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Journal of Chromatography A, 979 (2002) 425–429

JOURNAL OF CHROMATOGRAPHY A

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Separation and on-line concentration of bisphenol A and alkylphenols by micellar electrokinetic chromatography with cationic surfactant

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Abstract

The separation and on-line concentration of bisphenol A and three alkylphenols were investigated by micellar electrokinetic chromatography with cationic surfactant. Tetradecyltrimethylammonium bromide was used as surfactant and the separation conditions were optimized by the addition of the organic solvents and cyclodextrins to the running solution. The separation of hydrophobic analytes and 4-nonylphenol isomers was improved by the addition of 20% acetonitrile and 20 m*M* β-cyclodextrin to the running solution. When the sweeping with the running solution used as the on-line concentration procedure, 56-, 67- and 29-fold increase in detection sensitivity of bisphenol A, 4-*tert*.-butylphenol and 4-(1,1,3,3-tetramethylbutyl)phenol, respectively. The detection limits were 0.030, 0.098 and 0.159 mg/l, respectively.

Keywords: Buffer composition; Sweeping; Bisphenol A; Phenols; Alkylphenols; Endocrine disruptors

1. Introduction

Recently, several chemicals are considered to have endocrine disrupting effects [1]. The Environmental Agency of Japan arranged ‘‘Strategic Programs on Environmental Endocrine Disruptors ’98’’ (SPEED ’98) in May, 1998 [2]. In those programs, 67 suspected chemical groups are listed. For the accurate assessment of human exposure of these chemicals, the development of simple analytical methods was very important. At present, gas chromatography–mass spectrometry (GC–MS) is mainly used for their analysis. Bisphenol A (BPA) and alkylphenols (4-substituted, carbon number: 4~9) are included in SPEED ’98. These chemicals are consumed on a large scale for industrial use. Four of them, BPA, 4-*tert*.-butylphenol (4-tBP), 4-(1,1,3,3-tetramethylbutyl)phenol (4-tOP) and 4-nonylphenol (4-NP) have been frequently found in environmental waters in Japan [3]. BPA is mainly used for the production of polycarbonate or epoxy resin [4]. 4-NP is the raw material of nonylphenol polyethoxylates,

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)01404-8

which are used as nonionic surfactants. The mixtures of side chain isomers of 4-NP are widely used [5]. The regulated analytical method for BPA and alkylphenols determination is GC–MS with the derivatization of these compounds. However, the derivatization procedure is complex and time-consuming [6].

It is well known that micellar electrokinetic chromatography (MEKC) can provide higher resolution than that of high-performance liquid chromatography. We have been studying the analysis of nonvolatile or thermally degradable chemicals in water without any derivatization by MEKC [7–9]. We have achieved the simultaneous separation of BPA and three alkylphenols by MEKC with sodium dodecyl sulfate (SDS) [10]. The addition of β- or γ-cyclodextrin (CD) was necessary for the separation of nonylphenol isomers. However, the sensitivity of the method was poor, and the detection limit is still at the ppm level. Therefore, the effective concentration method should be applied to this system for the application to environmental samples. In MEKC, sweeping is an effective concentration method [11]. Kim et al. used cationic surfactants for the sweeping of negatively charged analytes [12]. Their retention factors and sweeping effects with cationic surfactants were higher than those obtained with SDS. BPA and alkylphenols are acidic substances and their separation with cationic surfactants has not been reported yet. In this paper, the separation conditions for BPA, 4-tBP, 4-tOP and 4-NP were optimized with a cationic surfactant, tetradecyltrimethylammonium bromide (TTAB). For their separation, different kinds of organic solvents and CDs as additives to the running solution in MEKC were investigated. Under the optimized conditions, the effect of sweeping was investigated.

2. Experimental

2.1. Apparatus

MEKC was performed with a CAPI-3000 CE system (Otsuka Electronics, Osaka, Japan). A 75 μm I.D. fused-silica capillary (GL Science, Tokyo, Japan) of 62 cm total length was used. The effective length to the detector was 50 cm. The instrumental control, data collection and analysis were performed with a PC-9801 personal computer (NEC, Tokyo, Japan). Conductivities were measured with a CM-5S conductivity meter (TOA Electronics, Tokyo, Japan).

2.2. Reagents

BPA and three alkylphenols were obtained from Tokyo Kasei (Tokyo, Japan). Tris(hydroxymethyl)aminomethane (Tris) and methanol were obtained from Kanto (Tokyo, Japan). TTAB was obtained from Wako (Osaka, Japan). Acetonitrile, β- and γ-CDs were obtained from Nacalai Tesque (Kyoto, Japan). All reagents were used without further purification.

2.3. Procedure

Running solutions were prepared by dissolving 50 m*M* TTAB in a mixture of 100 m*M* Tris–HCl buffer adjusted to pH 7.0. These solutions were filtered through a 0.45-μm pore size membrane filter prior to use. Stock solutions of phenols (1000 mg/l) were prepared in methanol. Sample solution for separation optimization was made by 10-fold dilution of the stock solutions with the running solution. Sample solutions for sweeping were prepared in running solutions without TTAB and they had conductivity similar to that of the running solution. When the running solution was changed, the capillary was rinsed with 1 *M* NaOH for 1 min using a vacuum at the detector reservoir, followed by subsequent rinses of distilled water for 3 min and running solution for 3 min. Sample injections were made by gravity (20 mm). The injection volume was about 8 nl for 30 s injection. The set-up voltage of the sample inlet side and temperature of the capillary cartridge were −20 kV and 30 °C, respectively, throughout all experiments. UV detection was performed at 230 nm. Triplicate measurements were made for all runs and the average values were obtained.

3. Results and discussion

3.1. Addition of organic solvents

The separation conditions were basically referred to Ref. [12]. It was necessary to use some additives

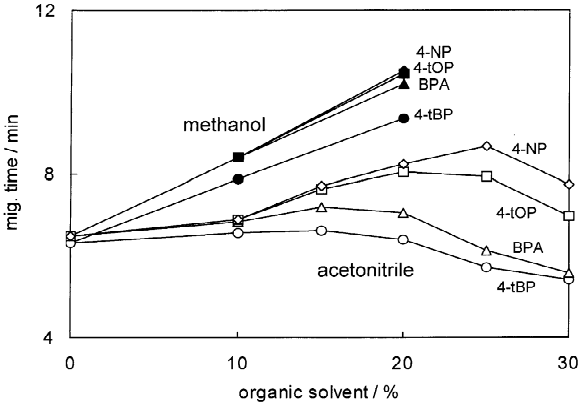

Fig. 1. Effects of the addition of organic solvents on migration behavior. Conditions: capillary, 57 cm (50 cm to the detector)×75 μm I.D.; migration buffer, 100 m*M* Tris–HCl (pH 7.0) containing 50 m*M* TTAB and organic solvents; injection, gravity, 20 mm, 30 s (ca. 8 nl); sample concentration, 100 mg/l; applied voltage, −20 kV; detection wavelength, 230 nm; temperature, 30 °C.

for the separation of hydrophobic analytes and 4-NP isomers. First, the use of organic solvent was investigated. The effects of acetonitrile and methanol on the migration times of the peaks are shown in Fig. 1. The separation of BPA, 4-tOP and 4-NP was not remarkably improved and the migration times were much longer by the addition of methanol. Their separation was improved by the addition of acetonitrile although the migration times were slightly longer. Therefore, acetonitrile was adopted as the additive. The effects of acetonitrile concentration to the peak resolutions are shown in Table 1. The addition of 25% acetonitrile was necessary for the complete separation of 4-tOP/4-NP pair, however, the separation of 4-tBP/BPA pair was the best at 20%. The addition of CD was expected to improve the separation of 4-tOP and 4-NP as well as isomers. So we fixed the concentration of acetonitrile to 20%.

Table 1
Dependence of resolution values on acetonitrile concentration[a]

	Acetonitrile (%)					
	0	10	15	20	25	30
R_s (4-tBP/BPA)	0.67	2.0	4.4	4.6	3.2	1.1
R_s (BPA/4-tOP)	0	0.35	3.3	6.3	10	8.1
R_s (4-tOP/4-NP)	0	0	0.45	0.83	1.8	2.0

[a] Other conditions as in Fig. 1.

Table 3
Dependence of resolution values on the concentration of γ-CD[a]

	γ-CD (m*M*)				
	1	2	5	7.5	10
R_s (4-tBP/BPA)	2.5	2.2	1.5	1.0	0.27
R_s (BPA/4-tOP)	4.0	3.5	1.7	0.65	0.53
R_s (4-tOP/4-NP)	1.1	1.0	2.7*	3.7*	2.8*

*Calculated from the first peak of 4-nonylphenol isomers
[a] Concentration of acetonitrile, 20%; other conditions as in Fig. 1.

3.2. Addition of cyclodextrins

The addition of CD improves both of the separations of hydrophobic solutes and isomers [13]. The selectivity of CD depends on its cavity size. We used β- or γ-CD for the separation, and the peak resolutions are shown in Tables 2 and 3, respectively. Isomers of 4-NP were partially separated using the running solution containing 5 m*M* β- or γ-CD. When β-CD was added, the complete separation of 4-tOP and 4-NP was achieved without deteriorating the separation of hydrophilic analytes. On the other hand, the separation of hydrophilic analytes deteriorated with use of γ-CD. Then, we chose β-CD as the second additive and investigated the effect of its concentration. This result is shown in Fig. 2. Iso-

Table 2
Dependence of resolution values on the concentration of β-CD[a]

	β-CD (m*M*)						
	2	5	10	15	20	25	30
R_s (4-tBP/BPA)	4.8	3.7	4.3	2.6	2.2	1.7	1.0
R_s (BPA/4-tOP)	7.3	6.9	5.4	6.2	4.8	3.1	2.3
R_s (4-tOP/4-NP)	1.6	2.9*	3.2*	3.7*	3.2*	1.7*	1.8*

*Calculated from the first peak of 4-nonylphenol isomers.
[a] Concentration of acetonitrile, 20%; other conditions as in Fig. 1.

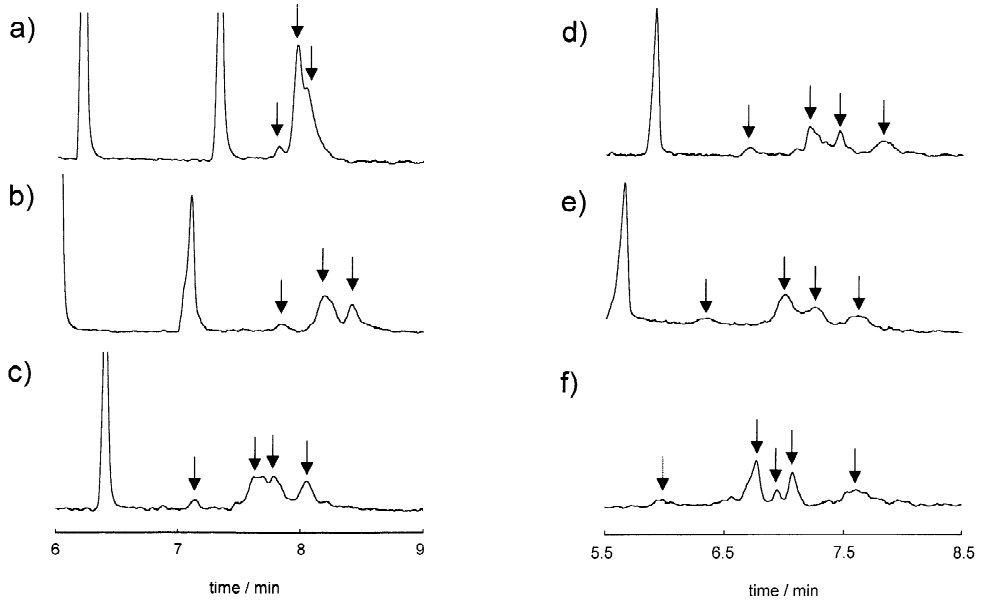

Fig. 2. Effects of the addition of β-CD on the separation of 4-NP isomers. Concentrations of β-CD: (a) 5 m*M*; (b) 10 m*M*; (c) 15 m*M*; (d) 20 m*M*; (e) 25 m*M*; (f) 30 m*M*. Other conditions as in Fig. 1.

meric separation was better as the concentration increased, while the separation of hydrophilic analytes worsened. Therefore, we set the concentration of β-CD to 20 m*M*. The limit of detections (LODs) of analytes under this condition were 0.6–3.2 mg/l except for 4-NP isomers.

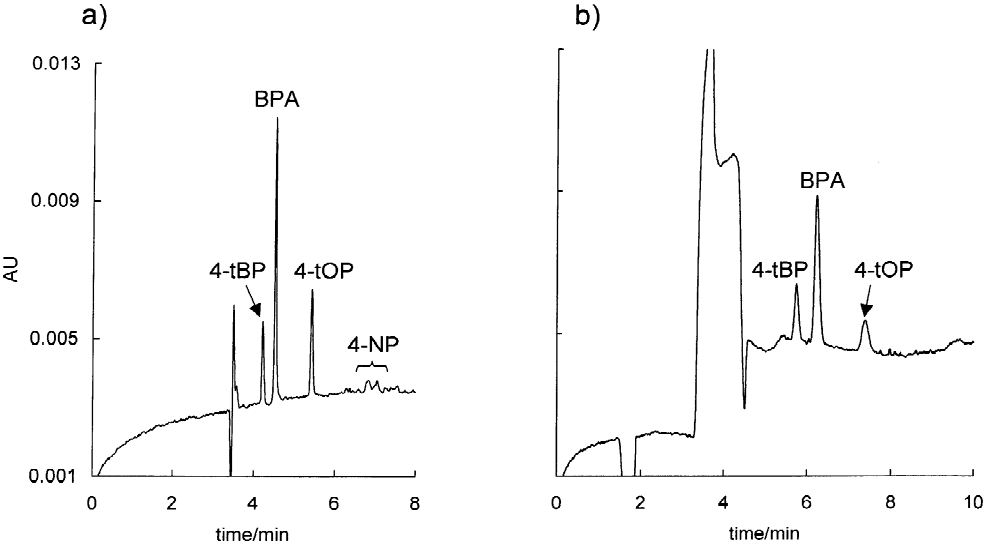

Fig. 3. Normal and sweeping MEKC analysis of bisphenol A and three alkylphenols. Conditions: migration buffer, 100 m*M* Tris–HCl (pH 7.0) containing 20 m*M* β-CD, 50 m*M* TTAB and 20% acetonitrile. Injection, (a) 30 s, (b) 2400 s; sample concentration, (a) 100 mg/l; (b) 1 mg/l. Other conditions as in Fig. 1.

Table 4
Limits of detections (LODs), relative standard deviations (RSDs), and sensitivity enhancement factors in terms of peak heights (SEF_{height}) in sweeping[a]

	4-tBP	BPA	4-tOP
Calibration line[b]	$y=1.87x-0.49$	$y=5.49x-1.14$	$y=2.55x-2.42$
Correlation coefficient	0.984	0.989	0.976
LOD ($S/N=3$) (mg/l)	0.098	0.030	0.159
RSD ($n=3$)			
Migration time (%)	1.9	2.0	2.5
Peak height (%)	5.0	4.1	12.1
SEF_{height}[c]	67	56	29

[a] Conditions as in Fig. 3.
[b] Calibration line: concentration (mg/l)=slope·peak height (mAU)+y-intercept.
[c] SEF_{height}=(peak height obtained with concentration/peak height obtained with usual MEKC injection)·dilution factor.

3.3. Sweeping of analytes

For the sensitivity enhancement of the method for phenols, sweeping was applied to the optimized separation system. The sample injection time was varied at 150, 600, 1200 and 2400 s for sweeping. In the case of 2400 s, 4-tBP, BPA and 4-tOP were detectable at 1 mg/l, while 4-NP was not detected as shown in Fig. 3. The peaks were broader compared to those of normal injection. In sweeping, the use of non-coated capillary brought the lower plate numbers of peaks in the presence of high electroosmotic flow [14]. Table 4 summarizes the results of the LODs, relative standard deviations (RSDs) and sensitivity enhancement factors in terms of peak heights (SEF_{height}) [12] obtained for the analytes. Effective sensitivity enhancement (>50-fold) and acceptable reproducibility (<5%) were achieved for 4-tBP and BPA by this method using sweeping.

4. Conclusion

The relatively high concentration ratios of 4-tBP and BPA were achieved by sweeping. However, further improvement was necessary for the separation and concentration of 4-NP isomers. The use of coating capillary and the comparison with the results obtained with anionic surfactants are now under investigation. The established method will be applied to the determination of bisphenol A and alkylphenols in environmental waters.

References

[1] T. Corborn, D. Dumanoski, J.P. Myers, Our Stolen Future, Penguin Books, New York, 1996.
[2] Strategic Programs on Environmental Endocrine Disruptors ’98 (SPEED ’98), Environmental Agency of Japan, Tokyo, 1998, (in Japanese).
[3] Research On the State of Environmental Endocrine Disruptors in Environmental Waters in Japan, in 1998 Summer, Environmental Agency of Japan, Tokyo, 1998, (in Japanese).
[4] R.E. Morrissey, J.D. George, C.J. Price, R.W. Tyl, M.C. Marr, C.A. Kimmel, Fundam. Appl. Toxicol 8 (1987) 581.
[5] C.D. Hager, in: D.R. Carsa (Ed.), Annual Surfactant Review, Sheffield Academic Press, Sheffield, 1998, p. 1.
[6] Provisional Manual of Analytical Methods For Environmental Endocrine Disruptors, Environmental Agency of Japan, Tokyo, 1998, (in Japanese).
[7] S. Takeda, S. Wakida, M. Yamane, K. Higashi, S. Terabe, J. Chromatogr. A 744 (1996) 135.
[8] S. Takeda, S. Wakida, M. Yamane, Z. Siroma, K. Higashi, S. Terabe, J. Chromatogr. A 817 (1998) 59.
[9] S. Takeda, S. Wakida, M. Yamane, A. Kawahara, K. Higashi, Anal. Chem. 65 (1993) 2489.
[10] S. Takeda, S. Iida, K. Chayama, H. Tsuji, K. Fukushi, S. Wakida, J. Chromatogr. A 895 (2000) 213.
[11] J.P. Quirino, S. Terabe, Science 282 (1998) 465.
[12] J.-B. Kim, J.P. Quirino, K. Otsuka, S. Terabe, J. Chromatogr. A 916 (2001) 123.
[13] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr. 516 (1990) 23.
[14] J.P. Quirino, S. Terabe, Anal. Chem. 71 (1999) 1638.