SEPARATION OF ENVIRONMENTAL TOXICANT ISOMER GROUP COMPONENTS BY γ-CYCLODEXTRIN MODIFIED/MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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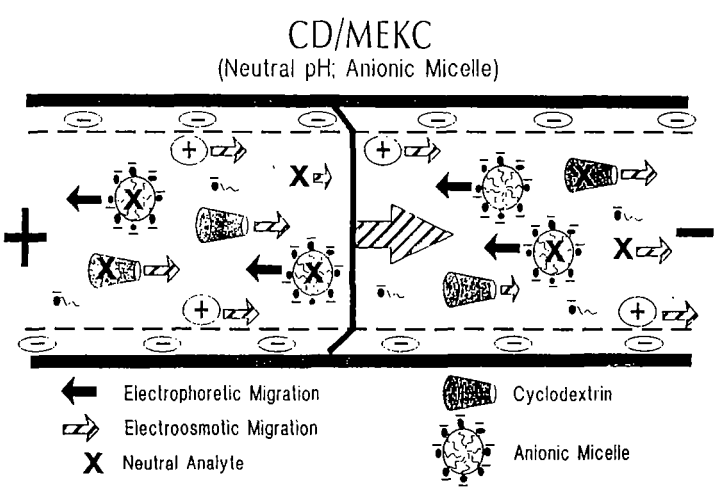
INTRODUCTION

Separation of neutral organic environmental toxicant mixtures and the identification and quantification of their component analytes has been the primary focus of environmental/analytical laboratories. Although the traditional approach has involved separations by gas chromatography and high performance liquid chromatography (HPLC), cyclodextrin-modified micellar electrokinetic chromatography (CD/MEKC)[1-8] has demonstrated the capacity for a formidable range of analyte separations since its introduction in 1989 by Terabe. High theoretical plate counts, picogram sample quantities and the broad range of variable parameters available for mixture component resolution make CD/MEKC the method of choice for an increasing number of applications. This method is particularly useful for separating mixtures of neutral analytes. For the environmental toxicant groups under consideration,

Figure 1. SEPARATION DYNAMICS FOR CYCLODEXTRIN MODIFIED MICELLAR ELECTROKINETIC CHROMATOGRAPHY.



separations for polyaromatic hydrocarbons[1,3,5,7] (PAHs) and trimethylbiphenyl isomers[1] have been reported by several groups of investigators. CD/MEKC separation methodology for organic neutal isomer/congener resolution has subsequently evolved as a very powerful chromatographic technique. Separations have been reported for complex PAH mixtures (generally 6 to 16 toxicant standard mixture components) and for chlorinated dibenzodioxins[10] and other environmental toxicant groups. Cyclodextrins, which function as isomer or congener selectors (based on the relative affinity of the analyte for CD inclusion complex formation) in the differential portitioning with sodium dodecylsulfate (SDS), have also been extensively utilized as chiral selectors.[4] In this work, γ-CD/MEKC is employed in the separation of mixture components for several environmental toxicant groups (coplanar biphenyls, dimethylnaphthalenes and mixtures of 22 and 31 polyaromatic hydrocarbons). The 100 mM borate buffer with 100 mM SDS at pH 9 appears to facilitate the separation of a broad class of neutral environmental toxicants, with specific group component resolution improved with cyclodextrin concentration variations.

EXPERIMENTAL

Analytes and Reagents. Dimethylnaphthalene isomers (Aldrich, Milwaukee, WI), the coplanar biphenyls (Accustandard, New Haven, CT) and polyaromatic hydrocarbons (Accustandard, New Haven, CT) were injected hydrodynamically in ethanol. Sodium borate, sodium dodecyl sulfate (SDS) and urea were obtained from Sigma (St. Louis, USA). Gamma-cyclodextrin (γ-CD) was obtained from Astec (Whippany, NJ, USA). Coplanar PCB congener structures were confirmed by GC/FTIR and ^{13}C NMR.

MEKC Instrumentation. Electrokinetic chromatography was performed on a Spectraphysics (Palo Alto, CA) Phoresis 1000 system with a variable temperature oven and a variable wavelength UV detector. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ) used for electrophoretic separations were 50 μ (id) X 44 cm. The 100 mM borate buffer (pH 9) contained 100 mM sodium dodecyl sulfate (SDS), 20 to 40 mM gamma-cylodexrin (r-CD) and 5 M urea to facilitate CD solubility. The analytes were injected hydrodynamically in ethanol solutions with ~20% 1,4-dioxane added to increase the solubility of more highly chlorinated congeners.

Spectroscopic Instrumentation. A Nicolet (Madison, WI) Model 170SX Fourier transform infrared (FTIR) spectrometer equipped with a mercury-cadmium-telluride (MCT) detector was used to generate infrared spectra. Chromatographic separations were performed by a Hewlett-Packard (Palo Alto, CA) model 5880A gas chromatograph containing a J & W Scientific (Rancho Cordova,CA) 30m X .52mm fused silica capillary column. A Varian (Palo Alto, CA) Model 300 XL NMR spectrometer was used to obtain ^{13}C NMR spectra for PCB congeners. Spectra were collected in acetone-d_6 at 30° C. Chemical shifts relative to TMS were calculated by referencing the residual acetone signal at 2.050 ppm.

RESULTS AND DISCUSSION

A diagram showing gamma-CD/MEKC analyte separaration dynamics is presented in Figure 1. Electrophrograms delineating the separation of environmental toxicant group components are presented in Figures 2-5. For the environmental toxicant groups examined, only coplanar polychlorinated biphenyl congener assignments were confirmed by off-line systematic techniques involving Fourier transform infrared (FTIR) and carbon-13 nuclear magnetic

resonance (^{13}C NMR) spectroscopy. Separation of Polyaromatic Hydrocarbons. Twenty of the 22 PAH mixture components are separated (Figure 2) using 22mM gamma-CD at 15kV and 15C. Determination of migration order in CD/MEKC can be a rather complex exercise involving the differentional partitioning between the two types of hydrophobic domains (micelles and cyclodextrins) as a function of partition coefficients, inclusion complex formation, hydrophobicities, and molecular geometries. Since all the PAHs are aromatic hydrocarbons (some with aliphatic substituents) with no other functional groups containing heteroatoms, one might generally project that smaller molecules with more linear geometries would encounter fewer barriers to cyclodextrin inclusion complex formation and exhibit shorter migration times than would more bulky fused aromatic skeletons. Figure 2 shows naphthalene, 1-methyl naphthalene, 2 methylene and trimethyl naphthalene are components 2, 5, 8, and 10 in the migration order, while the very bulky benzo(g,h,i)perelene molecule, acenaphthalene, acenapthene, anthracene, perelene and benzo(a)pyrene are components are 1, 3, 4, 6, 7, and 9 in the PAH mixture migration order.

Separation of Dimethylnaphthalenes and Polyaromatic Hydrocarbons.
Separation of a nine component dimethylnaphthalene (DMN) isomer mixture at 15 kV and 15°C (Figure 3) resulted in 8 baseline resolved peaks in 26 minutes. Under these conditions, all the DMN isomers except the 2,3- and 1,6-DMN isomers are separated. Separation of all nine DMN components in a 50μ X 70 mm column has been reported using the same buffer with 70 mM gamma cyclodextrin.[4] An electropherogram delineating the separation of a 31 component PAH mixture (containing the 22 PAH analytes shown in Figure 2 and the 9 DMN analytes shown in Figure 3) is presented in Figure 4. Twenty-seven peaks are observed for the 31 components with 2,3-DMN and 1,6-DMN comigrating as was observed in the DMN isomer mixture with pyrene and perylene comigrating as was observed in the PAH mixture.

Separation of Coplanar Polychlorinated Biphenyls
Seventeen peaks are observed (Figure 5) for the 18 component coplanar PCB mixture. Preliminary structural assignments for PCB congeners indicate that only 3,4'5-TrCBP and 3,3',4,4',5,5'-HxCBP comigrate under the conditions employed. Although structure/migration correlations appear to be complicated, the analytes with the higher migration velocities appear to contain one 3,5-dichlorinated ring while the analytes with lower migration velocities contain one 3,4-dichlorinated or one 4-chlorinated ring.

Figure 2. CD/MEKC SEPARATION OF A 22 COMPONENT POLYAROMATIC HYDROCARBON MIXTURE.

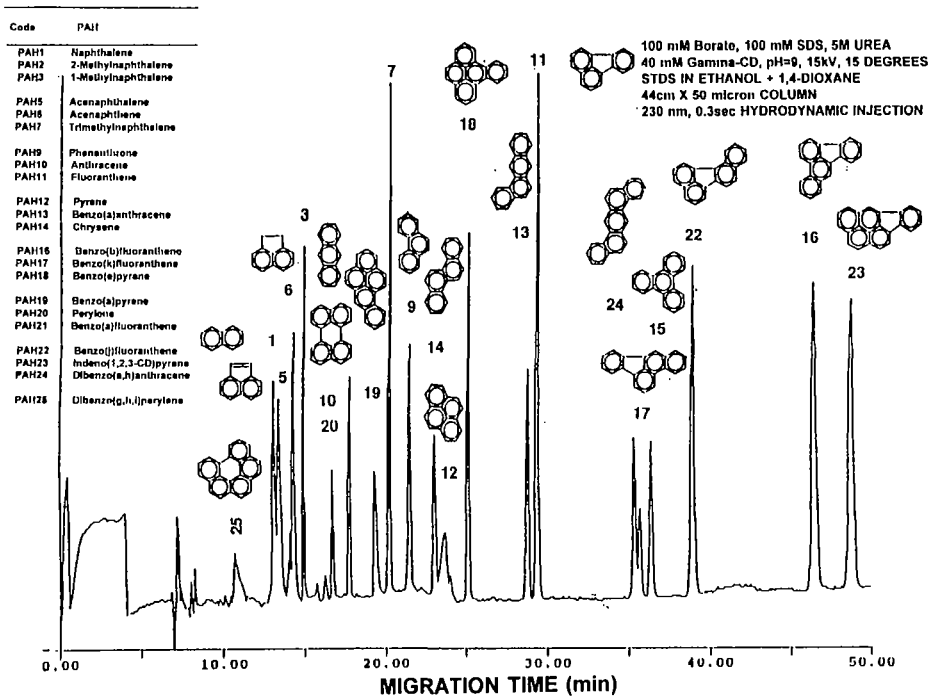


Figure 3. CD/MEKC SEPARATION OF A 9 COMPONENT DIMETHYLNAPHTHALENE MIXTURE.

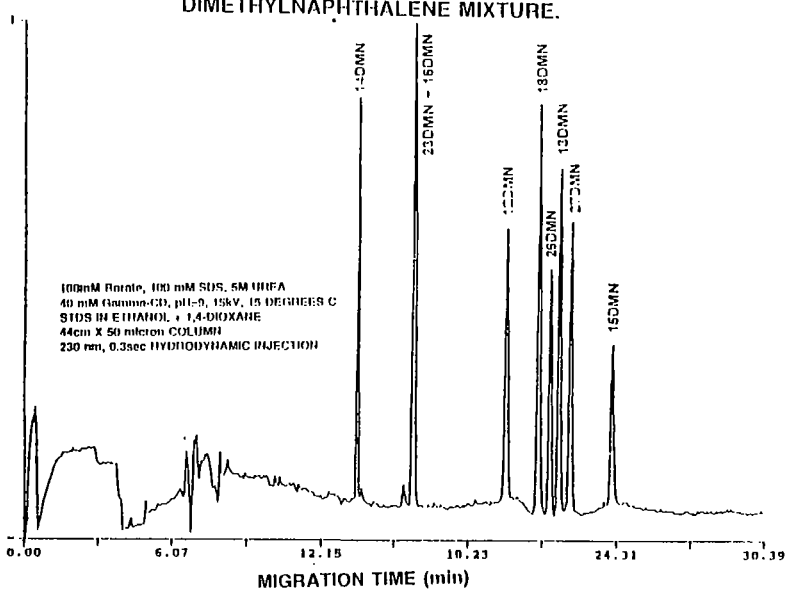


Figure 4. CD/MEKC SEPARATION OF A 31 COMPONENT POLYAROMATIC HYDROCARBON MIXTURE.

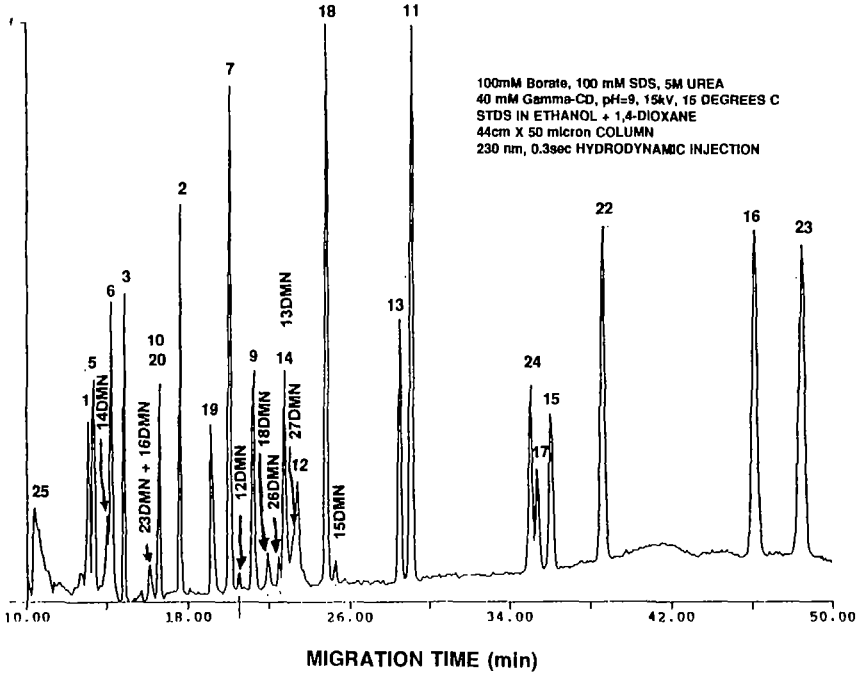
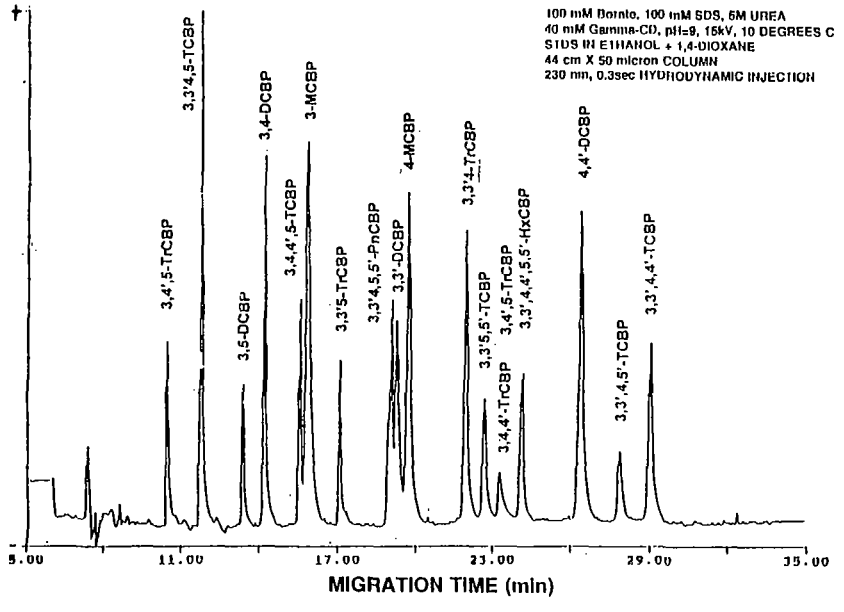


Figure 5. CD/MEKC SEPARATION OF AN 18 COMPONENT COPLANAR POLYCHLORINATED BIPHENYL MIXTURE.



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