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Stereoselective separation and detection of phenoxy acid herbicide enantiomers by cyclodextrin-modified capillary zone electrophoresis–electrospray ionization mass spectrometry

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Abstract

An application of on-line coupling of capillary electrophoresis and mass spectrometry (CE–MS) to the chiral separation of phenoxy acid herbicide enantiomers was investigated. As an ionization method, electrospray ionization (ESI) is used for a CE–MS interface. Generally, nonvolatile additives in separation solutions sometimes decrease the MS sensitivity and/or signal intensity. In this study, however, heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) was used as a chiral selector and it migrated directly into the ESI interface. By using the negative-ionization mode along with a methanol–water–formic acid solution as a sheath liquid and nitrogen as a sheath gas, stereoselective separation and detection of three phenoxy acid herbicide enantiomers were successfully achieved with a 20 m*M* TM-β-CD in a 50 m*M* ammonium acetate buffer (pH 4.6).

Keywords: Enantiomer separation; Pesticides; Phenoxy acid herbicides

1. Introduction

Recently, capillary electrophoresis (CE) has been widely used in various fields as a high-efficiency and high-resolution separation technique. In most commercially available CE instruments, UV absorbance is used as a detection scheme. Although a UV detector is easy to handle and maintain, and reliable, the relatively low sensitivity from the short optical path-length is an inherent disadvantage. The use of laser-induced fluorescence (LIF) detection can sometimes compensate for this problem. However, the applicability of LIF is limited to only compounds having fluorophores or derivatization sites. The use of mass spectrometry (MS) as a detection scheme in CE or on-line coupling of CE with MS (CE–MS) is an attractive technique in terms of high sensitivity and selectivity and molecular structural information. Several reviews on CE–MS appear [1–3], in which the electrospray ionization (ESI) technique is most widely used for the interface between CE and the MS detector.

Generally, the presence of nonvolatile components in a solution introduced into the ESI interface causes the deterioration of the MS signal and/or sensitivity and the increase of the background noise. However, Sheppard et al. [4] mentioned that the use of a buffer

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containing a cyclodextrin (CD) derivative in capillary zone electrophoresis (CZE)–MS showed no significant effect on the MS sensitivity. They achieved chiral separations and detection of terbutaline and ephedrine by CD-modified CZE (CD–CZE) using heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD) coupled with ESI–MS. They used a low pH or acidic running buffer to suppress the electroosmotic flow, and only a small amount of DM-β-CD was introduced into the ESI interface. Similarly, Lamoree et al. [5] reported on the chiral separation and detection of ropivacaine by CD–CZE–ESI–MS with DM-β-CD. In this instance, however, they described that the direct introduction of DM-β-CD into the ESI-interface caused significant deterioration of the sensitivity, and they used a special technique to prevent the direct injection of CD into the ESI interface.

Unfortunately, subsequent enantiomeric separation and detection by CE–ESI–MS has not been reported except for a few examples described by Fanali et al. [6] where vancomycin was used as a chiral selector in CZE–ESI–MS.

Chiral separation is one of the major objectives in CE as well as in chromatographic methods, and a number of papers on enantioseparations by CE have appeared, where one of the most popular techniques for achieving the chiral recognition is the use of CDs, including underivatized CDs, derivatized neutral CDs, and derivatized charged CDs, as chiral additives to the separation buffers [7–10] as mentioned above.

Analyzing various compounds of environmental pollutants is also an important application of CE, and there have been several papers available on this topic [11–18]. Among such compounds, herbicides are one of many important agricultural chemicals to be analyzed. Since Foret et al. [19] described the separation of some triazine herbicides by CZE in 1990, a number of papers on herbicide analyses by CE has been published [17,20]. As for the analysis of phenoxy acid herbicides, Nielen [21] reported on the separation and detection by CZE–ESI–MS with a rather acidic ammonium acetate buffer (pH 4.8) without any additives in the running solution. Some phenoxy acids, such as 2-(2,4-dichlorophenoxy)propionic acid (dichlorprop), 2-(4-chloro-2-methylphenoxy)propionic acid (mecoprop), and 2-(2,4,5-trichlorophenoxy)propionic acid (fenoprop), are optically active. These are well known compounds and, in each case, only the (+)-isomer is herbicidally active [22]. Garrison et al. [22] reported on the enantiomer separation of these three herbicides by CD–CZE with UV detection.

In this paper, the application of CE–MS on the enantiomeric separation of phenoxy acid herbicides is described. The purpose of this work is to examine the possibility of direct coupling of CD–CZE with ESI–MS as a complementary technique to UV detection. We used CD–CZE for chiral separation followed by ESI–MS detection without any technique for preventing the introduction of CD into the ESI interface. Although the result is preliminary, the stereoselective separation and detection of herbicide enantiomers were successfully achieved.

2. Experimental

2.1. Apparatus

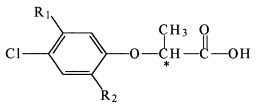
As CE instruments, a Beckman P/ACE System 2200 (Fullerton, CA, USA) equipped with a UV detector controlled by a System Gold software on MS-DOS and a Hewlett-Packard (HP) ^{3D}CE System (Waldbronn, Germany) equipped with a UV detector controlled by a ChemStation software on Windows NT, were used. For the mass spectrometer, a Finnigan LCQ ion-trap MS detector (San Jose, CA, USA) was used. An electrospray interface (Finnigan) was used for the coupling of each CE system and MS detector.

The separation capillaries were fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm I.D. and 375 μm O.D. The length of the capillaries were 980 mm in total with a 200-mm UV effective length for the Beckman system and 900 mm in total with a 200-mm UV effective length for the HP system. The capillary temperature was maintained at 25°C in both Beckman and HP systems.

A sheath liquid was delivered by a syringe pump equipped in the LCQ.

2.2. Procedure

As enantiomer samples, three optically active



Herbicide	R_1	R_2	pK_a	M_r
Dichlorprop	H	Cl	3.3	235.06
Fenoprop	Cl	Cl	3.2	269.51
Mecoprop	H	CH_3	3.4	214.65

Fig. 1. Structures and properties of phenoxy acid herbicides used.

phenoxy acid herbicides, such as dichlorprop, mecoprop, and fenoprop, were used. The structures of these herbicides are shown in Fig. 1 together with some chemical and physical properties. These herbicides were dissolved in acetonitrile at the concentration ca. 10^{-4} *M*.

Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) obtained from Sigma-Aldrich (St. Louis, MO, USA) was used for the chiral selector. The separation solutions were prepared by dissolving TM-β-CD in a 50 m*M* ammonium acetate buffer (pH 4.6). As a sheath liquid, a mixture of methanol and water (50:50, v/v) containing 1% (v/v) formic acid was normally used. All chemicals were used as received or without further purification.

3. Results and discussion

3.1. Selecting CD–CZE and MS conditions

Garrison et al. [22] reported the successful enantioseparation of optical isomers of fenoprop, mecoprop, and dichlorprop, by CD–CZE with TM-β-CD. They used a 50 m*M* acetate buffer (pH 4.45) containing 12.5 m*M* or 25 m*M* TM-β-CD. We examined similar conditions and found that 20 m*M* TM-β-CD in 50 m*M* ammonium acetate buffer (pH 4.6) was one of the promising conditions for CD–CZE (vide infra).

Then, we investigated the applicability of this condition to the ESI–MS detection. Under the positive-ion detection mode (ESI voltage =3.0 kV), however, we could not obtain clear MS signals for any herbicide, probably due to the lower ionizability of these anionic herbicides to the positively charged forms or $[M+H]^+$. On the other hand, we got a rather higher signal when using the negative-ion detection mode (ESI voltage=−3.0 kV). As Nielen reported [21], the $[M\text{-}H]^-$ ions can be detected under the negative-ion mode. To confirm the detection mass numbers or m/z ratios, the MS spectra of these herbicides were measured under the negative-ion detection mode in the absence of TM-β-CD, as shown in Fig. 2. Here, a sample solution containing these three herbicides was continuously delivered into the ESI interface by pressure using the HP CE system together with a sheath liquid of the mixture of methanol–water–formic acid (50:50:1, v/v/v). For mecoprop, whose nominal molecular mass is 214, the quasimolecular ion $[M\text{-}H]^-$ with m/z ratio of 213 was obtained, and also the isotopic effect from a chlorine atom can be seen at m/z=215. For dichlorprop (molecular mass=234), the quasimolecular ion of m/z=233 was detected, along with the isotopic effects from one and two chlorine atoms at m/z=235 and 237, respectively. Similarly, for fenoprop (molecular mass=268), the quasimolecular ion (m/z=267) and the isotopic effects from one to three chlorine atoms (m/z=269, 271, and 273, respectively) are observed. As typical m/z values for the quasimolecular ions of these herbicides, 213, 233, and 267 were chosen in the following experiments.

Under the CD–CZE conditions with 20 m*M* TM-β-CD in 50 m*M* ammonium acetate (pH 4.6), we briefly investigated the effects of the MS operating conditions on the sensitivity including the sheath liquid flow-rate and ESI capillary temperature, by using fenoprop as a sample and the full-scan mode with a m/z range 150–300 (ESI voltage=−3.0 kV). As a result, we found that a sheath flow-rate of 2.0 μl/min and ESI capillary temperature of 250°C were rather optimal, and we used these values. However, these parameters were not so critical under the CD–CZE conditions used.

3.2. Enantiomer separation and detection by MS

We then simply applied the above-mentioned MS conditions to the detection of CD–CZE enantioseparation of three herbicides. The CE system em-

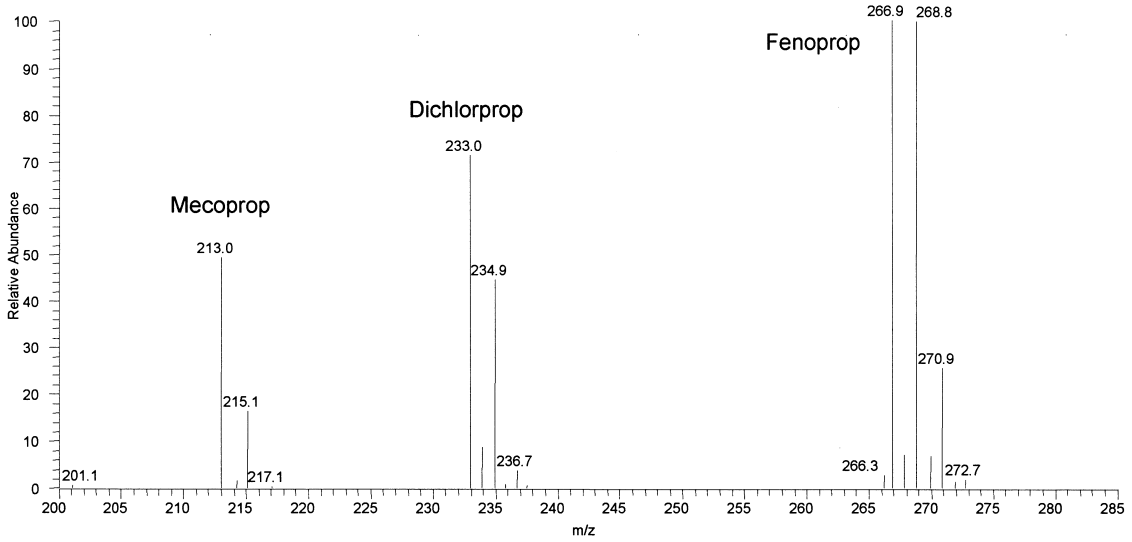


Fig. 2. Mass spectra of three herbicides by full-scan negative-ion ESI–MS. A sample solution mixed with a sheath liquid was continuously introduced into the ESI source by pressure. Conditions: sheath, methanol–water–formic acid (50:50:1, v/v/v) at 2.0 μl/min; ESI voltage, −3.0 kV; ESI capillary temperature, 250°C; sheath gas, nitrogen at 20 in an arbitrary value given by the LCQ software.

ployed was the Beckman instrument. Because of the long separation capillary, 980 mm total or from the injection to the ESI nozzle, a high applied voltage was required for a reduced separation time. After several adjustments of the separation conditions, successful enantiomer separation and detection could be achieved in a reasonable analysis time. A typical example of the enantioseparation of three herbicides is shown in Fig. 3. Here, the CE applied voltage was set at 27.5 kV, which means that the net voltage across the separation capillary was increased to 30.5 kV during the ESI–MS measurement. In both the UV (a) and ESI–MS (b) detection, three herbicides could be separated from each other, and each enantiomeric pair was almost completely resolved. Although the results are preliminary and we have not yet investigated the effect of the presence of TM–β-CD in the separation solution on the MS signal intensity and sensitivity along with the contamination in the ESI interface and/or inside of the MS detector, these results demonstrate one possibility of the direct coupling of the CD–CZE with ESI–MS without any special techniques suppressing the introduction of CD into the ESI interface.

Fig. 4 shows a reconstructed masspherogram (m/z range=266.5–267.5) of the chiral separation of fenoprop enantiomers (top) and mass spectra of corresponding enantiomers (center and bottom). The center portion is the mass spectrum of the first detected enantiomer, peak 1, (migration time of 17.98 min in the masspherogram), and the bottom portion is of the second enantiomer, peak 2, (18.57 min). Unlike the spectrum of fenoprop in Fig. 2, only one signal at $m/z=267$ appeared in both spectra, probably due to the reduced MS sensitivity compared with that in Fig. 2. However, this signal corresponds to the quasimolecular ion of fenoprop $[M\text{-}H]^-$ and hence, this strongly supports that these two peaks obtained from the CD–CZE separation of fenoprop correspond to the one and the other enantiomer. Similar results were observed for mecoprop and dichlorprop, and again we can conclude that the enantiomer separations of these herbicides were successfully achieved.

4. Conclusions

Although the results shown here are preliminary and there remains a requirement of further experiments and considerations, the direct coupling of CD–CZE with TM-β-CD with ESI–MS using no

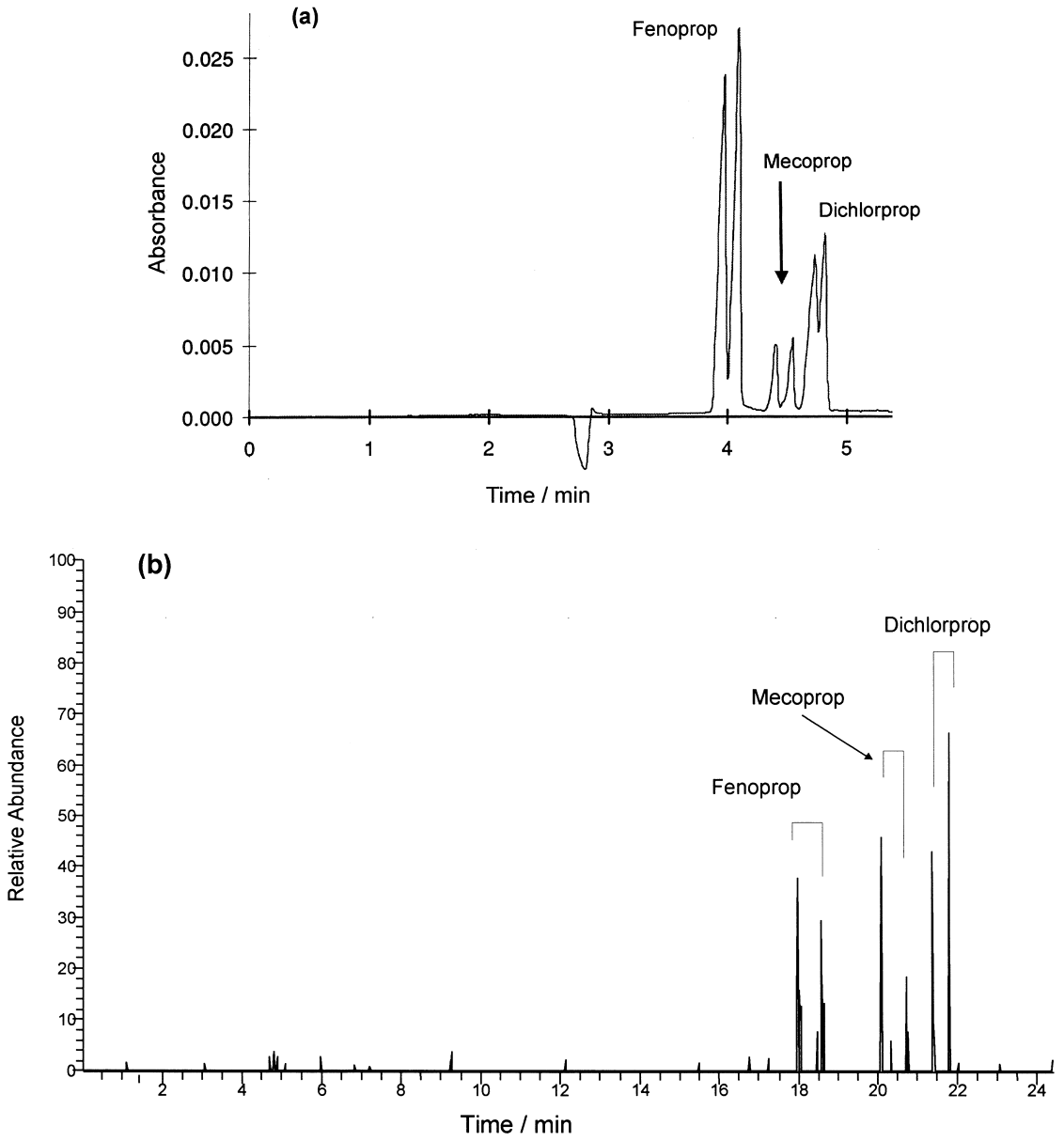


Fig. 3. Enantioseparation of fenoprop, mecoprop, and dichlorprop by CD–CZE–ESI–MS with TM-β-CD. (a) UV electropherogram, (b) reconstructed massperogram. CE conditions: separation solution, 20 m*M* TM-β-CD in 50 m*M* ammonium acetate (pH 4.6); capillary, 50 μm I.D.×375 μm O.D., 980 mm in total length, 200 mm in UV effective; total applied voltage, 27.5 kV (net voltage across the capillary, 30.5 kV); current, 16 μA; sample concentrations, ca. 10^{-4} *M* each in acetonitrile. MS conditions: massperogram reconstructed at $m/z=213\pm0.5$, 233 ± 0.5 and 267 ± 0.5. Other conditions as in Fig. 2.

special technique or under the continuous delivery of TM-β-CD into the ESI interface can be used for the chiral separation and detection of phenoxy acid herbicide enantiomers.

Effects of the presence of CD in the separation buffer on MS sensitivity and signal intensity and the contamination in the ESI interface should be examined. To suppress or decrease the background

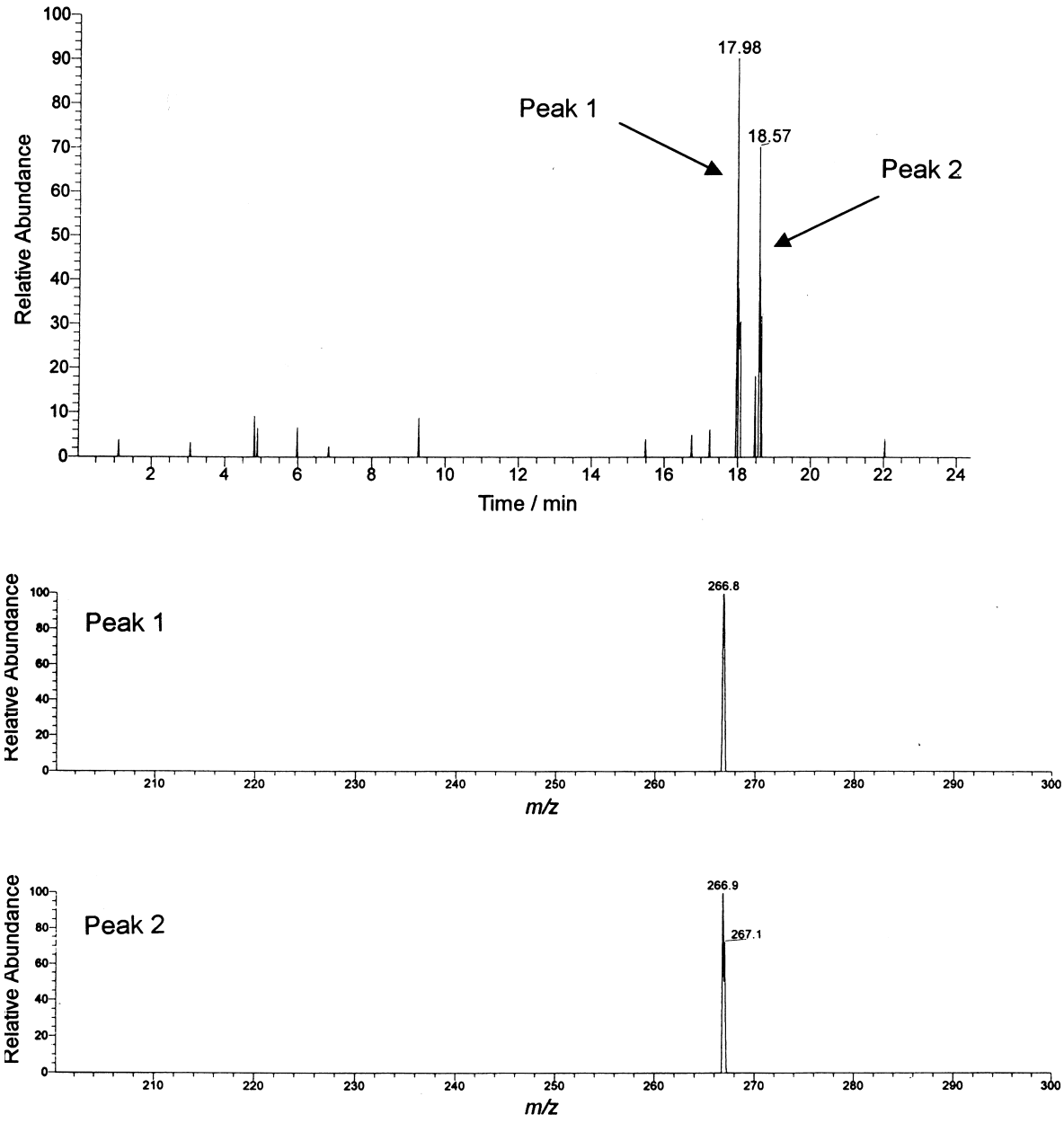


Fig. 4. Reconstructed masspherogram (top) of chiral separation of fenoprop enantiomers and mass spectra (center and bottom) corresponding to each enantiomer: center and bottom are of the mass spectra of the first and second peaks, respectively, in the top masspherogram. Masspherogram reconstructed at $m/z=267\pm0.5$. Other conditions as in Fig. 3.

noise, the use of nonaqueous buffer systems [23] containing CDs and other chiral selectors is also proceeding.

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