THE USE OF CYCLODEXTRIN MODIFIED MICELLAR ELECTROKINETIC CHROMATOGRAPHY FOR MONITORING HUMAN EXPOSURE TO ENVIRONMENTAL TOXICANTS

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INTRODUCTION

In micellar electrokinetic capillary chromatography, a micellar pseudo stationary phase, such as sodium dodecyl sulfate, and an aqueous buffer as the mobile phase are used to separate both charged and neutral molecules. An untreated fused silica capillary surface is negatively charged, so that the bulk electroosmotic flow is toward the negative electrode, whereas the micelles, formed from an anionic surfactant, migrate in the opposite direction, toward the positive electrode. However, when standard micellar electrokinetic chromatography (MEKC) conditions are used with anionic micelles, the dominant electroosmotic flow still drives the negatively charged micelles toward the negative end of the capillary.

Species having the same charge as that of the micelle do not interact with the micelle, while those having the opposite charge strongly interact with the micelle. The separation of charged species depends on the species' difference in electrophoretic mobility. On the other hand, the formation of micelles provides a unique chromatographic process for the separation of neutral molecules, whereby solute liquid-liquid differential partitioning between the micellar pseudo stationary phase and the electroosmotically pumped aqueous phase takes place[1]. Several published articles have described applications of MEKC for the separation of neutral molecules[2-4] and of charged species[5,6]. MEKC has become a popular microcolumn separation technique because of its high separation efficiency. Isotopically substituted compounds[7] and chiral molecules[8] have been separated by MEKC when organic modifiers were added to the mobile phase.

The high resolution of MEKC requires a small sample-injection volume. Small sample volumes, however, make the detection of low concentration samples difficult. For example, in the case of UV absorbance detection, typical concentration limits of detection (LOD) are on the order of 10^{-6} M,[9] which is inadequate for the analysis of low-concentration constituents in biological samples, such as dioxins in human serum.

Sample stacking of charged species has been used extensively in many areas of electrophoresis[9-14] to enhance the sensitivity of the measurements. A simple technique for on-column sample concentration of neutral molecules by using field-amplified sample stacking in MEKC media has produced a 75-85 fold increase in sensitivity for *tetrachlorodibenzo-p-dioxins*.[15] The addition of neutral gamma-cyclodextrin molecules (γ-CD) to the MEKC buffer system also greatly enhances the separation of highly hydrophobic neutral compounds such as PCDDs, PCDFs, PCBs, and PAHs.[3,16]

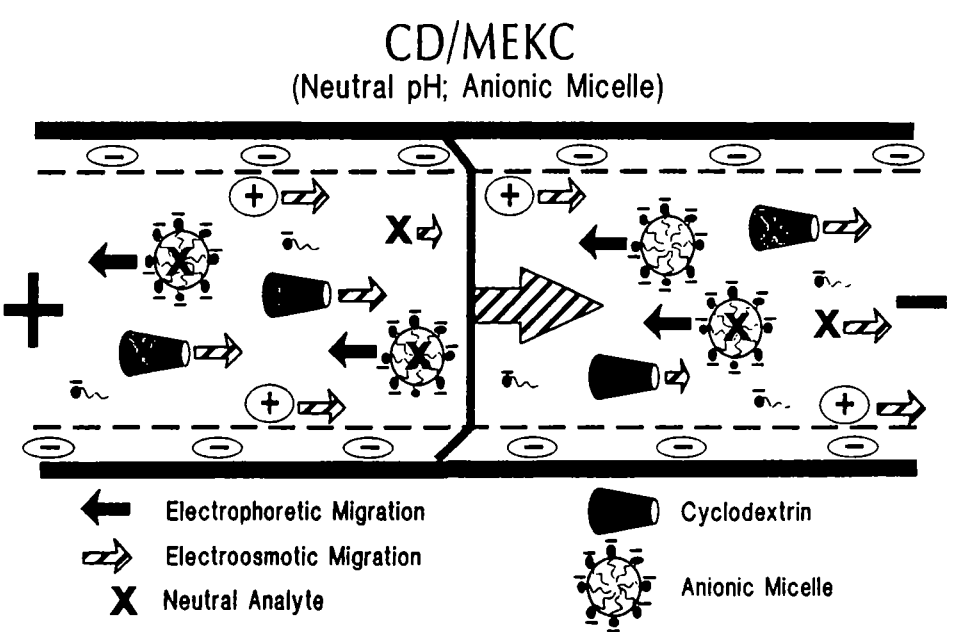
EXPERIMENTAL

An electrophoresis system similar to that described by Jorgenson and Lukacs[17] was constructed in our laboratory. The high-voltage power supply (0-60 kV) was from Glassman High Voltage Inc. (Whitehouse Station, N.J., USA). Each end of the electrophoretic capillary was placed in a small glass reservoir containing the appropriate buffer and a platinum electrode connected to the power supply. The two reservoirs must be level for normal running conditions. A CV[4] UV detector from Isco (Isco Inc., Lincoln, NB, USA) was operated at 230 nm, and the UV absorbance was recorded by a Shimadzu C-R3A integrator (Kyoto, Japan). An on-column optical detection cell was created by removing the polyimide coating from a short segment of the fused silica capillary (10 cm from one end). The system, except for the detector and the integrator, was enclosed in a Plexiglass box with an interlock switch to protect analysts from high voltages. Timing and switching of the applied high voltage were accomplished by using a timer and a high-voltage relay, which was enclosed in a lead-impregnated plastic box to protect the operator. For some separations a Beckman P/ACE 2100 or a Spectra-Physics Electrophoresis 1000 System with a UV absorbance detector was also used. Fused silica capillary columns (50-μm i.d. and 360-μm o.d.) were purchased from Polymicro Technologies (Phoenix, AZ, USA) and used without surface modification. Ultrapure sodium dodecylsulfate (SDS) was from Life Technologies Inc. (Gaithersburg, MD, USA). The polychlorinated dibenzo-p-dioxins (PCDDs) were synthesized in our laboratory[18]. All running and sample buffers were prepared with distilled water and filtered through a 0.45-μm disk filter (Alltech Associates, Inc., Dearfield, IL, USA).

RESULTS AND DISCUSSION

The principle of the CD/MEKC separation technique is shown in Figure 1. The neutral hydrophobic compounds are separated based on their partitioning coefficients among the aqueous, cyclodextrin, and micelle phases.[16]

FIGURE 1



The separations of nonchloro-through pentachlorodibenzo-p-dioxins by CD/MEKC are shown in Figures 2-5.

FIGURE 2

GAMMA-CD/MEKC SEPARATION OF DPD, MONO-, AND DICHLORINATED DIBENZO-p-DIOXIN ISOMERS USING 100 mM BORATE BUFFER

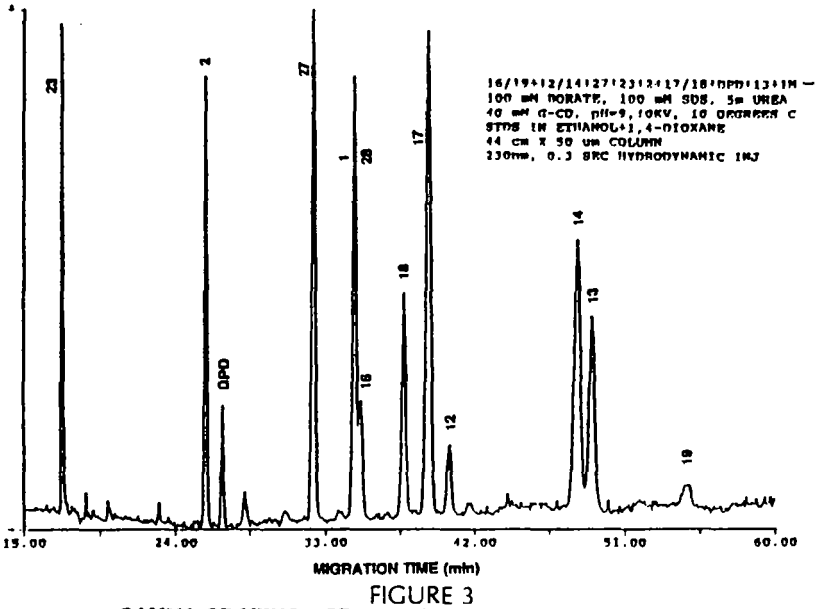


FIGURE 3

GAMMA-CD/MEKC SEPARATION OF TRICHLORINATED DIBENZO-p-DIOXIN ISOMERS USING 100 mM BORATE BUFFER AT 15kV AND 18 DEGREES

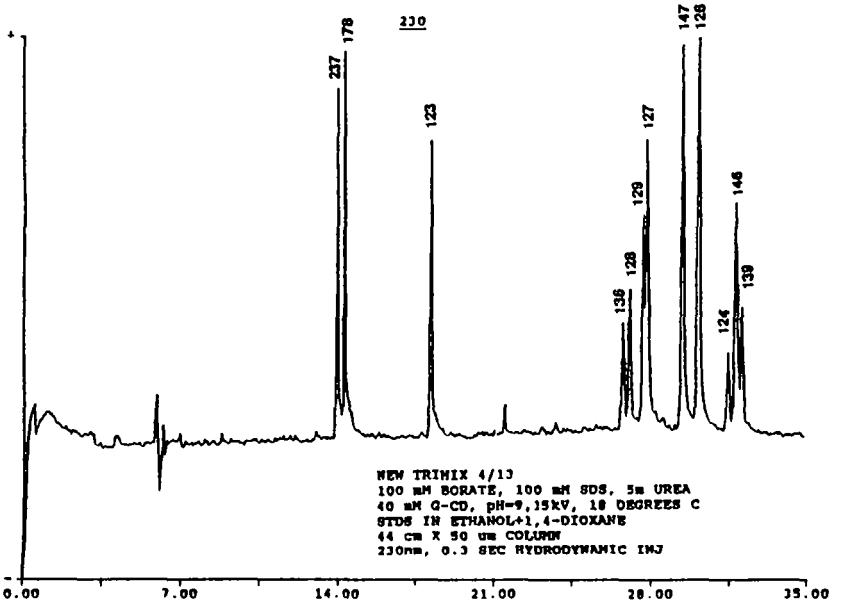


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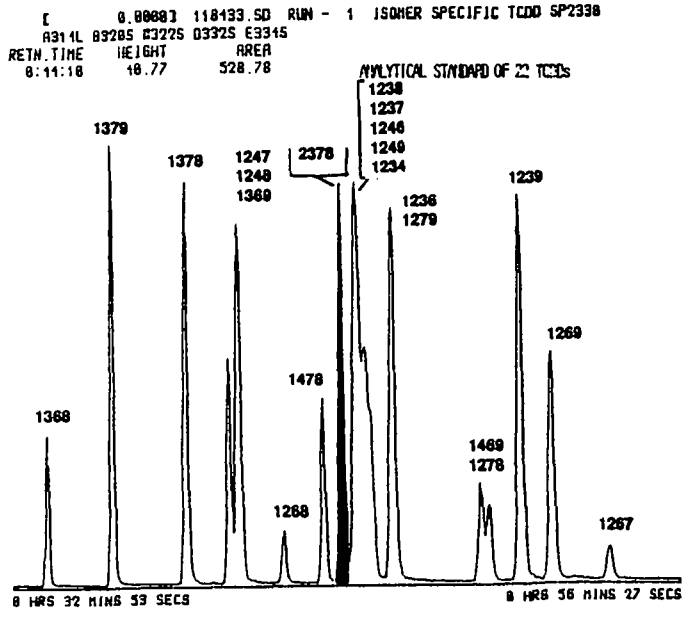
FIGURE 4
COMPARISON OF A GC/MS RECONSTRUCTED CHROMATOGRAM FOR THE 22 TCDD ISOMERS WITH A GAMMA-CD/MEKC ELECTROPHEROGRAM



GAMMA- CD/MEKC SEPARATION OF TETRACHLORO-DIBENZODIOXIN ISOMERS USING 100 mM BORATE BUFFER

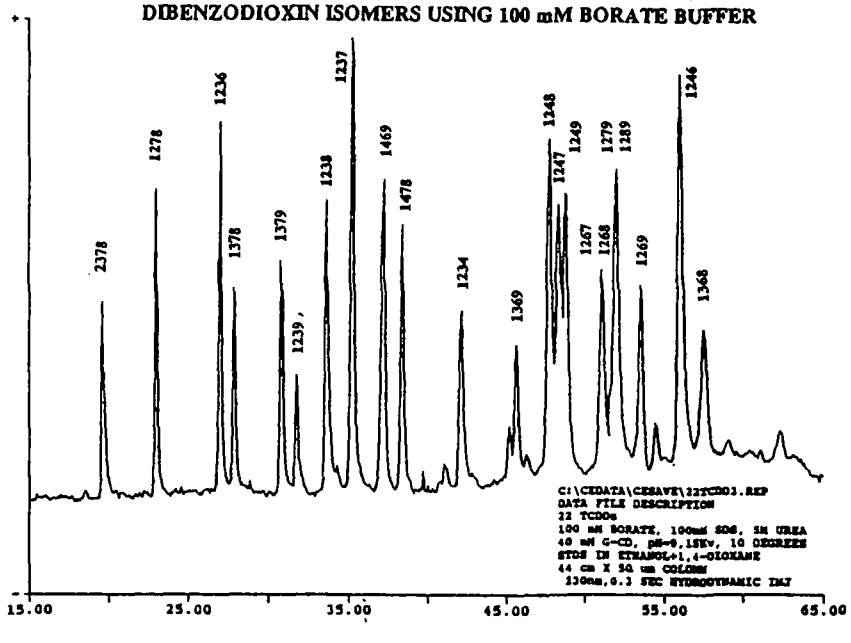
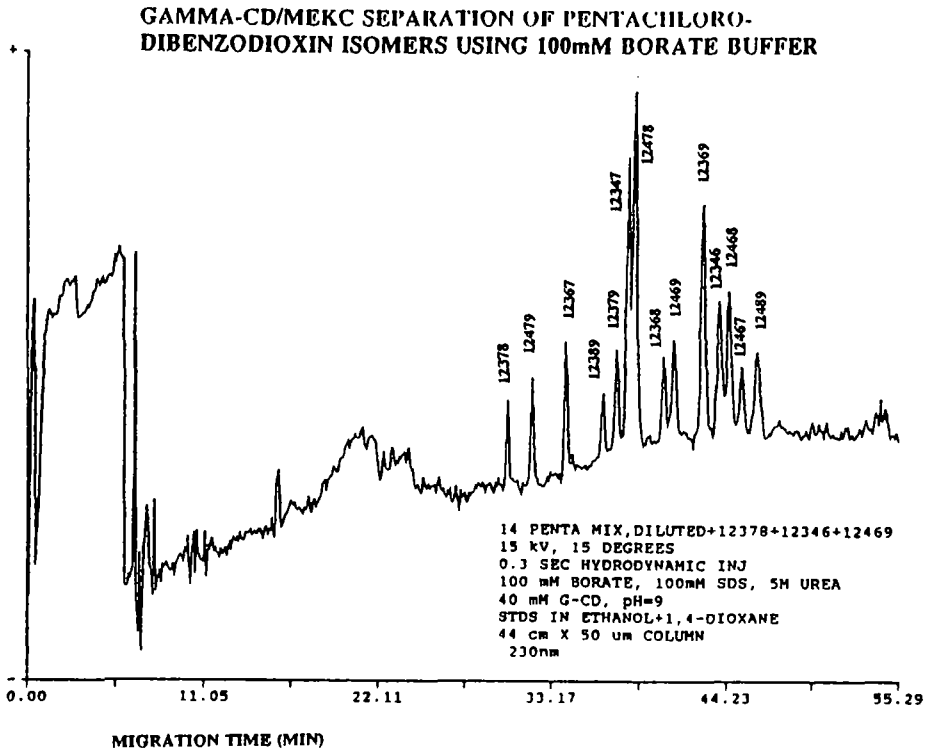


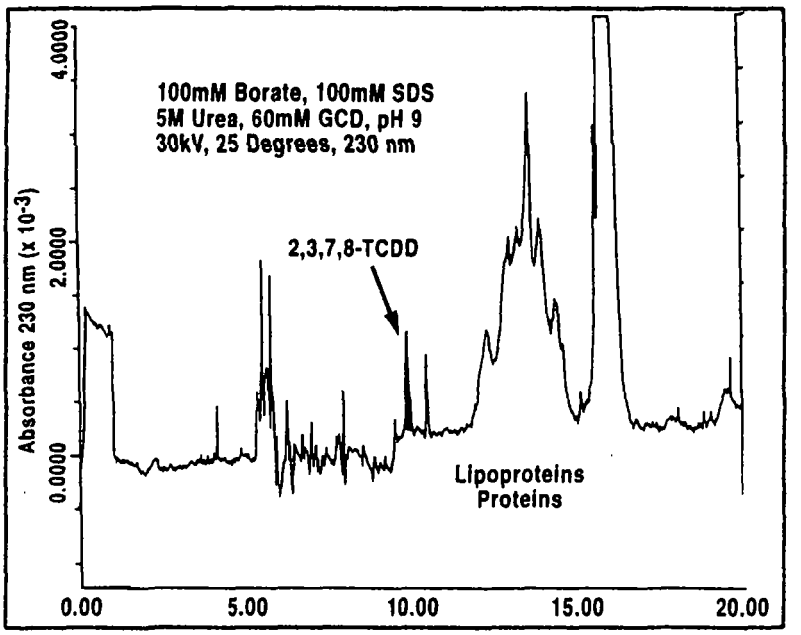
FIGURE 5



The nonchloro and two monochlorodibenzo-p-dioxin congeners are separated under the conditions shown in Figure 2. The ten dichloro- congeners are separated into 8 peaks with two unresolved pairs. Figure 3 shows that 12 of the 14 trichloro congeners are separated while 20 of the 22 tetrachloro congeners are separated as shown in Figure 4. All 14 of the pentachloro congeners are separated by the conditions shown in Figure 5. The number of resolved peaks using CD/MEKC is much larger than by any single capillary GC column.[19] There are 20 TCDD peaks by CD/MEKC (Figure 4) as compared to 12 peaks on a DB-5 and 15 peaks on a SP-2331 capillary GC column. The CD/MEKC separation of the PnCDDs produced 14 peaks (Figure 5) while the DB-5 and SP-2331 GC columns produced 11 peaks. In addition, the 2,3-DCDD; 2,3,7-TrCDD; 2,3,7,8-TCDD; and 1,2,3,7,8-PnCDD congeners are each separated isomer specifically and elute first in their respective congener groups (Figures 2-5).

Serum can be injected directly onto the capillary column without any cleanup using the CD/MEKC technique. The isomer specific determination of 2,3,7,8-TCDD spiked human serum by direct injection onto the capillary column with UV detection is shown in Figure 6. The 2,3,7,8-TCDD peak represents 50 pg injected on-column and elutes isomer specifically in about 10 minutes in a window just ahead of the serum protein/lipoprotein components. We are currently developing laser induced fluorescence detection and a mass spectrometer interface to improve the sensitivity of the technique. The CD/MEKC technique shows great promise for the rapid analysis of biological samples for environmental toxicants such as PCDDs, PCDFs, PCBs, and PAHs.

FIGURE 6
Direct Injection of 2,3,7,8-TCDD Spiked Human Serum by CD/MEKC



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