NEUTRAL CYCLODEXTRIN MODIFIED/MICELLAR ELECTROKINETIC CHROMATOGRAPHIC (CD/MEKC) SEPARATION OF POLYCHLORINATED DIBENZO-P-DIOXINS (PCDDS) AND POLYCHLORINATED BIPHENYLS (PCBS)

S. Terabe, Y. Miyashita, and O. Shibata
Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Sakyo-ku, Kyoto 606, Japan

D.G. Patterson Jr., E.R. Barnhart, and L.R. Alexander
Division of Environmental Health Laboratory Sciences, Centers for Disease Control, Atlanta, Georgia 30333

B.L. Karger
Barnett Institute, Northeastern University, Boston, Massachusetts 02115

K. Hosoya and N. Tanaka
Department of Polymer Science and Engineering, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606, Japan

ABSTRACT

Cyclodextrin modified micellar electrokinetic chromatography was developed for the separation of highly hydrophobic compounds. The technique has been able to separate isomers which coelute by other techniques.

KEYWORDS: PCDDs, PCBs, Electrokinetic Chromatography

INTRODUCTION

In 1989 a technology exchange project (no. 01044081) funded by the Monbusho International Scientific Research Program was initiated under the direction of Dr. Nobuo Tanaka. We have pursued areas of mutual interest involving the separation, identification, and quantification of compounds which are of environmental concern and which potentially pose health risks to the general public. These areas of investigation include the use of newly synthesized aromatic stationary phases for reversed-phase liquid chromatography as well as electrokinetic chromatography (EKC) with fused silica capillary tubing for separating closely related groups of compounds. It is the latter area of EKC which will be the focus of this presentation.

Electrokinetic chromatography was developed and named by Shigeru Terabe (1,2) and micellar EKC (1,3-6), cyclodextrin EKC (2), ligand-exchange EKC (7), borate-complex EKC (8,9), and ion-exchange EKC (2,10) have been reported. The EKC technique is well suited to the separation of highly hydrophobic neutral compounds. The purpose of this study was to examine the applicability of the CD/MEKC technique to the separation of hydrophobic compounds such as PCBs, PCDDs, PAHs, and other environmental toxicants.

EXPERIMENTAL

Electrokinetic Chromatographic Apparatus

The apparatus consisted of microbore vitreous silica tubing [650 mm, 0.05 mm (i.d.)], a regulated high voltage power supply, and a spectrophotometric UV detector which was modified with a slit (0.05 mm x 0.75 mm) for the accommodation of the capillary tube. Each end of the capillary tube was placed in separate buffered micellar-electrophoretic solutions. The vials of solutions were covered with silicone-rubber caps, each with two small holes, one for a platinum electrode and the other for the capillary tube. On-column UV detection (220 mm) occurred at a position 150 mm from the negative end of the capillary by removing the polymer coating at this point.

Sample Injection

The capillary tube is filled with sodium dodecylsulfate (SDS) solution with a microsyringe and the ends of the capillary placed in their respective vials of solution. The vial containing the sample to be analyzed is held 4 cm above the level of the terminal negative electrode vial. The positive end of the capillary is then removed from its solution vial and inserted into the sample vial. The sample solution is siphoned into the capillary for about 30 seconds. This sampling time was adequate for aromatic compounds (5-7 ng/uL) and resulted in a sample injection volume of 1 nL (the total volumn of the capillary is about 1 uL). The positive end of the capillary is then removed from the sample vial and placed in its respective solution vial. The voltage is then applied to the capillary (typically 15 kV) and the electrokinetic chromatogram recorded.

Principle of the Separation Method

The principles involved in the separation of neutral compounds using the various modifications of EKC have been described in detail by Professor Terabe in several articles (1,2,11) and only a brief description will be given here. The inside walls of fused silica capillaries under neutral PH conditions will carry a negative charge. Consequently, as is illustrated in Figure 1, the liquid adjacent to the negative charge will have a net positive charge (when an anionic surfactant is used) and will migrate to the negative electrode (electroosmotic flow). The anionic micelle (SDS) will migrate to the positive electrode (electrophoretic flow).

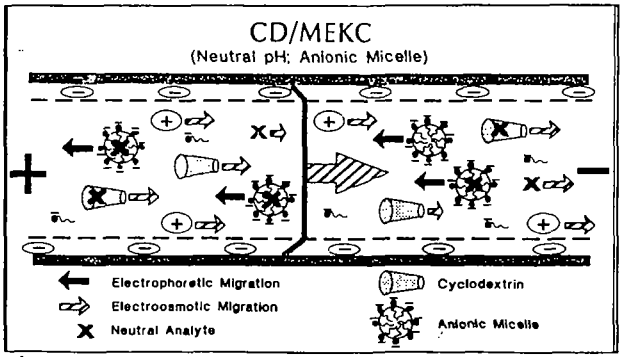

Figure 1. An Illustration of the Separation Principle of Cyclodextrin Modified/Micellar Electrokinetic Chromatography with Neutral PH Conditions and an Anionic Micelle

If near neutral pH conditions are used, the electroosmotic flow is stronger than the electrophoretic velocity of the anionic micelle and all solutes will move toward the negative electrode. However, the anionic micelle migrates electrophoretically with a different velocity from the bulk solution. In MEKC the separation is based on the differential partitioning of the solute between the micelle and the aqueous phase and successful separations have been achieved using this technique (1,3-6). However, compounds which are extremely hydrophobic are usually completely incorporated into the micelle and no separation is possible. A modification of the MEKC technique involving the addition of neutral cyclodextrin (CD) to the micellar solution has been successful in separating highly hydrophobic compounds (11). The neutral CD has a hydrophilic outside surface and will not interact with the micelle but instead will migrate with the bulk solution. Hydrophobic compounds will partition between CD and the micelle instead of between the micelle and the aqueous phase. Molecular size and hydrophobicity are important considerations in the formation of inclusion-complexes with CD and structurally similar compounds can be separated using this technique.

RESULTS AND DISCUSSION

The CD/MEKC technique has successfully separated all twelve of the mono to hexachlorobenzenes, as well as congeners of PCDDs, PCBs, PCNs, and PAHs (11). In this short abstract a brief description of the PCDD and PCB separations will be presented.

Separation of Polychlorinated Dibenzo-p-dioxins

As a preliminary experiment to investigate the selectivity of CD/MEKC for closely related isomers, we analyzed several pairs of TCDDs which are not separated by capillary column GC. The 1246/1249-TCDD pair was easily separated by the conditions shown in Figure 2 as well as the 1236/1237 and 1267/1289-TCDD pairs. Other PCDDs were also separated but cannot be presented here.

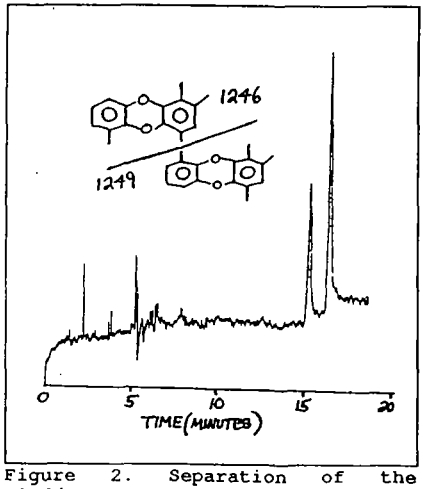

Figure 2. Separation of the 1246/1249-TCDD pair by γ-CD/MEKC. Capillary, 650 mm(Scientific Glass Engineering); separation solution, 60 mM γ-CD, 100mM SDS and 2 M urea in 100 mM borate buffer (PH8.0), applied voltage 18.0 kV, 56.5 μA.

Separation of Polychlorinated Biphenyls

Mono, di, tri, and tetrachlorobiphenyls have been separated by CD/MEKC. An example of the separation that can be achieved by this technique is illustrated in Figure 3. Eleven trichlorobiphenyls were completely separated using γ-CD modified MEKC.

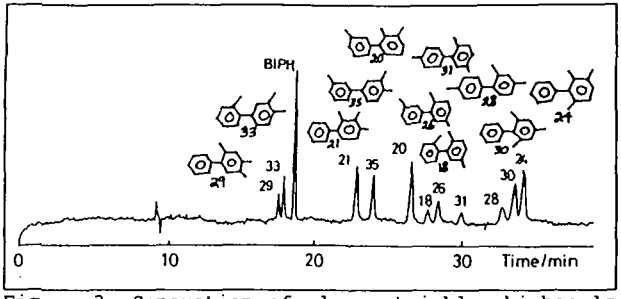

Figure 3. Separation of eleven trichlorobiphenyls by γ-CD/MEKC. Capillary, 650mm (Scientific Glass Engineering); separation solution, 60mM γ-CD, 100mM SDS and 2M urea in 100mM borate-50mM phosphate buffer (PH 8.0), applied voltage 15.4 kV, 50μA.

SUMMARY AND CONCLUSIONS

The CD/MEKC technique shows good potential as a microanalytical method for highly hydrophobic compounds. Studies to determine what factors affect the separations as well as applications to other environmental toxicants remains to be done. We have plans to interface this technique with our VG 4-sector mass spectrometer.

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