEF_{height}	11	10	10	8

Conditions as in Fig. 4(B).
[a] See Table 1.

detection sensitivity or SEF_{height}. The results are summarized in Table 2. The LOD for each peak, which was determined by the calibration curve along with the signal-to-noise ratio (S/N) as 3, ranging 0.8–3.8 ppm or 2.8–13 μM. The repeatabilities in the migration time, corrected peak area, and peak height are similar to those obtained in aforementioned sweeping–CD–MEKC system. Also SEF_{height} attained are factor of 8–10, which are comparable with the sweeping–CD–MEKC system.

4. Conclusion

Enantiomeric separations of triadimenol were successfully achieved by EKC techniques employed a nonionic CD derivative or HP-γ-CD and an ionic one or HS-β-CD as chiral selectors. In the former case or CD–MEKC system, sweeping was applied to enhance the concentration detection sensitivity, whereas in the latter case or CDEKC system, SRMP was applied. In both cases, around 10-fold increases in detection sensitivity in terms of obtained peak height were achieved for each stereoisomeric peak. The SRMP–CDEKC system, however, overall shows better enantioseparation and detectability than the sweeping–CD–MEKC system. Although the enhancement factor is not sufficient and much improvement is required to achieve the highly sensitive detection, the present study can be recognized as a preliminary introduction of the on-line concentration techniques to the enantioselective separation of agricultural interests by EKC with CD derivatives.

Generally, it is often said that on-line preconcentration of less hydrophobic compounds is difficult and the enhancement factor of the detection sensitivity is usually small in several EKC techniques, compared to much hydrophobic compounds. Further investigations to enhance the detection sensitivity, especially for hydrophilic compounds, are currently continued.

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