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

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Abstract

Separation and on-line concentration of bisphenol A and three alkylphenols were investigated by micellar electrokinetic chromatography with the anionic surfactant, sodium dodecyl sulfate. The separation conditions were optimized by the simultaneous addition of the organic solvent and cyclodextrin to the running solution. The separation of hydrophobic analytes and 4-nonylphenol isomers was improved by the addition of 10% methanol and 5 mM β -cyclodextrin to the running solution. When the sweeping with the running solution was used as the on-line concentration procedure, 69-, 48-, 55- and 41-fold increases in detection sensitivity were obtained for bisphenol A, 4-*tert*-butylphenol and 4-(1,1,3,3-tetramethylbutyl)phenol, and the second peak of 4-nonylphenol isomers, respectively. The detection limits were 0.0071, 0.0065, 0.021 and 0.055 mg/l, respectively. These results were better than those with the cationic surfactant, tetradecyltrimethylammonium bromide.

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1. Introduction

Recently, several chemicals were thought to have endocrine disrupting effects [1]. The Environmental Agency of Japan published “Strategic Programs on Environmental Endocrine Disruptors ’98” (SPEED ’98) in May 1998 [2]. In the programs, 67 chemical groups are listed as the chemicals suspected to have

endocrine disrupting effects. For the accurate assessment of human exposure of these chemicals, it is important to develop simple analytical methods for those chemicals. Bisphenol A (BPA) and alkylphenols (4-substituted, carbon number: 4–9) are included in the SPEED ’98. These chemicals are consumed on a large scale for their industrial use. Four of them, BPA, 4-*tert*-butylphenol (4-*t*BP), 4-(1,1,3,3-tetramethylbutyl)phenol (4-*t*OP) and 4-nonylphenol (4-NP) have been frequently found in environmental waters in Japan [3]. BPA is mainly used for the

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production of polycarbonate or epoxy resin [4]. 4-NP is the raw material of nonylphenol polyethoxylates, which are used as non-ionic surfactants. The mixtures of side chain isomers of 4-NP are widely used for their manufacturing [5]. The regulated analytical method for BPA and alkylphenols is gas chromatography–mass spectrometry (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 non-volatile or thermally degradable chemicals in water by MEKC without any derivatization procedure [7–9]. The separation of BPA and three alkylphenols was examined 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 in the ppm level. Therefore, it is necessary to develop an effective concentration method for the application of MEKC to the determination of these chemicals in environmental samples. In MEKC, sweeping is one of the effective concentration methods [11]. Kim et al. used cationic surfactants for the sweeping of negatively chargeable analytes [12]. We investigated the separation and concentration of BPA and three alkylphenols with cationic surfactant, tetradecyltrimethylammonium bromide (TTAB) [13]. The simultaneous addition of organic solvent and CD was effective for the separation. Under optimized conditions, the effect of sweeping was investigated. For BPA, 4-*t*BP and 4-*t*OP, a 29–67-fold increase in detection sensitivity was obtained. However, the isomers of 4-NP could not be detected because their peak shapes were broadened. It could be presumed that the phenomenon was caused by the suppression of the reversed electroosmotic flow (EOF) at the sample zone. When cationic surfactant was used, reversed EOF was generated by the absorption of cationic surfactant to the inner capillary wall. On the other hand, normal EOF was not almost affected by anionic surfactant. Therefore, the separation and concentration for BPA and three alkylphenols including the isomer of 4-NP was investigated by MEKC

with SDS as anionic surfactant. The simultaneous addition of organic solvent and CD was also investigated.

2. Experimental

2.1. Apparatus

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

2.2. Reagents

BPA and three alkylphenols were obtained from Tokyo Kasei (Tokyo, Japan). Methanol was obtained from Kanto (Tokyo, Japan). SDS, acetonitrile, β - and γ -CD were obtained from Nacalai Tesque (Kyoto, Japan). All reagents were used without further purification.

2.3. Procedure

Running solutions were prepared by dissolving 20 mM SDS in a mixture of 20 mM borate-phosphate buffer adjusted to pH 8.0. These solutions were filtered through a 0.45- μ m pore size membrane filter prior to use. Stock solutions of phenols (1000 mg/l) were prepared in methanol. Sample solutions used for the separation optimization were prepared by 10-fold dilution of the stock solutions with the running solution. Sample solutions used for sweeping were prepared in running solutions without SDS in order to have a similar conductivity with 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. Samples were injected into the capillary by gravity (20 mm). The

Table 1
Dependence of resolution values (R_s) of 4-*t*BP and BPA on organic solvent and CD^a

	Acetonitrile (%)		Methanol (%)	
	5	10	10	20
β -CD				
5 mM	1.80	0.47	2.00	0.92
10 mM	0	0	1.39	0.52
15 mM	0	0	0.99	0
γ -CD				
5 mM	0.70	1.21	0.45	0
10 mM	0.85	0	0	0
15 mM	0	0	0	0

^a Other conditions as in Fig. 1.

injection volume was about 8 nl for a 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. The UV detection was performed at 210 nm. Triplicate measurements were made for all runs and the average values were obtained.

3. Results and discussion

3.1. Optimization of peak separation

We already investigated the separation of BPA and three alkylphenols with SDS and either organic solvent or CD [10]. However, simultaneous addition of organic solvent and CD has not been investigated. We chose acetonitrile or methanol as organic solvent, and β -CD or γ -CD as CD. The concentrations were 5% or 10% for acetonitrile, 10% or 20% for methanol, and 5, 10 or 15 mM for CDs according to our previous paper [10]. These 24 additive combinations were applied for the separation. We investigated three separation criteria: (1) complete peak separation for 4-*t*BP/BPA pair and BPA/4-*t*OP pair, i.e. the resolution values were more than 1.5; (2) two or more peaks for 4-NP (isomeric separation); and (3) peak sharpness and reproducibility.

The resolution values of 4-*t*BP/BPA under the 24 combinations are shown in Table 1. Their complete separation was achieved under the only two conditions. One was the simultaneous addition of 5% acetonitrile and 5 mM β -CD, and the other was that of 10% methanol and 5 mM β -CD. The complete separation of BPA/4-*t*OP was achieved and three 4-NP isomer peaks were obtained under both conditions. The comparison of theoretical plate numbers and relative standard deviation of peak area for the two conditions are shown in Table 2. Considering these results, we chose the simultaneous addition of 10% methanol and 5 mM β -CD for the separation.

3.2. Sweeping of analytes

For the sensitivity enhancement of the method for phenols, sweeping was applied to the optimized separation system. The sample injection period was varied at 150, 300, 600, 1200 and 2400 s for sweeping. In the case of the 2400-s injection, complete peak separation of 4-*t*BP and BPA was not obtained. Therefore we chose the case of the 1200-s injection as sweeping. The comparison of normal and sweeping analysis is shown in Fig. 1. The injection volumes for normal and sweeping injection were about 8 and 340 nl, respectively. Theoretical plate numbers for the first peak were 2.6×10^4 and 3.5×10^4 , respectively. All phenols, including 4-NP were detectable at 1 mg/l in sweeping in contrast with the case of TTAB used for surfactant. The limit of detection, relative standard deviation and sensitivity enhancement factors in terms of peak heights (SEF_{height}) [12] in sweeping are summarized in Table 3. Effective sensitivity enhancement and ac-

Table 2
Theoretical plate numbers (N) and relative standard deviations (RSDs) of peak area for the two conditions^a

Condition	N (10^4)			RSD (%)		
	4- <i>t</i> BP	BPA	4- <i>t</i> OP	4- <i>t</i> BP	BPA	4- <i>t</i> OP
5% Acetonitrile and 5 mM β -CD	0.14	2.8	2.9	2.2	2.6	7.4
10% Methanol and 5 mM β -CD	5.5	15	7.2	1.9	3.2	2.0

^a Other conditions as in Fig. 1.

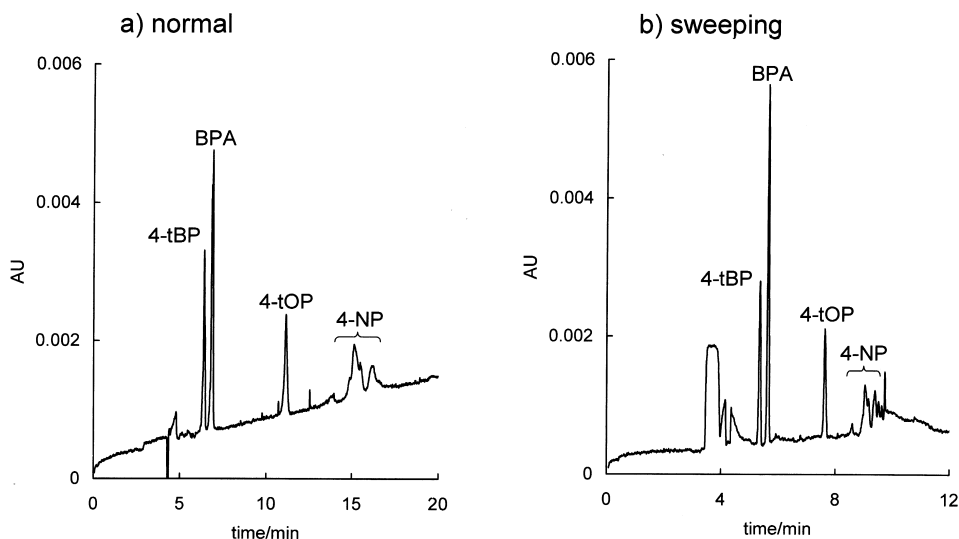


Fig. 1. Normal and sweeping MEKC analysis of BPA and three alkylphenols. Conditions: capillary, 62 cm (50 cm to the detector) \times 75 μ m I.D.; migration buffer, 20 mM borate-phosphate (pH 8.0) containing 20 mM SDS, 10% methanol and 5 mM β -CD; injection, gravity, 20 mm; applied voltage, 20 kV; detection wavelength, 210 nm; temperature, 30 $^{\circ}$ C. Injection: (a) 30 s, (b) 1200 s; sample concentration: (a) 50 mg/l; (b) 1 mg/l.

ceptable reproducibility were achieved for BPA and alkylphenols by this method.

As for the limit of detection, lower values were obtained than those in the case of TTAB [13]. One reason is in the difference between the detection wavelength used in the case of SDS and that used in the case of TTAB. TTAB absorbs at 210 nm. The adsorption intensities for BPA and three alkylphenols at 210 nm were much higher than those at 230 nm. Another reason is that the peak broadening was not observed in the case of SDS. It was proved that the

peak broadening for 4-NP in the case of TTAB was caused by the suppression of reversed EOF as explained in the Introduction.

4. Conclusion

The relatively low limit of detection of BPA and three alkylphenols was obtained by sweeping with SDS. In the sweeping of these phenols, SDS has more advantages than TTAB. The established meth-

Table 3

Limit of detection (LOD), relative standard deviation (RSD), and sensitivity enhancement factors in terms of peak heights (SEF_{height}) in sweeping^a

	4- <i>t</i> BP	BPA	4- <i>t</i> OP	4-NP ^b
Calibration line ^c	$y = 2.3x + 0.014$	$y = 5.3x + 0.11$	$y = 1.5x - 0.0021$	$y = 0.54x - 0.013$
Correlation coefficient	1.000	0.995	0.999	0.993
LOD ($S/N=3$) (mg/l)	0.0071	0.0065	0.021	0.055
RSD ($n=10$)				
Migration time (%)	0.5	0.5	1.0	1.6
Peak height (%)	2.5	3.5	9.2	9.8
SEF_{height} ^d	48	69	55	41

^a Conditions as in Fig. 1.

^b The highest peak of 4-NP isomer peaks (the second peak).

^c Calibration line: concentration (mg/l) = slope \times peak height (mAU) + y-intercept.

^d $SEF_{\text{height}} = (\text{peak height obtained with concentration} / \text{peak height obtained with usual MEKC injection}) \times \text{dilution factor}$.

od will be applied to the determination of BPA and alkylphenols in environmental waters.

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