Separation of Chiral Polychlorinated Biphenyls by Cyclodextrin Modified Micellar Electrokinetic Chromatography.

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1. Introduction

The toxicity of polychlorinated biphenyls (PCBs) to humans and animals (they are persistent environmental pollutants) has prompted an increasing interest in developing analytical methodologies to determine PCBs in the environment. From the 209 congeners of the PCBs, 78 possess axial chirality but only nineteen containing three or four chlorine atoms in the ortho positions are stable to enantiomerization at room temperature[1]. The fact that enantiomers may have different activities, toxicities or metabolic pathways supports the need to determine each enantiomer proportion to assess PCBs actual toxic capability in samples[2]. The interest for PCBs enantiomeric separation methods is reflected in the works appeared in literature on the subject. So far, Gas Chromatography (GC), usually employed to separate PCB congeners, has also been used for chiral PCB separations[3, 4]. These separations require the use of enantioselective GC with cyclodextrin derivatives as chiral stationary phases.

Cyclodextrin Modified Micellar Electrokinetic Chromatography (CD-MEKC) is an alternate technique for the separation of chiral compounds. In this mode of Capillary Electrophoresis, a cyclodextrin is added into the micellar separation buffer[5]. In CD-MEKC, sodium dodecyl sulphate (SDS) as surfactant and β- or γ-cyclodextrins are normally used. Although its potential for the separation of chiral compounds of environmental interest has been reported[6], CD-MEKC applicability to separate chiral PCBs is yet to be described.

The aim of this work is to achieve chiral separations of PCBs in multicomponent mixtures by CD-MEKC using β- and γ-cyclodextrin mixtures as additives in the separation buffer.

2. Experimental

All reagents employed were of analytical grade. Urea, β-cyclodextrin and γ-cyclodextrin were purchased from Fluka Chemie AG (Buchs, Switzerland). 2-(N-cyclohexylamino)-ethanesulphonic acid (CHES) was from Sigma Chemical Co (St. Louis, MO, USA). Sodium hydroxide and sodium docecyl sulphate (SDS) were from

Merck (Darmstadt, Germany). Dimethylformamide (DMF) was from Scharlau (Barcelona, Spain).

PCBs studied in this work were purchased from Dr. Ehrenstorfer Reference Substances (Augsburg, Germany) and were the following (Ballschmiter nomenclature[7]): 2,2',3',6'-tetrachlorobiphenyl (PCB 45); 2,2',3,3',6'-pentachlorobiphenyl (PCB 84); 2,2',3',4',6'-pentachlorobiphenyl (PCB 88); 2,2',3,4',6-pentachlorobiphenyl (PCB 91); 2,2',3,5',6-pentachlorobiphenyl (PCB 95); 2,2',3,3',4,6'- hexachlorobiphenyl (PCB 132); 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136); 2,2',3',4,4',6'-hexachlorobiphenyl (PCB 139); 2,2',3,4',5',6-hexachlorobiphenyl (PCB 149); 2,2',3,3',4,4',6'-heptachlorobiphenyl (PCB 171); 2,2',3',4,4',5,6'-heptachlorobiphenyl (PCB 183); 2,2',3,3',4,4',5,6'-octachlorobiphenyl (PCB 196). Standard solutions in cyclohexane (PCBs 45 and 91) and in isooctane (PCBs 84, 88, 95, 132, 136, 139, 149, 171, 183, and 196) at a concentration of 10 mg L^{-1} for each PCB were concentrated by solvent evaporation and redissolved in DMF to obtain a concentration for each PCB standard solution of about 100 mg L^{-1}. This final DMF solution was used for direct injection. Synthetic mixtures were prepared as before by concentrating a mixture of standard solution of PCBs in cyclohexane and isooctane and using DMF as final solvent. Final concentrations of 100 mg L^{-1} of each PCB were also obtained in the multicomponent mixture.

The instrumentation consisted of a programmable injector for capillary electrophoresis model Prince, a Lambda 1000 UV-detector and a high voltage power supply, all purchased from Lauer Labs (The Netherlands). The integrator employed was a HP3394 from Hewlett Packard (Avondale, PA, USA). The capillary temperature was 45°C and detection was carried out at 235 nm. A fused-silica capillary tube (50 µm I.D.; 375 µm O.D.) from Polymicro Technologies (Phoenix, AZ, USA) was employed. The total length was 65 cm and the effective length was 50.5 cm.

Buffer concentrated stock solutions were prepared by adding a 0.3 M NaOH solution to a 0.3 M CHES solution, until the pH arised to 10.0. Separation buffers were prepared by dissolving the appropriate amount of surfactant, urea, β-cyclodextrin and γ-cyclodextrin in a diluted CHES buffer. The final concentration of CHES was obtained by diluting appropriate volumes of buffer concentrated stock solutions with water.

3. Results and Discussion

In order to achieve the separation of the enantiomers of PCBs 45, 84, 88, 91, 95, 132, 136, 139, 149, 171, 183 and 196, CD-MEKC was used with cyclodextrins as chiral selectors. SDS was employed as micellar system and β- and γ-cyclodextrins were used as additives in the separation buffer. Mixtures of these two cyclodextrins were employed due to the possible increase in selectivity that it could be obtained. CHES was used as buffer because its organic nature has shown to be favourable for the solubilization of lipophilic compounds in aqueous media[8]. A pH = 10 value was chosen to obtain a high electroosmotic flow. Urea was also added to the separation buffer in order to increase the cyclodextrin and solute solubility.

A low concentration of CHES at pH = 10 (about 0.06 M), which generated a high electroosmotic flow, was tested in order to separate each PCB in its two enantiomers. Since an increase in the cyclodextrin concentration was shown to increase selectivity, a high concentration of β-cyclodextrin (about 0.07 M) was employed first but no resolution of the enantiomers was obtained. Then, γ-cyclodextrin

was also added to the same separation buffer and its concentration was increased until separation was achieved. A concentration of γ-cyclodextrin of about 0.02 M was sufficient to obtain chiral separation of all PCBs. As in a previous work[9], SDS and urea concentrations in the separation buffer were kept constant and equal to 0.11 M and 2 M, respectively. Chiral separations of PCBs 84, 171, 183 and 132 in an analysis time close to 25 min were achieved in these conditions. This analysis time is similar to that obtained for these separations when only γ-cyclodextrin was used in the separation buffer[9] but shorter than the time required by GC for chiral separation of enantiomers of PCBs 84 and 132[4].

Although the above-mentioned experimental conditions were adequate for chiral separation of individual PCBs, the separation of multicomponent mixtures could not be possible. In this case, one PCB enantiomer overlapped with an enantiomer of another different PCB. Since an increase in the CHES concentration reduces the electroosmotic flow and improves the selectivity for multicomponent separation when the concentrations of the other buffer additives are kept constant, the concentration of CHES was increased. The cost of this resolution enhancement is an increase in the analysis time. Figure 1 shows the electropherogram corresponding to the separation of a mixture of eight chiral PCBs (45, 88, 91, 95, 136, 139, 149 and 196) in their sixteen enantiomers in 35 min using a CHES concentration of about 0.09 M.

4. Conclusion

This work shows that CD-MEKC is a promising alternative to GC with chiral stationary phases for chiral separations. In the particular case of PCBs, the chiral separation of multicomponent mixtures previously reported by GC needs a longer analysis time (110 min for a separation of five PCBs[4]) than those achieved in this work (35 min for eight PCBs). From this point of view, CD-MEKC can be advantageous with respect to GC. However, low sensitivity is a serious drawback of CD-MEKC techniques for PCB analysis.

5. References

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6. Acknowledgements

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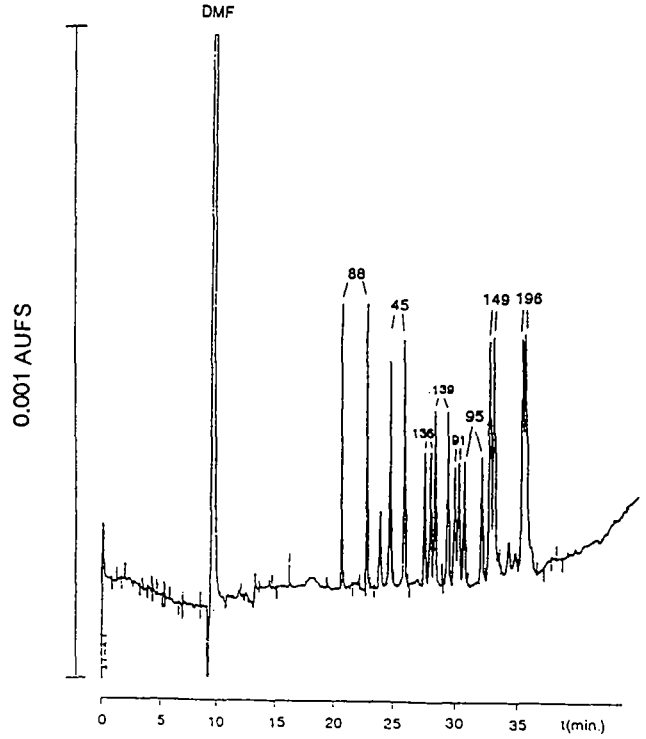

Figure 1. Electropherogram corresponding to the separation of a mixture of eight chiral PCBs. For PCB identification see Experimental. Separation buffer: 0.092 M CHES (pH = 10.0), 2 M urea, 0.11M SDS, 0.073 M β-cyclodextrin and 0.022 M γ-cyclodextrin. Current 42 μA. Injection by pressure, 0.02 min at 20 mbar. UV detection at 235 nm. Applied voltage, 15 kV. Temperature 45°C.