ELSEVIER

Journal of Chromatography A, 752 (1996) 265–270

JOURNAL OF CHROMATOGRAPHY A

Separation of chiral polychlorinated biphenyls by micellar electrokinetic chromatography using β- and γ-cyclodextrin mixtures in the separation buffer

M.L. Marina[a,*], I. Benito[a], J.C. Díez-Masa[b], M.J. González[b]

[a]*Departamento de Química Analítica, Facultad de Ciencias, Universidad de Alcalá de Henares, 28871 Alcalá de Henares, Madrid, Spain*

[b]*Instituto de Química Orgánica General (C.S.I.C.), C/ Juan de la Cierva 3, 28006 Madrid, Spain*

Received 28 July 1995; revised 19 March 1996; accepted 24 May 1996

Abstract

Chiral polychlorinated biphenyls (PCBs) 45, 84, 88, 91, 95, 132, 136, 139, 149, 171, 183 and 196 were separated each in its two enantiomers by cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC). Mixtures of β- and γ-cyclodextrins were used as chiral modifiers in a 2-(N-cyclohexylamino)ethanesulphonic acid (CHES) buffer containing urea and sodium dodecyl sulphate (SDS) micelles. Separations of multicomponent mixtures of PCBs into their enantiomers were also performed. A mixture of PCBs 45, 88, 91, 95, 136, 139, 149 and 196 was separated into all sixteen enantiomers in an analysis time of approx. 35 min.

Keywords: Enantiomer separation; Polychlorinated biphenyls; Cyclodextrins

1. Introduction

The toxicity of polychlorinated biphenyls (PCBs) to humans and animals has been studied for the past 25 years. They are persistent environmental pollutants and some of them are suspected to have toxic properties similar to other very toxic pollutants such as polychlorinated dibenzo-*p*-dioxins (PCDDs) whose toxicity (they cause neurologic and psychiatric problems among others) strongly depends on their molecular structure [1]. This 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 19 containing three or four chlorine atoms in the ortho positions are stable to enantiomerization at room temperature [2]. Since there is evidence of the important role played by chirality of atropisomeric PCBs in many recognition events associated with their enzymatic degradation processes [3,4], the need for analytical methods allowing the separation of chiral PCBs is obvious. Furthermore, 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 [3]. The interest for PCBs enantiomeric separation methods is reflected in the literature. Gas chromatography (GC), usually employed to separate PCB congeners, has also been used for chiral PCB separations. The

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00506-7

results of these works include separation of PCBs 45, 95, 139 [5], PCBs 84, 91, 95, 132, 136, 149 [6,7] and PCBs 45, 84, 88, 91, 95, 131, 132, 135, 136, 139, 149, 174, 175, 176 and 183 [8] (Ballschmiter nomenclature [9]). 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 (CE), a cyclodextrin is added into the micellar separation buffer [10,11]. In CD-MEKC, sodium dodecyl sulphate (SDS) as surfactant and β- or γ-cyclodextrins are normally used. The larger size of the cavity of these cyclodextrins compared to α-cyclodextrin makes them more useful for the separation of large molecules due to the formation of inclusion complexes between solutes and cyclodextrins [12]. CD-MEKC usefulness for enantiomeric separations has recently been shown [13,14]. Although its potential for the separation of chiral compounds of environmental interest has been reported [15], application of CD-MEKC to separation of chiral PCBs has yet to be described.

The migration time of a solute in a CD-MEKC system can be modified by changing either the surfactant's or the cyclodextrin concentration in addition to the buffer concentration. The use of expensive cyclodextrin derivatives in CD-MEKC is possible due to the small volume of electrophoretic solution required. This fact coupled with the high efficiency obtained in CE should enable CD-MEKC to become one of the major and most versatile techniques for analytical enantiomeric separation in the near future [13].

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

2.1. Chemicals

All reagents employed were of analytical grade. Urea, β-cyclodextrin and γ-cyclodextrin were purchased from Fluka (Buchs, Switzerland), 2-(N-cyclohexylamino)-ethanesulphonic acid (CHES) from Sigma (St. Louis, MO, USA), sodium hydroxide and SDS from Merck (Darmstadt, Germany) and dimethylformamide (DMF) from Scharlau (Barcelona, Spain).

The PCBs studied in this work were purchased from Dr. Ehrenstorfer Reference Substances (Augsburg, Germany) and were the following (Ballschmiter nomenclature [9]):
[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 iso-octane (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 described above) by concentrating a mixture of standard solutions of PCBs in cyclohexane and iso-octane and using DMF as final solvent. Final concentrations of 100 mg l^{-1} of each PCB were also obtained in the multicomponent mixture.

2.2. Apparatus

The instrumentation consisted of a programmable injector for CE Model Prince, a Lambda 1000 UV detector and a high-voltage power supply, all purchased from Lauer Labs (Emmen, 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. The electrolyte solutions were degassed in an ultrasonic system Transsonic 460 (Elma, Germany). A 654 pH meter (Metrohm, Herisau, Switzerland) was employed to measure separation buffer pH. Standard solutions of PCBs were concentrated in a Reacti-Vap (Pierce, IL, USA).

2.3. Procedure

Buffer concentrated stock solutions were prepared by adding a 0.3 *M* NaOH solution to 0.3 *M* CHES solution, until the pH reached 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 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 a micellar system and β- and γ-cyclodextrins were used because they are more effective in enantiomeric separation of large molecules than α-cyclodextrin [12]. Mixtures of these two cyclodextrins were employed due to the possible increase in selectivity that could be obtained [13]. CHES was used as buffer because its organic nature is favourable for the solubilization of lipophilic compounds in aqueous media [16]. A pH 10 value was chosen to obtain a high electroosmotic flow. Urea was added to the separation buffer to increase the cyclodextrin and solute solubility [17].

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 into its two enantiomers. Since an increase in the cyclodextrin concentration increases selectivity [18], a high concentration of β-cyclodextrin (about 0.07 *M*) was employed at 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 [19], SDS and urea concentrations in the separation buffer were kept constant and equal to 0.11 and 2 *M*, respectively. Fig. 1 shows the chiral separations of PCBs 84, 171, 183 and 132. Fig. 1a shows the separation of a mixture of PCBs 84 and 171 and Fig. 1b and 1c show the separation of the enantiomers of PCBs 183 and 132, respectively. An analysis time close to 25 min was needed to achieve these separations. This analysis time is similar to that obtained for these separations when only γ-cyclodextrin was used in the separation buffer [19] but shorter than the time required by GC for chiral separation of enantiomers of PCBs 84 and 132 [8].

Although the above-mentioned experimental conditions were adequate for chiral separation of individual PCBs, the separation of multicomponent mixtures was not possible. In this case, one PCB enantiomer overlapped with an enantiomer of another 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. As an example of the effect of increasing CHES concentration, Table 1 shows the separation factor for two enantiomers of PCBs 95 and 149 (one enantiomer of each PCB) and the same for two enantiomers of PCBs 136 and 139. In both cases, one enantiomer of a PCB overlapped with an enantiomer of the other PCB at a CHES concentration of 0.06 *M*. Table 1 shows that separation factors for these especially difficult separations increased when increasing CHES concentration. The cost of this resolution enhancement is an increase in the analysis time. Fig. 2a 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 16 enantiomers in 35 min using a CHES concentration of about 0.09 *M*.

As shown in Fig. 2a the two enantiomers of PCBs 149 and 196 are not completely resolved with this kind of cyclodextrin mixture. An increase in the γ-cyclodextrin concentration improves chiral separation of individual highly hydrophobic PCBs [19]. Fig. 2b shows the multicomponent separation of the

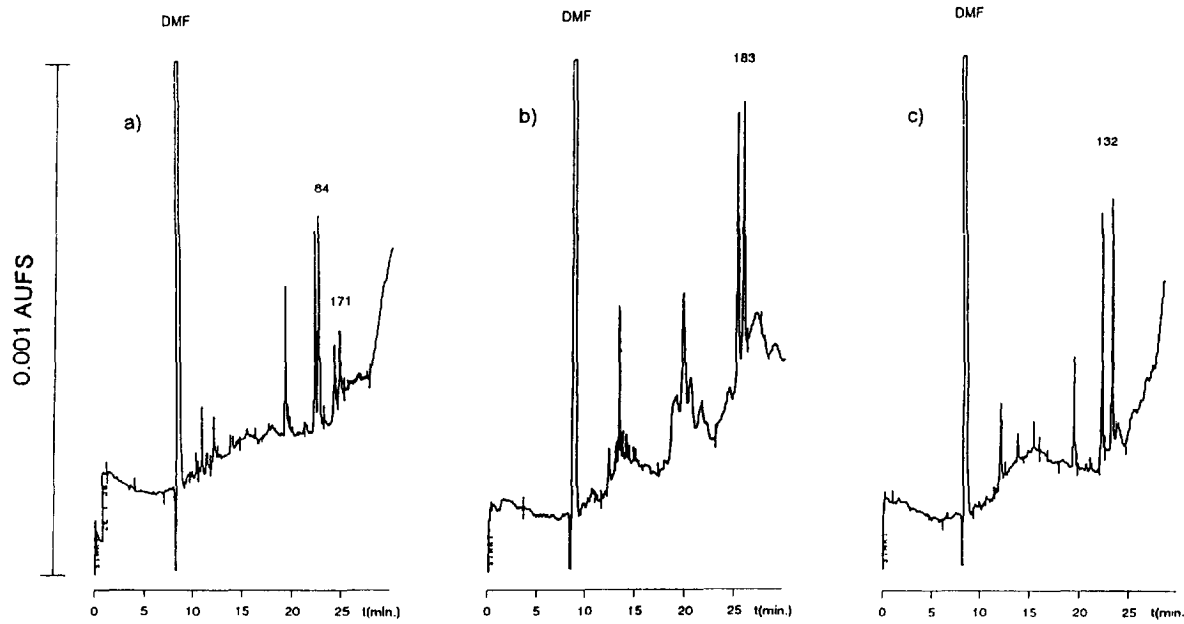

Fig. 1. Electropherogram corresponding to the chiral separation of (a) a mixture of PCBs 84 and 171, (b) PCB 183 and (c) PCB 132. For PCB identification see Section 2. Separation buffer: 0.060 *M* CHES (pH 10.0), 2 *M* urea, 0.11 *M* SDS, 0.073 *M* β-cyclodextrin and 0.022 *M* γ-cyclodextrin. Capillary, 65 cm total length, 50.5 cm effective length, 50 μm I.D. Injection by pressure, 0.02 min at 20 mbar. UV detection at 235 nm. Applied voltage 15 kV. Current 41 μA. Temperature 45°C.

sixteen enantiomers corresponding to the same mixture of eight PCBs using a 0.079 *M* β-cyclodextrin and 0.024 *M* γ-cyclodextrin mixture in the separation buffer. In this case, a better chiral resolution in PCBs 149 and 196 can be observed. β-Cyclodextrin concentration was also increased because otherwise the resolution be deteriorated.

The only change in selectivity observed when using mixtures of β- and γ-cyclodextrins to achieve multicomponent separations of PCBs compared to the use of γ-cyclodextrin alone in the separation buffer [19] is the reversal in the elution order for PCBs 136 and 139. Different selectivities have been obtained in CD-MEKC with different cyclodextrins and the results have been used to confirm the presence of specific impurities [20].

Table 1
Separation factors for two pairs of enantiomers of different PCBs at different CHES concentrations

	0.060 *M* CHES	0.084 *M* CHES	0.092 *M* CHES
α_1^a	1.00	1.05	1.07
α_2^b	1.14	1.20	1.26

[a] α_1: Separation factor for one enantiomer of PCB 136 and one enantiomer of PCB 139.
[b] α_2: Separation factor for one enantiomer of PCB 95 and one enantiomer of PCB 149.

The results obtained in this work show that CD-MEKC techniques are an interesting alternative to GC in enantiomeric separations. The use of β- and γ-cyclodextrin mixtures reduces the cost of the technique compared to γ-cyclodextrin modified MEKC. However, the use of γ-cyclodextrin alone as modifier in MEKC to perform chiral separation of PCBs seems to have better possibilities in the separation of multicomponent mixtures or the separation of some individual highly hydrophobic PCBs in their enantiomers [19]. With regard to CD-MEKC sensitivity, for instance, the minimal concentration detectable for PCB 88 at a wavelength value of 240 nm is about 40 mg l^{-1}. This result shows that, as could be expected, the sensitivity of this technique is lower than that obtained by GC techniques (which is in the μg l^{-1} range). An attempt to decrease detection limits for PCB analysis in CD-MEKC is under study in our laboratory.

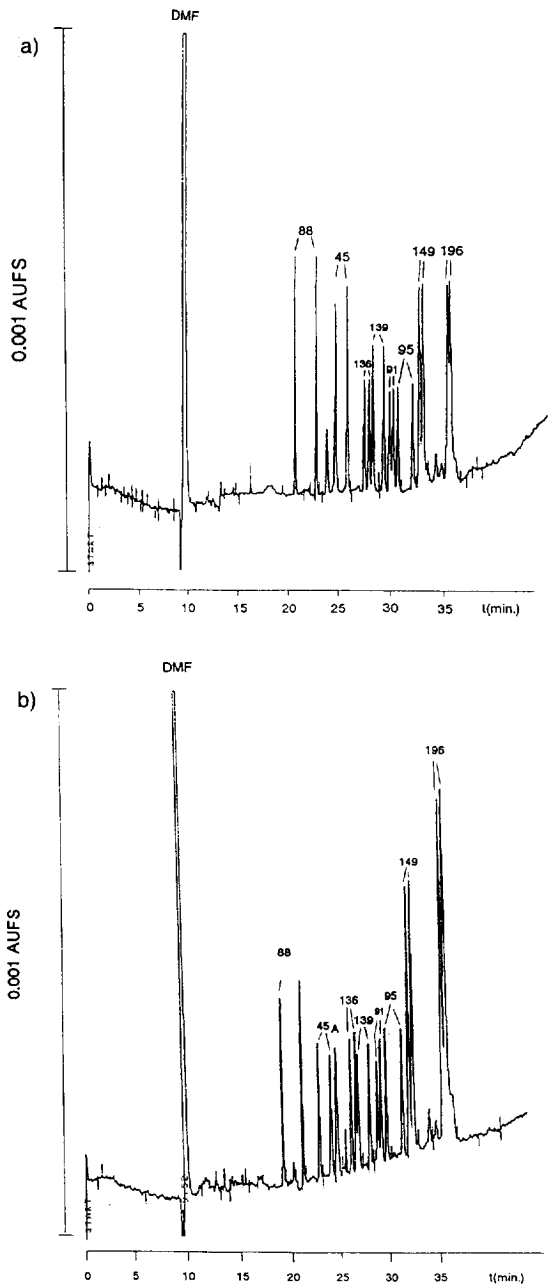

Fig. 2. Electropherogram corresponding to the separation of a mixture of eight chiral PCBs. For PCB identification see Section 2. A: Unknown peak. (a) Separation buffer: 0.092 *M* CHES (pH 10.0), 2 *M* urea, 0.11 *M* SDS, 0.073 *M* β-cyclodextrin and 0.022 *M* γ-cyclodextrin. Current 42 μA. (b) Separation buffer: 0.090 *M* CHES (pH 10.0), 2 *M* urea, 0.11 *M* SDS, 0.079 *M* β-cyclodextrin and 0.024 *M* γ-cyclodextrin. Current 45 μA. Other experimental conditions as in Fig. 1.

4. Conclusions

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 [8]) than those achieved in this work (35 min for eight PCBs). From this point of view, CD-MEKC has an advantage over GC. However, low sensitivity is a serious drawback of CD-MEKC techniques for PCB analysis.

Acknowledgments

Drs M.L. Marina and M.J. González thank the Comunidad Autónoma de Madrid for grant COR0010/94. Dr. Díez-Masa thanks the CICYT (Spain) for grant ALI94-0737.

References

[1] S. Safe, CRC Crit. Rev. Toxicol., 21 (1990) 51.
[2] M. Püttmann, F. Oesch, L.W. Roberston and A. Mannschreck, Chemosphere, 15 (1986) 2061.
[3] L.E. Rodman, S.I. Shedlofsky, A. Mannschreck, M. Püttmann, A.T. Swin and L.W. Roberston, Biochem. Pharmacol., 41 (1991) 915.
[4] H. Hühnerfuss, B. Pfaffenberger, B. Gehrcke, L. Karbe, W.A. König and O. Landgraff, Organohalogen Compounds, 21 (1994) 15.
[5] W.A. König, B. Gehrcke, T. Runge and C. Wolf, J. High Resolut. Chromatogr., 16 (1993) 376.
[6] V. Schurig and A. Glausch, Naturwissenschaften, 80 (1993) 468.
[7] A. Glaush, G.J. Nicholson, M. Fluck and V. Schurig, J. High Resolut. Chromatogr., 17 (1994) 347.
[8] I.H. Hardt, C. Wolf, B. Gehrcke, D.H. Hochmuth, B. Pfaffenberger, H. Hühnerfuss and W.A. König, J. High Resolut. Chromatogr., 17 (1994) 859.
[9] K. Ballschmiter and M. Zell, Fresenius Z. Anal. Chem., 302 (1980) 20.
[10] K. Otsuka and S. Terabe, Trends in Anal. Chem., 12 (1993) 125.
[11] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr. A, 666 (1994) 295.
[12] H.J. Isaaq, Instrumentation Sci. and Technology, 22 (1994) 119.

[13] S. Terabe, Y. Miyashita, Y. Ishihama and O. Shibata, J. Chromatogr., 636 (1993) 47.
[14] W.C. Brumley and W.J. Jones, J. Chromatogr. A, 680 (1994) 163.
[15] M.W.F. Nielen, in D. Barceló (Editor), Environmental Analysis: Techniques, Applications and Quality Assurance, Elsevier, Amsterdam, 1993, Ch. 17, p. 607.
[16] M.A. García, M.L. Marina and J.C. Díez-Masa, J. Chromatogr. A, 732 (1996) 345.
[17] H. Nishi and M. Matsuo, J. Liq. Chromatogr., 14 (1991) 973.
[18] I. Benito, J.M. Saz, J.C. Díez-Masa, M.J. González and M.L. Marina, submitted.
[19] M.L. Marina, I. Benito, J.C. Díez-Masa and M.J. González, Chromatographia, 42 (1996) 269.
[20] M.W.F. Nielen, J. Chromatogr., 637 (1993) 81.