ISOMER-SPECIFIC ISOLATION OF 2,3,7,8-TCDD FROM SPIKED HUMAN SERUM BY CYCLODEXTRIN/MICELLAR CAPILLARY ELECTROPHORESIS

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INTRODUCTION

The development and application of analytical methods for measuring environmental toxicants in humans are important for biomonitoring and risk assessment. Cyclodextrin-modified micellar electrokinetic chromatography (CD/MEKC) is useful for the separation of electrically neutral, highly hydrophobic compounds including persistent organic pollutants. The separation of such substances is generally difficult by conventional electrophoretic techniques.

In micellar electrokinetic capillary chromatography (MEKC), a micellar pseudo stationary phase, such as sodium dodecyl sulfate(SDS), and an aqueous buffer as the mobile phase are used to separate both charged and neutral molecules. 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. 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]. The addition of neutral gamma-cyclodextrin molecules (γ-CD) to the MEKC buffer system greatly enhances the separation of highly hydrophobic neutral compounds such as PCDDs, PCDFs, PCBs and PAHs[2,3]. The neutral hydrophobic compounds are separated based on their partitioning coefficients among the aqueous, cyclodextrin and micelle phases [3]. Dioxins are very hydrophobic molecules. When exposed to aqueous buffer, dioxins migrate into the micelles and cyclodextrins just as in serum where they associate with the lipoprotein fraction.

Our goal was the electrophoretic separation of the most toxic dioxin isomer, 2,3,7,8-TCDD, from serum protein/lipoprotein constituents which would enable screening exposed populations if detection limits can be lowered through laser-induced fluorescence, sample stacking or high-resolution mass spectrometric detection.

EXPERIMENTAL

Reagents

The polychlorinated dibenzo-p-dioxins (PCDDs) were synthesized in our laboratory. Standards were prepared in ethanol (Pharmco, USA) or a 1:1 ethanol/1,4-dioxane mix. Serum was from a pool obtained from 300 Atlanta, Georgia residents acquired from the local Red Cross. It has been characterized by mass spectrometric techniques and the 2,3,7,8-TCDD level was below 10 parts-per-quadrillion which is well below detection limits by UV.

Apparatus

The experiments were performed with a Beckman P/ACE or a SpectraPhysics 1000 Capillary Electrophoresis system with UV detection at 230-nm using a fused-silica capillary (44 cm X 50-um I.D., 36.5 cm from the buffer inlet reservoir to the detector). Fused silica capillary (50-um i.d. and 360-um o.d.) was purchased from Polymicro Technologies (Phoenix, AZ, USA) and used without surface modification. Injections were hydrodynamic at 0.3 second (0.5 psi). All experiments were run at 10^0 or 25^0 C in constant-voltage mode at 15kv and 30kv.

Background electrolyte

All run buffers were prepared with distilled water and filtered through a 0.2-um Nalgene disk filter (Baxter Diagnostics, GA,USA). A 50 and 100 mM Borate buffer (Sodium tetraborate decahydrate, Aldrich, USA) prepared with distilled water was used for all experiments. A 50 mM Boric acid solution (Sigma Chemical Company, USA) was used to adjust the pH to 9. Urea (5M, Sigma Chemical Co.) and Sodium Dodecyl Sulfate (SDS, 100mM, Sigma Chemical Co.) were added to all buffer solutions. The following γ-CD (Advanced Separation Technologies, NJ, USA) concentrations were also added to the mixed buffer: 15mM, 40mM, 50mM and 60mM.

Procedure

The capillary was pretreated overnight with a 1.5% solution of FL-70 which is a laboratory detergent (Fisher Scientific, USA) that has a basic pH. The FL-70 (2 ml)is diluted in water (98 ml) and isopropanol (33 ml). Before beginning work, the capillary is rinsed with water for 2 minutes, then with 1 N sodium hydroxide solution for 2 minutes, 0.1 N sodium hydroxide solution for 2 minutes and water again for 2 minutes. The capillary is rinsed between runs with the FL-70 solution for 2 minutes. At the beginning of a run there is a 2 minute water rinse followed by a 2 minute buffer rinse to fill the capillary with run buffer.

RESULTS AND DISCUSSION

Serum can be injected directly onto the capillary column without any cleanup using the CD/MEKC technique. The optimum γ-CD concentration for separation of the 22 TCDD isomers is 40mM (Figure 1) in 100 mM borate, 100 mM sodium dodecyl sulfate, and 5 M

urea at pH 9. When serum spiked with 2,3,7,8-TCDD is run at a lower γ-CD concentration (15 mM, Figure 2) and slightly different conditions, the 2,3,7,8-TCDD migrates after the serum protein/lipoprotein components. We wanted to adjust conditions so that the 2,3,7,8-TCDD migrated before the serum protein/lipoprotein components. If that were possible, the run could be halted at any point after the detection of the 2,3,7,8-TCDD and the column rinsed in preparation for the next run thus decreasing run time. Increasing voltage and temperature did not change the migration pattern. We then began adjusting the γ-CD concentration of the buffer. Modifying the γ-CD concentration resulted in no change in the mobility of the serum components while all the TCDDs migrated at a slower or faster rate. The migration pattern of the TCDDs was condensed so that more co-migrated but none interfered with the 2,3,7,8-TCDD. While developing this method we used the next eluting isomer, 1,2,7,8-TCDD due to cost, availability and safety concerns. In Figure 3 the 1,2,7,8-TCDD is seen co-eluting with serum proteins at a γ-CD concentration of 40 mM. By increasing the γ-CD concentration to 50 mM (Figure 4) the peak migrated faster relative to the serum proteins, which did not change. When we found that a concentration of 60 mM γ-CD gave us the 1,2,7,8-TCDD peak migrating before the serum proteins(Figure 5), we switched back to spiking the serum with 2,3,7,8-TCDD. Using 60mM γ-CD, 2,3,7,8-TCDD migrates isomer-specifically in about 10 minutes in a window just ahead of the serum protein/lipoprotein components(Figure 6). The 2,3,7,8-TCDD peak represents 50 pg injected on-column. These experiments were conducted with higher levels of 2,3,7,8-TCDD than have been reported in human serum when other methods were used.

CONCLUSIONS

Cyclodextrin-modified/micellar electrokinetic chromatography can be used to separate 2,3,7,8-TCDD from human serum by modifying the cyclodextrin content of the buffer. Direct analysis for 2,3,7,8-TCDD spiked serum samples is possible and may be feasible for screening exposed populations if detection limits can be lowered using laser-induced fluorescence, sample stacking or high-resolution mass spectrometric detection.

REFERENCES

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2. S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R.Alexander, D.G. Patterson, Jr., B. L. Karger, K. Hosoya, and N. Tanaka, *J. Chromatogr.*, 516(1990) 23.
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Tetrachlorinated Dibenzo-p-dioxins

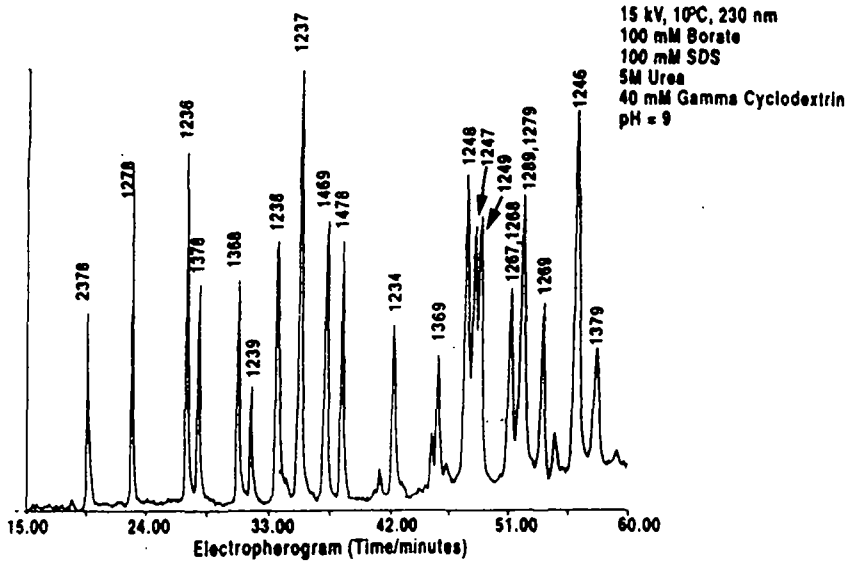

Figure 1

Blank Serum +2,3,7,8-TCDD

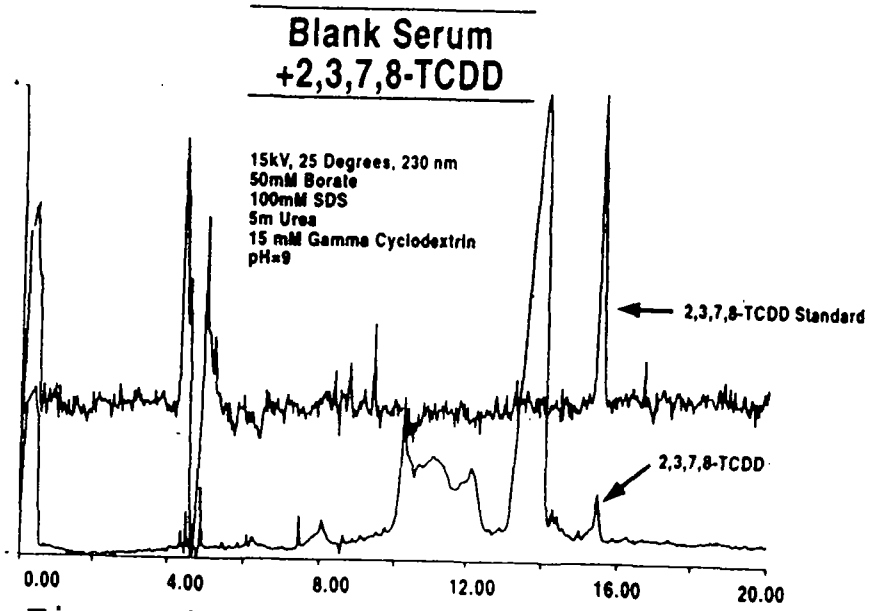

Figure 2

CD/MEKC of 1,2,7,8-TCDD Spiked Serum by Direct Serum Injection

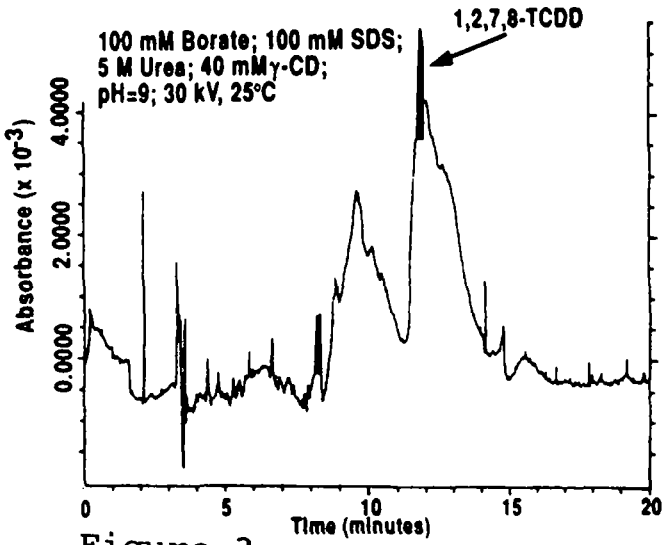

Figure 3

CD/MEKC of 1,2,7,8-TCDD Spiked Serum by Direct Serum Injection

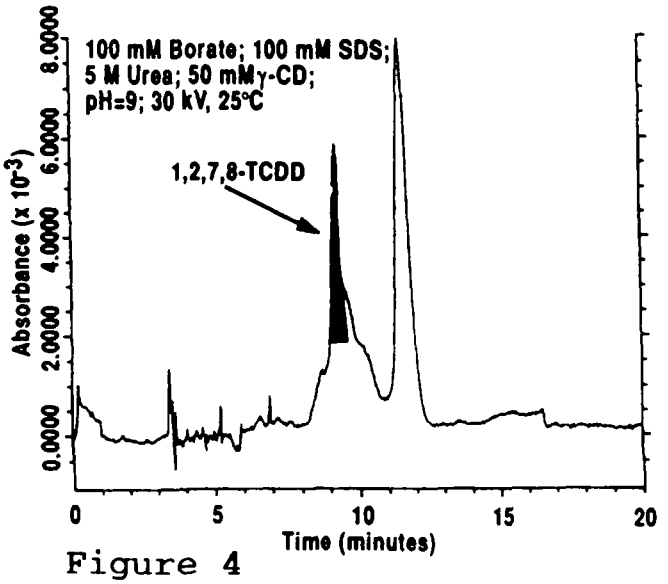

Figure 4

CD/MEKC of 1,2,7,8-TCDD Spiked Serum by Direct Serum Injection

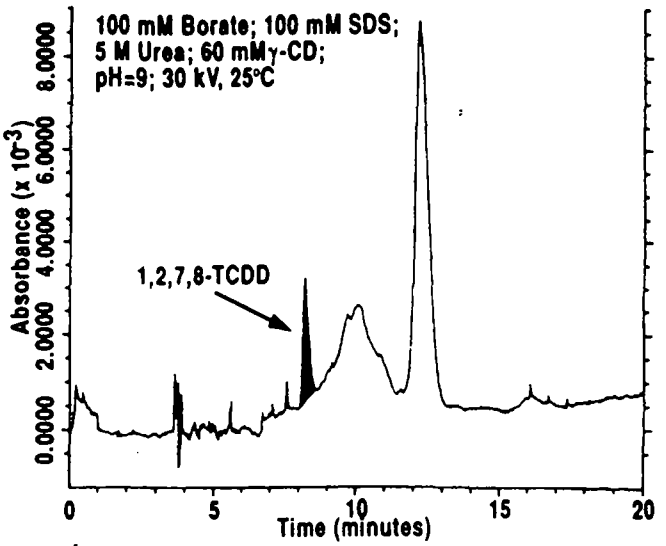

Figure 5

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

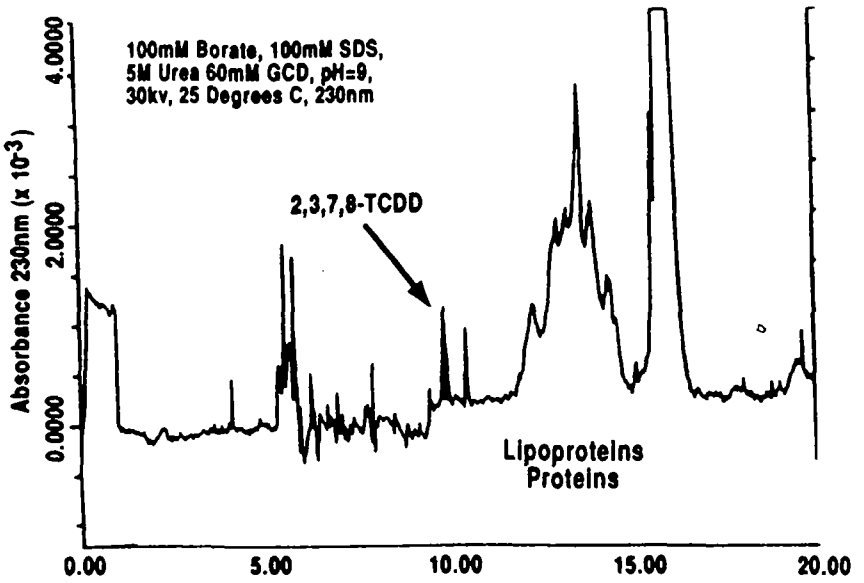

Figure 6