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Separation of highly hydrophobic compounds by cyclodextrin-modified micellar electrokinetic chromatography

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ABSTRACT

Cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC), in which CD is added to the micellar solution, was developed for the separation of electrically neutral, highly hydrophopbic compounds. The separation of such substances is generally difficult by electrophoretic techniques. In CD-MEKC, a water-insoluble hydrophobic solute is partitioned between the micelle and CD. When the solute is included in the CD cavity it migrates with the electroosmotic velocity, and when it is incorporated into the micelle it migrates with the micellar velocity. Accordingly, the differential partition of the solute between CD and the micelle enables a separation to be achieved. Highly hydrophobic and closely related compounds such as chlorinated benzene congeners, polychlorinated biphenyl congeners, tetrachlorodibenzo-*p*-dioxin isomers and polycyclic aromatic hydrocarbons have been successfully separated.

INTRODUCTION

Electrokinetic chromatography (EKC)[1] is a branch of both high-performance capillary electrophoresis (HPCE) and high-performance liquid chromatography (HPLC). From the viewpoint of electrophoresis, EKC is especially effective for the

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separation of electrically neutral substances, which cannot be separated in principle by conventional HPCE. Micellar EKC (MEKC)[2–4], in which an ionic micelle migrates electrophoretically with a different velocity from that of the bulk solution, is the most popular technique among various forms of EKC. The separation principle of MEKC is based on the differential partition of the solute between the micelle and the surrounding aqueous phase, as micellar chromatography, but it has an extremely high efficiency in comparison with HPLC. Therefore, MEKC is a powerful technique for the separation of various neutral substances[5–9]; further, it also improves the selectivity of ionic substances in contrast with conventional HPCE[10–14]. Another useful application of MEKC is optical resolution with a mixed micelle of a chiral compound[15,16] or with a chiral micelle[17,18].

In addition to MEKC, cyclodextrin (CD) EKC[1,19], ligand-exchange EKC[20], borate-complex EKC[21,22] and ion-exchange EKC[1,23] have been reported. Each of these methods has extended the applicability of HPCE to the analysis of various types of compounds. However, the separation of non-polar and highly hydrophobic compounds has not been reported, except for one paper[24], in which a tetraalkylammonium ion was used as a carrier of EKC (electrophoretically migrating phase)[1] in an aqueous organic solvent to separate some aromatic hydrocarbons.

As a preliminary experiment, we tried to separate polycyclic aromatic hydrocarbons (PAHs) by MEKC at the early stage of the development of EKC[25], but PAHs were so hydrophobic that they were almost totally incorporated into the micelle. The use of aqueous organic solvents increased the distribution of PAHs to the aqueous phase but still the resolution was not very successful because of tailed peaks[25].

Some PAHs whose molecular weights were less than 200 were successfully separated by CD-EKC with a β-CD derivative having a 2-amino ethylamino group[1,26], but aqueous organic solvents such as 50% aqueous methanol had to be used in order to increase the solubility of the PAHs. The separation of larger PAHs seemed difficult because of the extremely low solubility into solvents compatible with CD-EKC.

This paper describes CD-modified MEKC (CD-MEKC), in which a neutral CD is added to the micellar solution. CD itself is electrically neutral and its outside surface is hydrophilic; accordingly, it will not interact with the micelle. Therefore, CD in the micellar solution should behave as another phase against the micelle, and migrate with an identical velocity with the bulk solution. When a highly hydrophobic substance, which is insoluble in water, is injected into the CD-MEKC system, it will distribute itself between the micelle and CD, as shown in Fig. 1. Such a hydrophobic solute is incorporated by either the micelle or CD, but it does not exist in the aqueous medium. It should be noted that the aqueous phase in MEKC is seemingly displaced by CD in CD-MEKC for hydrophobic compounds and that water is an inert solvent only for the micelle and CD and not for the analytes.

The capacity factor, $\tilde{k}'$, of a highly hydrophobic solute in CD-MEKC is given by

$$\tilde{k}' = \frac{n_{mc}}{n_{CD}} = K \cdot \frac{V_{mc}}{V_{CD}} \tag{1}$$

where n_{CD} and n_{mc} are the total amounts of the solute included by CD and those of the solutes incorporated by the micelle, respectively, V_{CD} and V_{mc} are the volumes of CD

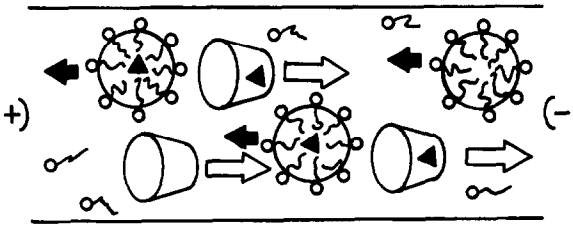

Fig. 1. Schematic illustration of the separation principle of CD-MEKC. Filled arrows indicate the electrophoretic migration of the micelle and open arrows the electroosmotic migration of CD.

and the micelle, respectively, and K is the distribution coefficient. The capacity factor is proportional to the phase (volume) ratio of the micelle to CD. The distribution coefficient means the relative affinity of the solute between CD and the micelle. The ratio of the solute incorporated in the micelle depends on its hydrophobicity but the inclusion-complex formation of the solute with CD depends on the concordance of the solute molecular size with the cavity diameter of CD in addition to the hydrophobicity. Consequently, the selectivity in CD-MEKC will be mostly determined by the tendency of the solute to form an inclusion complex with CD.

The purpose of this study was to explore the applicability of CD-MEKC to the separation of highly hydrophobic and closely related compounds, such as polychlorinated biphenyls (PCBs), tetrachlorodibenzo-*p*-dioxin (TCDD) isomers and PAHs. These compounds are generally separated by capillary gas chromatography (GC) or by HPLC. HPCE and EKC have advantages common to either of capillary GC or HPLC: (1) they require small amounts of samples as in capillary GC; (2) they give highly efficient resolution as in capillary GC; and (3) they are compatible with the analysis of biological fluids as in HPLC. Consequently, HPCE and EKC are considered to be very useful techniques for the analysis of minute amounts of biological fluids or environmental samples if sensitive detectors such as mass spectrometers or laser-induced fluorescence spectrophotometers become readily available.

EXPERIMENTAL

Apparatus

Fused-silica capillary tubes from two different commercial sources were employed to obtain different electroosmotic velocities: Scientific Glass Engineering (Ringwood, Victoria, Australia) (0.05 mm I.D., 0.25 mm O.D.) and Polymicro Technologies (Phoenix, AZ, U.S.A.) (0.05 mm I.D., 0.37 mm O.D.). The total length was 650 or 700 mm and the effective length to the detector was 500 mm for either tube.

The separation of PCBs and TCDD isomers was exclusively performed at the Toxicology Branch of the Center for Disease Control (CDC) on Chamblee Campus. A regulated high-voltage power supply laboratory-made in Northeastern University, which was operated under either constant voltage or current conditions, an ISCO (Lincoln, NE, U.S.A.) V^4 spectrophotometric detector, the cell holder of which was modified to accommodate the capillary tube for on-column detection, and a Shimadzu (Columbia, MD, U.S.A.) C-R3A data processor were employed to build an HPCE

instrument. The detector was operated at 220 nm. An HPLC system, which consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A pump, a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector and a Waters Assoc. Model 990 multi-channel absorbance detector, was used for the HPLC separation of trichlorobiphenyl isomers with Cyclobond I (β-CD bonded phase) and Cyclobond II (γ-CD bonded phase) (Advanced Separation Technologies, Whippany, NJ, U.S.A.).

The EKC separation of chlorinated benzenes and PAHs was mainly performed at Kyoto University using essentially the same apparatus as described previously[2,3]. UVIDEC-100-IV and V spectrophotometric detectors (JASCO, Tokyo, Japan), which were operated at 210 nm, and HRS-24p and HeppLL-30N regulated high-voltage power supplies (Matsusada Precision Devices, Kusatsu, Shiga, Japan), which were operated under constant-voltage conditions, were employed to construct the apparatus.

Reagents

All of the PCB congeners employed were purchased from Ultra Scientific (Kingstown, RI, U.S.A.) and used without further purification. TCDD isomers were synthesized at the Toxicology Branch of CDC[27]. Chlorinated benzene congeners and CDs from Nacalai Tesque (Kyoto, Japan) were used as received. PAHs were obtained from Accustandard (New Haven, CT, U.S.A.). Other reagents used for the preparation of the separation solutions or as solvents for the samples were of analytical-reagent grade. All of the micellar solutions were filtered through a membrane filter of 0.45-μm pore size prior to use.

Procedure

Most solutes were injected as methanol solutions by the siphoning method. When a sample was poorly soluble in methanol, tetrahydrofuran (THF) or dimethyl sulphoxide (DMSO) was added to methanol in an appropriate proportion to give a sample solution. Hexachlorobenzene was dissolved in ethanol. The capillaries were washed with DMSO or THF and with borate buffer that was used for the preparation of the micellar solution when the peaks became broad and tailed.

RESULTS AND DISCUSSION

Separation of chlorinated benzene congeners

The total number of chlorinated benzene congeners is twelve: three each for di-, tri- and tetrachlorobenzene isomers and one each for mono-, penta- and hexachlorobenzene. Mono-, di- and trichlorobenzenes were not very hydrophobic and consequently they were not totally incorporated into the sodium dodecyl sulphate (SDS) micelle as shown in Fig. 2. Three isomers of dichlorobenzenes were partially resolved, but those of trichlorobenzenes were not, although they were separated from other polychlorinated benzenes. Tetra-, penta- and hexachlorobenzenes were eluted at migration times similar to that of the micelle. The slightly lower efficiencies observed in Fig. 2, except for chlorobenzene, can probably be ascribed to slow kinetics in the partition equilibrium of the solutes[4].

The addition of 40 m*M* γ-CD to the separation solution employed in Fig. 2 permitted the separation of all of the chlorinated benzene congeners, as shown in Fig.

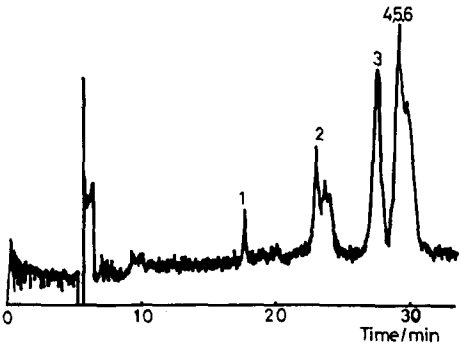

Fig. 2. MEKC separation of chlorinated benzene congeners: 1 = mono-, 2 = di-, 3 = tri-, 4 = tetra-, 5 = penta- and 6 = hexachlorobenzene. Capillary, 700 mm (Scientific Glass Engineering); separation solution, 100 m*M* SDS in 100 m*M* borate buffer (pH 8.0) containing 2 *M* urea; applied voltage, 18 kV; current 30 μA.

3. The difference in electroosmotic velocities between Fig. 2 (1.6 mm s^{-1}) and Fig. 3 (0.93 mm s^{-1}) was due to both applied voltages and fused-silica materials. Capillary tubes from Polymicro Technologies usually generated a weaker electroosmotic flow than those from Scientific Glass Engineering. When the former tubes were used under the conditions shown in Fig. 2, the migration times increased significantly and elution of peaks 3–6 was hardly observed.

The isomer separation shown in Fig. 3 can evidently be ascribed to the differential partition of the isomers to the γ-CD cavity, because these isomers are not resolved in the absence of γ-CD, as shown in Fig. 2. An isomer having a shorter migration time is included more strongly in the CD cavity than an isomer having a longer migration time, *e.g.*, 1,2,3,5-tetrachlorobenzene forms the most stable complex among the three tetrachlorobenzene isomers. As all of the congeners are not totally incorporated into the SDS micelle, the migration order observed in Fig. 3 does not always agree with the order of the stability of the inclusion complexes.

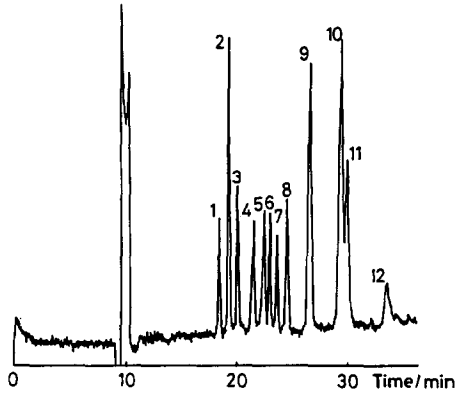

Fig. 3. γ-CD-MEKC separation of chlorinated benzene congeners: 1 = 1,2,3,5-tetra-, 2 = 1,2,3-tri-, 3 = 1,3,5-tri-, 4 = 1,2-di-, 5 = 1,2,4,5-tetra-, 6 = mono-, 7 = 1,3-di-, 8 = 1,2,4-tri-, 9 = 1,2,3,4-tetra-, 10 = penta-, 11 = 1,4-di- and 12 = hexachlorobenzene. Capillary, 700 mm (Polymicro Technologies); separation solution, as in Fig. 1 but containing an additional 40 m*M* γ-CD; applied voltage, 15 kV; current, 23 μA.

Although the cavity diameter (0.95 nm)[28] of γ-CD seems slightly too large for chlorinated benzenes, all of the congeners are included by γ-CD. In particular, tetra- and trichlorobenzenes are strongly included. The discrepancy between the cavity and molecular sizes is explained by taking into consideration that an SDS molecule is probably co-included with a cholorobenzene congener. Hexachlorobenzene, which should have the largest molecular size among the congeners, migrates with the slowest velocity, but is still included to a considerable extent, as easily judged from the fact that it was not eluted in the absence of γ-CD, as mentioned above.

Separation of trichlorobiphenyl isomers

Among 24 possible isomers of trichlorobiphenyls, eleven isomers were available at CDC for the preparation of sample solutions for this study. Although HPCE and EKC have high mass sensitivities even with a UV detector, their concentration sensitivities are not very high beause of the small detection volume. Therefore, concentrations higher than 0.1 mg ml^{-1} are required for each solute.

All of the isomers of trichlorobiphenyls migrated with the same velocity as that of the micelle in MEKC. In other words, trichlorobiphenyls were too hydrophobic to be separated by MEKC. γ-CD-MEKC allowed the complete separation of the eleven isomers, as shown in Fig. 4. The separation using β-CD instead of γ-CD was not very successful, but the HPLC separation of the isomers was better with a β-CD bonded phase column (Cyclobond I) than with a γ-CD bonded phase column (Cyclobond II). A chromatogram with Cyclobond I is shown in Fig. 5, under which conditions biphenyl had a similar retention time to peak 35 at 36.5 min.

In CD-MEKC, the elution order should agree with the tendency of the isomers to form inclusion complexes with CD in the presence of SDS; on the other hand, in HPLC using a CD-bonded phase, the isomer that interacts more strongly with CD should have a longer retention time. Consequently, the elution order must be reversed between the two separation methods. However, the observed orders are not exactly reversed between Figs. 4 and 5. This can probably be explained in terms of three factors: the difference in the cavity size of the CDs, the presence of SDS in EKC and methanol in HPLC and possible extra retention mechanisms in HPLC such as hydrophobic interactions with the spacer or linkage part of CD.

Other PCBs such as mono-, di- and tetrachlorobiphenyls and commercial PCB products were also subjected to the γ-CD-MEKC separation under the same

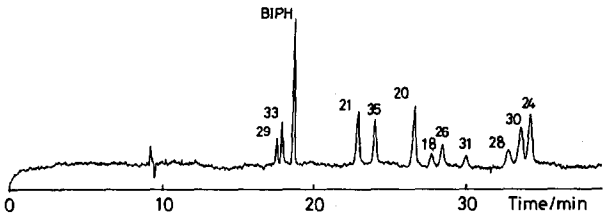

Fig. 4. Separation of eleven trichlorobiphenyl isomers by γ-CD-MEKC. Peaks are identified with the IUPAC number: 18 = 2,2',5-, 20 = 2,3,3'-, 21 = 2,3,4-, 24 = 2,3,6-, 26 = 2,3',5-, 28 = 2,4,4'-, 29 = 2,4,5-, 30 = 2,4,6-, 31 = 2,4',5-, 33 = 2',3,4- and 35 = 3,3',4-trichlorobiphenyl; BIPH = biphenyl. Capillary, 650 mm (Scientific Glass Engineering); separation solution, 60 m*M* γ-CD, 100 m*M* SDS and 2 *M* urea in 100 m*M* borate–50 m*M* phosphate buffer (pH 8.0); applied voltage, 15.4 kV; current, 50 μA.

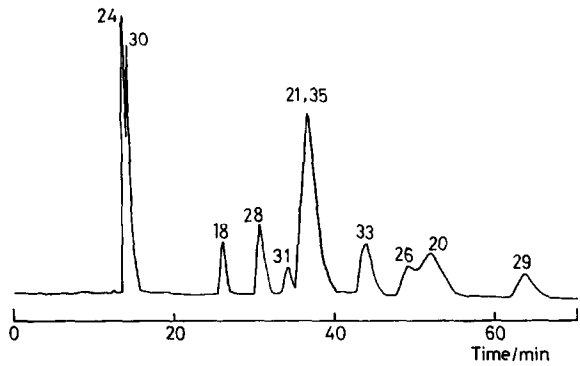

Fig. 5. HPLC separation of the trichlorobiphenyl isomers with a β-CD bonded phase column. See Fig. 4 for peak identification. Column, Cyclobond I (250 m × 4.6 mm I.D.); mobile phase, 50% (v/v) aqueous methanol; flow-rate, 0.5 ml min^{-1}; detection wavelength, 210 nm.

conditions and excellent resolution was achieved, although each peak was not identified. As the purpose of this work was to confirm the applicability of the proposed method to the separation of highly hydrophobic and closely related compounds such as PCBs, we did not study extensively PCB analysis by CD-MEKC.

Separation of tetrachlorodibenzo-p-dioxin isomers

Three pairs of TCDD isomers that were hardly resolved even by capillary GC were employed as samples together with some other TCDD isomers to investigate the selectivity of closely related compounds by CD-MEKC. Examples of the TCDD pair separations are shown in Fig. 6. The complete separation of each pair was easily achieved under the same conditions as employed for the PCB analysis. In particular, 1,2,4,6- and 1,2,4,9-TCDD isomers were extremely difficult to resolve by capillary GC. Although the peaks are broad and tailed in Fig. 6c, sharp and symmetrical peaks were obtained when the capillary was washed with THF. The washing with THF increased the electroosmotic velocity and hence reduced the migration times but with slightly poorer resolution.

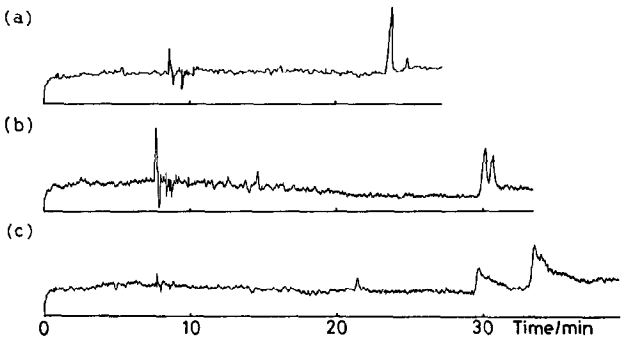

Fig. 6. γ-CD-MEKC separation of three pairs of closely related TCDD isomers: (a) 1,2,3,6- and 1,2,3,7-TCDD; (b) 1,2,6,7- and 1,2,8,9-TCDD; (c) 1,2,4,6- and 1,2,4,9-TCDD. Each peak was not identified. Conditions as in Fig. 4.

The identification of each peak was impossible because one or other compound of each pair was not available in an isolated form. 1,2,3,4- and 1,4,7,8-TCDD isomers were eluted at close migration times of 27.0 and 27.7 min under the conditions shown in Fig. 6. The most toxic isomer of TCDD, 2,3,7,8-TCDD, was poorly soluble in most organic solvents that were miscible with water, such as methanol, DMSO and THF, and therefore it was impossible to analyse it by CD-MEKC.

Some polychlorinated naphthalenes were also subjected to CD-MEKC, although extensive analyses were not performed. With TCDD isomers or polychlorinated naphthalenes, γ-CD generally gave better results than β-CD.

Separation of polycyclic aromatic hydrocarbons

Preliminary experiments on the separation by CD-MEKC of sixteen PAHs included in the list of priority pollutants showed a chromatogram which had more than fourteen peaks. Fig. 7 shows the separation of a mixture of naphthalene and four tricyclic and three tetracyclic aromatic hydrocarbons by γ-CD-MEKC. The elution order means that smaller PAHs are more easily included in the CD cavity or the larger PAHs are incorporated by the SDS micelle to a greater extent.

CONCLUSION

As this is preliminary work, we have not yet extensively studied the separation conditions for optimizing the resolution. The separations shown are not always satisfactory for the purpose of environmental or biological analysis. However, the results strongly suggest the potential of CD-MEKC as a microanalytical method for highly hydrophobic compounds.

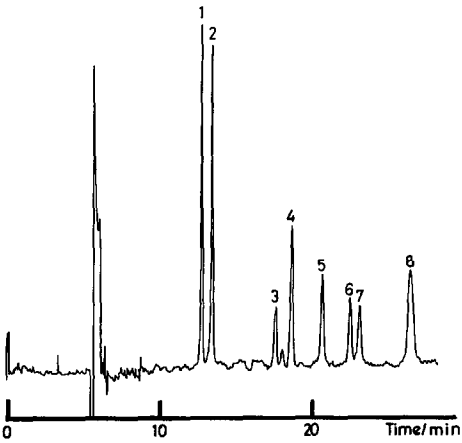

Fig. 7. γ-CD-MEKC separation of a mixture of naphthalene and four tricyclic and three tetracyclic aromatic hydrocarbons: 1 = naphthalene; 2 = acenaphthene; 3 = anthracene; 4 = fluorene; 5 = phenanthrene; 6 = chrysene; 7 = pyrene; 8 = fluoranthene. Capillary, 700 mm (Polymicro Technologies); separation solution, 30 m*M* γ-CD, 100 m*M* SDS and 5 *M* urea in 100 m*M* borate buffer (pH 9.0); applied voltage, 20 kV; current, 41 μA.

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