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Separation of bisphenol A and three alkylphenols by micellar electrokinetic chromatography

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Abstract

Analytical conditions of pH, surfactants, and additives were investigated for the simultaneous separation of bisphenol A and alkylphenols by micellar electrokinetic chromatography. Reproducibility of migration time was improved at higher pH (pH 8.0). When five surfactants having linear alkyl chains or four bile salts were used, the separation of hydrophobic phenols and 4-nonylphenol isomers was not achieved. In order to improve the separation, the use of additives with sodium dodecyl sulfate solution was investigated. The separation of hydrophobic phenols was improved by the addition of organic solvents, however, isomers were not separated. Their separation was achieved by the addition of β- or γ-cyclodextrin.

Keywords: Buffer composition; Alkylphenol; Phenols; Bisphenol A

1. Introduction

Recently, several chemicals are suspected that they 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 the programs, sixty-seven suspected chemical groups are listed. For the accurate assessment of human exposure of these chemicals, development of their easy analytical methods was very important. At present gas chromatography–mass spectrometry (GC–MS) is mainly regulated as their analytical method.

Bisphenol A (BPA) and alkylphenols (4-substituted, carbon number: 4~9) are listed in SPEED ’98. They are consumed in large volume 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 relatively high detection frequency from environmental waters in Japan [3]. BPA is mainly used for the production of polycarbonate or epoxy resin [4]. 4-NP are the raw material of nonylphenol polyetoxylates, those are widely used nonionic surfactants and used as the complex of branched sidechain isomers [5]. The regulated analytical method of BPA and alkylphenols is GC–MS with derivatization because of their polar hydroxyl groups, however, derivatization procedure is complex and time-consuming [6].

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We have studied the analysis of non-volatile or thermally degradable chemicals in water by micellar electrokinetic chromatography (MEKC) which can provide higher resolution than that of high-performance liquid chromatography [7–9]. As far as we know, the simultaneous separation of BPA and alkylphenols have not been reported. In this paper, as the preliminary study, the optimization of analytical conditions for the separation of BPA, 4-tBP, 4-tOP and 4-NP was demonstrated. The effect of the buffer pH, surfactants and additives in MEKC was investigated. Five anionic surfactants having linear alkyl chains were used. They have different selectivities for ionic or hydrophobic compounds [7,8]. Four bile salts were also used as surfactants. Methanol, acetonitrile and cyclodextrins (CDs) were used as additives.

2. Experimental

2.1. Apparatus

MEKC was performed with a P/ACE 5010 CE system (Beckman, CA, USA). A 50 μm I.D. fused-silica capillary (GL Science, Tokyo, Japan) of 57 cm total length was used. The effective length was 50 cm to the detector. The instrument control, data collections and analysis were performed with a Compaq Deskpro personal computer (Compaq, TX, USA).

2.2. Reagents

BPA and three alkylphenols were obtained from Tokyo Kasei (Tokyo, Japan). Sodium dodecylsulfate (SDS), sodium *N*-lauroyl sarcosinate (SLN), sodium taurocholate (STC), α-, β- and γ-CD were obtained from Nacalai Tesque (Kyoto, Japan). Sodium cholate (SC) and sodium deoxycholate (SDC) were obtained from Wako (Osaka, Japan). Sodium taurodeoxycholate (STDC) was obtained from Sigma (MO, USA). Sodium *N*-myristoyl sarcosinate (SMN), sodium *N*-lauroyl-*N*-methyltaurate (LMT) and sodium laurylsulfoacetate (LSA) were donated by Nikko (Tokyo, Japan). AO-10-dodecylbromide (AO-10) obtained from Dojin Kagaku (Kumamoto, Japan) was used as the tracer of micelle. All reagents were used without further purification.

2.3. Procedure

Running solutions were prepared by dissolving 20 m*M* surfactants in a mixture of 20 m*M* sodium dihydrogenphosphate solution and 20 m*M* sodium tetraborate solution adjusted to pH values were 6.0, 7.0 and 8.0. These solutions were filtered through a 0.45 μm pore size membrane filter prior to use. Stock solutions of phenols were prepared in methanol. Sample solution was made by twenty-fold dilution of the stock solutions with running buffer. Each concentration of solute was 50 mg/l except especially described.

When the running solution was changed, the capillary was rinsed with 1 *M* NaOH for 1 min using 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 pressure (0.5 p.s.i., 5 s; p.s.i.=6894.76 Pa). The injection volume was about 3.0 nl. The set-up voltage and temperature were 20 kV and 30°C, respectively, throughout all experiments. The UV detection was performed at 214 nm (for SDS and LSA) or 280 nm (for other surfactants). Three times measurement were made for all runs and the average values in the paper.

As the surfactants, five surfactants having linear alkyl chains, SDS, SLN, SMN, LMT and LSA were used. AO-10 was added to the sample as the tracer of micelle. The use of four bile salts, SC, SDC, STC and STDC was investigated. They form less hydrophobic micelles than those formed by the surfactants having linear alkyl chains, therefore they were effective in the separation of relatively hydrophobic solutes [10].

For the simultaneous separation of four phenols including isomers, addition of organic solvent or cyclodextrin (CD) was investigated in order to improve the separation of hydrophobic phenols. Methanol (10%), acetonitrile (5%) or three types of CD (α-, β-, γ-, 10 m*M*) were used as additives. The interaction of hydrophobic solutes is decreased by their addition [9,11].

Table 1
Migration time and resolution values for surfactants having linear alkyl chains

Surfactant	Migration time/min					Resolution	
	4-tBP	BPA	4-tOP	4-NP	AO-10	4-tOP/4-NP	4-NP/AO-10
SDS	14.1	20.1	24.4	24.8	25.6	0.98	2.32
LSA	12.2	16.8	19.3	19.7	20.0	1.38	0.63
LMT	15.5	21.9	23.8	24.5	24.5	0.86	0
SLN	14.2	21.5	24.1	24.9	24.9	0.90	0
SMN	13.3	15.8	16.3	16.5	16.5	0.65	0

3. Results and discussion

3.1. Effect of pH

The effect of pH was investigated using borate-phosphate buffers with different pH values. The relative standard deviation of migration time at pH 6.0 and 7.0 exceeded 10%, while at pH 8.0, The deviation is within 3%. Therefore, a pH 8.0 buffer was used for the investigation of surfactants and additives.

3.2. Effect of surfactants

The migration time and resolution values of phenols are shown in Table 1. When SMN was used, 4-tOP, 4-NP and AO-10 were not separated. For SLN and LMT, 4-NP and AO-10 were not separated. They were partially separated with SDS and LSA as shown in Fig. 1. Calculated retention factors for each surfactant were shown in Table 2. SMN has the longest alkyl chain among the five surfactants, therefore the values are largest among them. This indicates that interaction of phenols and SMN micelles are too strong. For SDS and LSA, the values were smaller than those for other surfactants. The interaction of their micelles with phenols are moderate.

Four bile salts used for the improvement of the

Table 2
Retention factors for surfactants having linear alkyl chains

Surfactant	4-tBP	BPA	4-tOP	4-NP
SDS	4.5	14	64	86
SLN[a]	6.1	38	129	–
SMN[a]	9.4	165	456	–
LMT[a]	6.7	37	116	–
LSA	3.9	27	58	99

[a] 4-NP and AO-10 (micelle's tracer) are not separated.

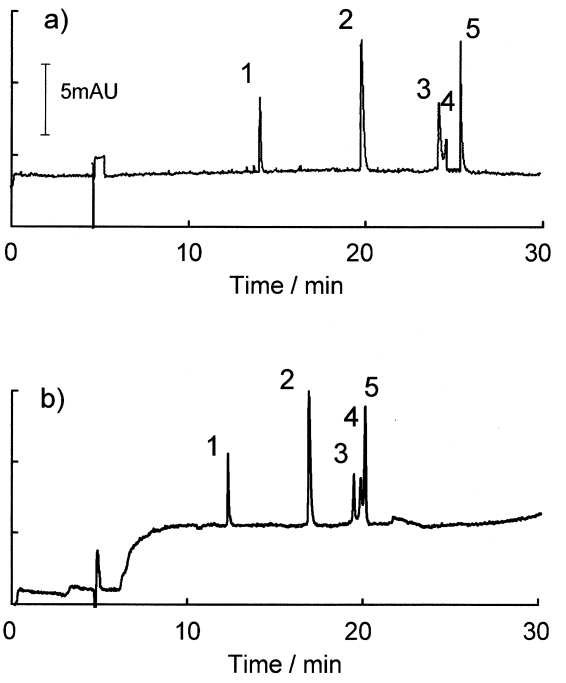

Fig. 1. Electropherograms of phenols using anionic surfactants with linear alkyl chains, (a) SDS and (b) LSA. Other conditions: Capillary, 57 cm (50 cm to the detector)×50 μm I.D.; concentration of surfactants, 20 mM; running buffer, 20 m*M* borate-phosphate (pH 8.0); applied voltage, 20 kV; detection wavelength, 214 nm; temperature, 25°C. Peak identification: 1=4-tBP; 2=BPA; 3=4-tOP; 4=4-NP; 5=AO-10.

Table 3
Migration time and resolution values for bile salts

Surfactant	Migration time/min				Resolution,
	4-tBP	BPA	4-tOP	4-NP	4-tOP/4-NP
SC	7.06	8.31	11.7	11.7	0
SDC	7.21	8.06	11.4	11.6	0.75
STC	6.66	8.17	11.6	12.0	0.74
STDC	7.58	8.95	12.3	12.6	0.83

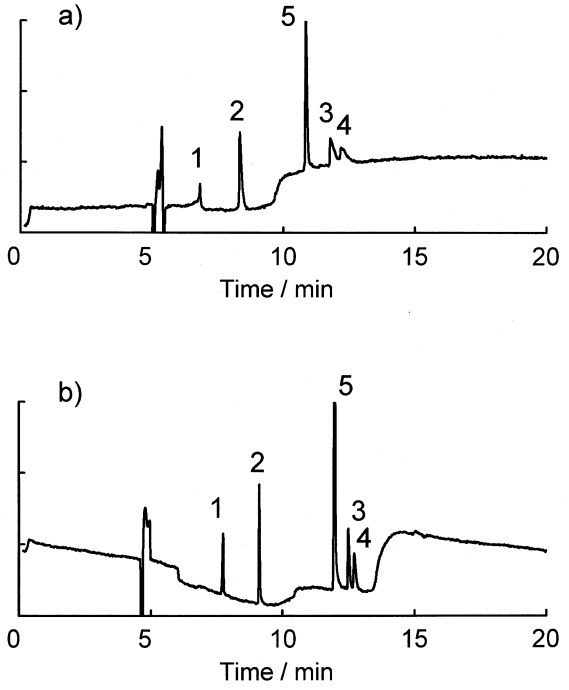

Fig. 2. Electropherograms of phenols using bile salts,(a) STC and (b) STDC. Other conditions as in Fig. 1.

separation of hydrophobic solutes, 4-tOP, 4-NP and its isomers. The migration time and resolution values of phenols are shown in Table 3. 4-NP isomers were not separated with any bile salts. The pherograms with STC and STDC are shown in Fig. 2. In case of these bile salts, AO-10 migrated faster than 4-tOP and 4-NP. This shows AO-10 is not suitable for the tracer of bile salt micelle.

We chose SDS as the surfactant using for further investigation because of the better separation among the surfactants, baseline stability and having no absorbance at 214 nm.

3.3. Effect of additives

The migration time and resolution values of phenols are shown in Table 4. The pherograms with SDS solution added organic solvent is shown in Fig. 3. Addition of methanol or acetonitrile, the separation of 4-tOP and 4-NP was improved, however, their migration time is much longer because of the decrease of electroosmotic velocity. 4-NP isomers were not separated. The addition of CD improve both of the separations of hydrophobic solutes and isomers. The selectivity of CD depends on its cavity size. He et al. demonstrated the separation of 4-NP isomers by using SDS solution with HP-β-CD [12]. We used three types of CDs having different cavity sizes for the separation, and the results are shown in Fig. 4. When α-CD was added, similar chromatogram as used SDS only was obtained. This shows the interaction of α-CD and phenols is slight and α-CD has no effect in the improvement of the separation. On the contrary, 4-NP isomers were separated by the addition of β- or γ-CD. Their cavity size is suitable

Table 4
Migration time and resolution values using additives

Additive	Migration time/min				Resolution	
	4-tBP	BPA	4-tOP	4-NP	4-tOP/4-NP	4-tBP/BPA
Methanol (10%)	14.8	21.3	36.7	39.5	1.91	10.5
Acetonitrile (5%)	14.3	20.0	30.6	32.2	1.03	5.53
α-CD (10 m*M*)	12.7	15.5	17.6	17.9	0.45	3.56
β-CD (10 m*M*)	5.06	5.23	6.47	7.63[a]	14.5	0.70
γ-CD (10 m*M*)	5.84	6.23	6.58	7.95[a]	4.87	1.67

[a] The value of isomer peak most closed to 4-tOP.

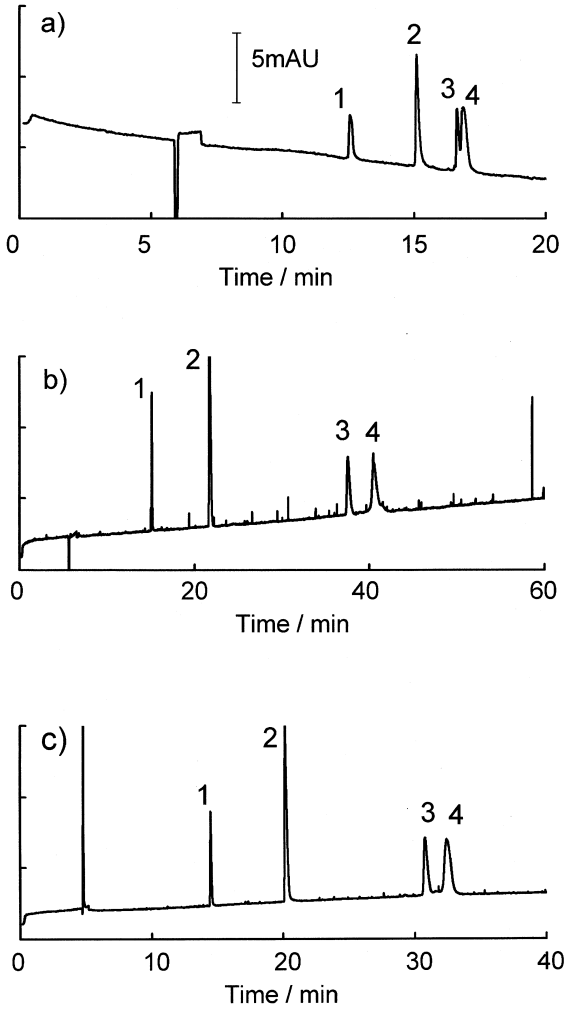

Fig. 3. Electropherograms of phenols using SDS with organic solvents. (a) SDS only, (b) 10% methanol and (c) 5% acetonitrile. Other conditions as in Fig. 1.

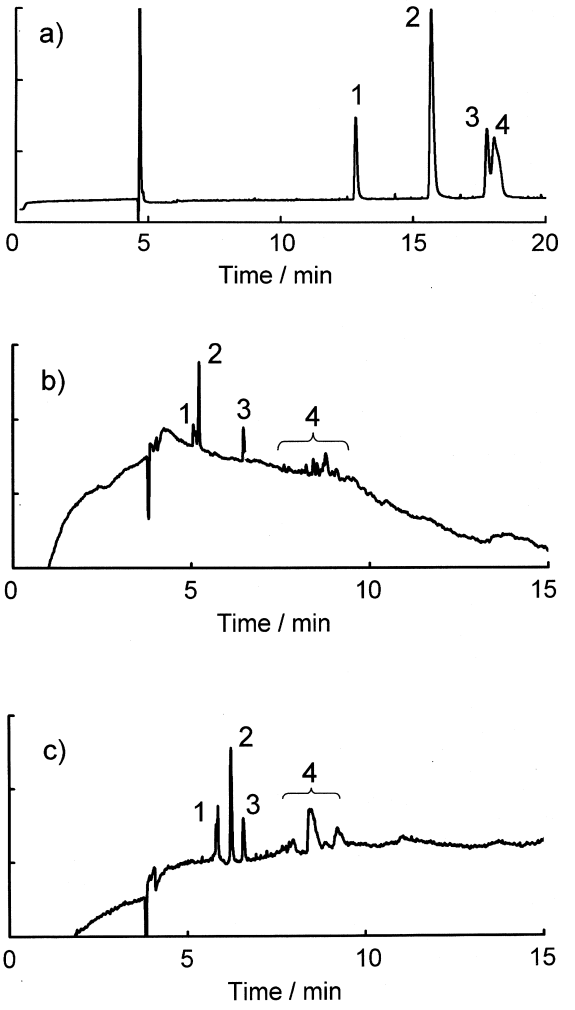

Fig. 4. Electropherograms of phenols using SDS with CDs. (a) α-CD, (b) β-CD and (c) γ-CD. Concentration of CD: 10 m*M*. Other conditions as in Fig. 1.

for the separation of isomers. When β-CD was used, 4-NP isomers were separated into more than ten peaks, however, the separation of 4-tBP and BPA was incomplete. When γ-CD was used, 4-tBP and BPA were separated and 4-NP isomers were separated into three peaks. More improvement of both separations of four phenols and 4-NP isomers is now under investigation. The reproducibility of the peak areas of 4-NP isomers became poor than that of other phenols because of peak separation. Combination of some concentration method is necessary for the application of this method to real environmental samples.

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