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On-chip micellar electrokinetic chromatographic separation of phenolic chemicals in waters

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Abstract

This paper describes on-chip micellar electrokinetic chromatography (MEKC) separation of bisphenol A and 3 kinds of alkylphenols, which have been recently recognized as endocrine disrupting chemicals for fish by the Japanese government, using microchip capillary electrophoresis with UV detection. We successfully obtained high-speed separation of the phenolic chemicals within 15 s as optimizing in microfluidic controls and MEKC separation conditions. We obtained fairly good linearity with correlation coefficient of over 0.98 from 0 to 50 mg/l phenolic chemicals except for 4-nonylphenol, which sample is the mixture of many geometrical isomers ($r = 0.86$). The values of the relative standard deviation for peak height in 50 mg/l phenolic chemicals were less than 8% except for bisphenol A (11.0%). The limits of detection obtained at a signal-to-noise ratio of 3 were from 5.6 to 20.0 mg/l. To realize on-site monitoring, we described strategy for on-chip MEKC analysis of the phenolic chemicals in waters using a portable analyzer based on microfluidic devices.

Keywords: Water analysis; Endocrine disruptors; Phenols; Microchip capillary electrophoresis; Buffer composition; Microfluidic controls; Strategy for on-site monitoring

1. Introduction

Several chemicals have been suspected of having endocrine disrupting effects since the 1990s [1]. Among the 67 candidate for endocrine disruptors (EDs) postulated in the strategic program of SPEED '98 [2], only 3 kinds of phenolic chemicals such as 4-nonylphenol (4-NP), 4-(1,1,3,3-tetramethylbutyl)phenol (4-tOP) and bisphenol A (BPA) were postulated to have strong endocrine disrupting effects on fish at the concentrations believed to be present in the environment in Japan, as shown from results of typical EDs screening using the vitellogenin assay for Medaka fish and the over a two-generation full-life-cycle study for Medaka fish [3]. The other phenolic chemical in the candidate EDs, 4-tert-butylphenol (4-tBP) is under investigation for possible study of endocrine disrupting effects in detail. For the assessment of ecosystem exposure to the above-mentioned phenolic chemicals, the development of analytical methods for these phenolic chemicals has become more important recently.

In Japan, the four phenolic chemicals, 4-NP, 4-tOP, BPA and 4-tBP have been often found in environmental waters although chemical pollution by the suspected EDs has been much controlled every year since the SPEED '98 program. The latest data on the phenolic chemicals in the environment are disclosed in the official data of Ministry of the Environment in Japan [4] and are summarized in Table 1. The detected concentration-range was from N.D. (less than 0.1 μg/l) to several μg/l for the phenolic chemicals. At present, gas chromatography/mass spectrometry (GC/MS) is generally used as the analytical method [5], however, phenolic chemicals, which are non-volatile or thermally degradable, cannot be analyzed directly using GC/MS. The derivatization procedure necessary for GC/MS is well known to be complex and time-consuming.

Capillary electrophoresis (CE) has high separation efficiency and can be easily applied for the analysis of non-volatile or

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Table 1
Detection frequency of the phenolic chemicals in waters in Japan (FY 2003)

	River	Lake	Under-ground	Sea	Total	Detected range (μg/l)
4-NP	42% (21/50)	40% (2/5)	20% (2/10)	0% (0/10)	33% (25/75)	[a]N.D.–2.9
4-tOP	60% (30/50)	0% (0/5)	10% (1/10)	20% (2/10)	44% (33/75)	[b]N.D.–0.47
BPA	84% (42/50)	60% (3/5)	20% (2/10)	50% (5/10)	69% (52/75)	[b]N.D.–0.40
4-tBP	30% (15/50)	0% (0/5)	10% (1/10)	0% (0/10)	21% (16/75)	[b]N.D.–1.9

[a] N.D. is less than 0.1 mg/l.
[b] N.D. is less than 0.01 mg/l.

thermally degradable chemicals [6–8]. In particular, micellar electrokinetic chromatography (MEKC) can provide higher resolution than that of high-performance liquid chromatography (HPLC) for phenols [9–14]. We [15,16] and several groups [17–19] have studied the separation of the phenolic chemicals suspected as EDs using MEKC mode.

For on-site monitoring of chemicals in waters, microfluidic devices based on CE (microchip CE, MCE) have attracted much attention, because of the relatively small instrumentation capability with the advantage of high-throughput screening and μl level of sample and waste volumes [20]. We have successfully demonstrated dissolved organic carbon in waters using MCE [21], however, as far as we know, MCE separation for the phenolic chemicals has not been reported in the literature.

We investigated a preliminary separation of the phenolic chemicals, BPA and three alkylphenols using MCE/UV detection without any derivatization procedure in the first study. Our final goal of the on-going research is to design on-site analysis for phenolic chemicals using a portable microfluidic system based on MCE. The objectives of the study are to demonstrate high-speed separation of the phenolic chemicals in standard solution to optimize in microfluidic controls for MCE and MEKC separation conditions, and also to examine the calibration curves, reproducibility, and limit of detection (LOD) to make a strategy for on-site monitoring based on on-chip MEKC analysis for the phenolic chemicals in waters.

2. Experimental

2.1. Chemicals and solutions

Bisphenol A and three alkylphenols, 4-NP, 4-tOP and 4-tBP were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Methanol was purchased from Kanto Chemical (Tokyo, Japan). Sodium dodecyl sulfate (SDS), acetonitrile, and β-cyclodextrin (CD) were purchased from Nacalai Tesque (Kyoto, Japan). Other reagents were of analytical grade. All reagents were used without further purification. Water was purified with a Millipore Q system (Nihon Millipore, Tokyo, Japan).

Running buffer solutions were prepared by dissolving 20 mM SDS and 5 mM β-CD in a mixture of 20 mM borate-phosphate (pH 8.0) adding 10% (v/v) methanol. A HM-60V pH meter (DKK-TOA, Tokyo, Japan) was used for pH adjustment of the borate-phosphate buffer. The phenolic chemicals solutions (50 mg/l) used for separation optimization were prepared by 20-fold dilution of the stock solutions with the running buffer solution, after stock solutions of the phenolic chemicals (1000 mg/l) were prepared.

2.2. Apparatus and microchip

On-chip MEKC was performed with a MCE-2010 system (Shimadzu, Kyoto, Japan). A Type U microchip (Shimadzu; 12.5 mm × 35.0 mm × 1.25 mm) was a cross-type with a sample injection channel (50 μm × 20 μm × 7.5 mm), a separation channel (50 μm × 20 μm × 33 mm, effective separation channel length of 25 mm) and four reservoirs (ca. 2.2 mm diameter at the top) at the ends of each channel as described in detail elsewhere [22]. The microchannels on the base plate (0.25 mm thick) were made using a conventional wet etching process for quartz glass micromachining technology [23]; the shallow channel shapes are 50 μm wide at the top 20 μm deep at the center. The optical slit (sputterd Si) was formed on the reverse side of the base plate. The Pt/Ti contact electrode was sputtered at the resevoirs on the cover plate (1.0 mm thick). The data on electric currents in each of the microchannels were automatically monitored using the MCE 2010 system for all operations.

The electrophoretic sample injection and separation was performed by applying a voltage to the four sample and buffer reservoirs using computer-programmed sequencing of the MCE-2010 system. The separation behavior was observed using direct UV detection at 210 nm for the whole separation channel, using a 1024-point linear photodiode array detector.

To compare the conventional CE, MEKC separation for the phenolic chemicals were performed with a CAPI-3200Q CE system (Otsuka Electronics, Osaka, Japan) equipped with a photodiode array UV detection system. The fused silica capillaries (GL Science, Tokyo, Japan) used were 50 cm long (37.8 cm effective length) with an inner diameter of 75 μm.

2.3. Procedure of MCE and CE

On-chip MEKC separation was performed by microfluidic voltage control, with a pinched injection (ca. 80 pl of sample injection volume) using the MCE 2010 system, after which the microchip and sample inlet tube and valves were rinsed with pure water for 5 min and running buffer solution for 5 min automatically.

To compare the conventional MEKC separation using a CAPI-3200Q CE system, the sample solutions (2 mg/l) prepared

were injected into the capillary by gravity injection (20 mm, 30 s), which corresponds to the sample injection volume of ca. 8 nl, after which the capillary was rinsed with 1 M NaOH for 1 min using a vacuum at the detector reservoir, followed by rinsing with pure water for 3 min and running buffer solution for 3 min. The MEKC separation with UV detection at 210 nm was performed at constant 20 kV as the applied voltage to the sample inlet side at a temperature of 30 °C in the capillary cartridge.

3. Results and discussion

3.1. Microfluidic controls

To demonstrate high-speed separation of the phenolic chemicals in the limited effective separation channel (25 mm length), we initially examined an Ohm's law plot, an electric current against electric field strength plot under a MEKC buffer condition using the MCE system. When the voltage applied was up to 1.8 kV (the safety limit for the MCE system), the electric current in the separation channel showed good correlation ($r = 0.999$) with in the applied voltage from 0 to 1.8 kV, which corresponded to electric field strengths from 0 to ca. 450 V/cm in the separation channel filled with the optimized running buffer solution. The results indicated that we can only apply 1.5-fold higher electric field strength for on-chip separation compared with conventional MEKC separation, because the values of maximum electric fields in MCE and conventional CE were ca. 450 and 320 V/cm [15], respectively.

We selected a conventional pinched injection to demonstrate complete separation of the phenolic chemicals among the various kinds of microfluidic controls in sample injection [24]. The pinched injection has the advantage in separation of a precisely controlled sample plug, however, it has a disadvantage in concentration sensitivity due to the small sample injection volume.

We investigated optimization of the microfluidic voltage control and peak separation at the maximum applied voltage of 1.8 kV (ca. 450 V/cm) for the separation channel in MCE. The other applied voltage conditions for each reservoir were optimized as follows; in sample loading mode, 0.30, 0.00, 0.18 and 0.25 kV were applied to sample reservoir, sample waste reservoir, buffer reservoir and buffer waste reservoir for 25 s, respectively, and in sample separation mode, 0.90, 0.90, 1.80, 0.00 kV were applied to sample reservoir, sample waste reservoir, buffer reservoir and buffer waste reservoir for 13 s, respectively.

3.2. Optimization of peak separation

We have already investigated the MEKC separation of the phenolic chemicals, BPA and three alkylphenols with several kinds of organic solvents or CDs using conventional CE [15]. Under the above-mentioned CE conditions, we examined on-chip MEKC separation for a relatively high sample concentration, 50 mg/l phenolic chemicals, which concentrations were different from those as described in [15]. We had disappointing results with no repeatable peaks in the separation using MCE under the CE conditions. In particular, the peak of the most hydrophobic 4-NP was not frequently obtained. As we investigated the addition of methanol to the sample solution to improve the solubility of phenolic chemicals, we observed effective repeatability in the on-chip separation. From a practical point of view regarding the volatility of the sample solution in the sample reservoir, the optimized methanol concentration in the phenolic chemicals was found to be relatively high at 68% from amongst the several methanol concentrations investigated.

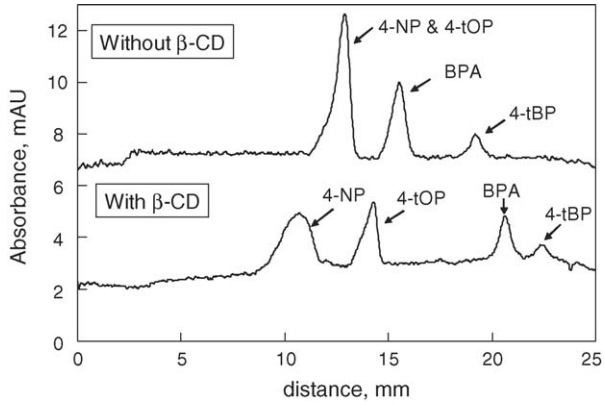

Fig. 1. On-chip MEKC separation of the phenolic chemicals using whole-channel UV detection, with and without β-CD addition. Conditions: 13-s-separation; 50 mg/l phenolic chemicals.

In the CE separation, we obtained several peak separations for the 4-NP isomers using β-CD [15]. From a practical viewpoint, the peak separation of the 4-NP isomers is not useful for on-site monitoring. We examined the additive effect of β-CD on the on-chip separation for the phenolic chemicals. On-chip MEKC separation for the phenolic chemicals, with and without β-CD addition are shown in Fig. 1. In β-CD addition, we obtained the complete separation for the peaks of BPA and three alkylphenols at ca. 23, 21, 14 and 11 mm from the cross channel to the separation channel using the MCE with the whole channel UV detection. The β-CD addition was observed to be greatly effective for separation of 4-tOP and 4-NP. Concerning the additive effect of β-CD on the separation of phenolic chemicals, we obtained different effects from conventional CE separation [15] and MCE separation. In the former CE separation, we obtained several peak separations for the 4-NP isomers. On the contrary, in the latter MCE separation we obtained overlapped peak for the 4-NP isomers. The different effects might have resulted from the difference in the effective separation length and the applied voltage fields.

We successfully demonstrated high-speed on-chip MEKC separation in 13 s. As preliminary MEKC separation was achieved within 17 min [15], we obtained ca. 75 times more rapid separation. Using similar conditions in the MCE separation, we successfully achieved complete separation within 9 min using conventional MEKC separation with a relatively short capillary column (50 cm) with relatively high 400 kV/cm electric field strength to obtain high-speed separation. Using similar conditions, ca. 40 times more rapid separation was

Table 2
Linearity of calibration curves, relative standard deviations (RSDs) and limits of detection (LOD) of peak height for the phenolic chemicals

Phenolic chemicals	4-NP	4-tOP	BPA	4-tBP
Correlation coefficient[a]	0.86	0.98	0.99	0.99
RSD (%)[b]	7.7	7.0	11.0	7.4
LOD (mg/l)[c]	5.6	8.0	6.7	20.0

[a] Calculation from linear calibration curves of peak height.
[b] $n = 24$.
[c] S/N = 3.

achieved in spite of ca. 15 times shorter in effective separation length.

3.3. Calibration curves, reproducibility, LOD in on-chip MEKC separation

We examined validation, calibration curves, reproducibility, and LOD for the on-chip MEKC separation. The calibration curves of the phenolic chemicals were obtained from 0 to 50 mg/l from the peak heights. Except for 4-NP, we obtained fairly good linearity (correlation coefficient $r > 0.98$). In 4-NP, a lower correlation coefficient ($r = 0.86$) was obtained. It may be explained that 4-NP is a mixture of many geometrical isomers. In conventional MEKC separation using the optimized on-chip MEKC condition, we observed over ten peaks assigned as 4-NP isomers.

The linearity of calibration curves, reproducibility, and LOD obtained are summarized in Table 2. We obtained good repeatability with less than 8% relative standard deviation (RSD), except for BPA (11.0%). In our experimental experience, we can much improve the repeatability using the internal standard method. We also obtained ca. 5 to 20 mg/l of LOD (S/N = 3). As we expected, there are serious issues regarding the low concentration sensitivity. To realize on-site monitoring for the phenolic chemicals in waters, we should make much improvement in concentration sensitivity to achieve the minimum target values of 0.1 μg/l.

4. Conclusion

We successfully obtained high-speed separation for the phenolic chemicals within 15 s with optimized microfluidic controls and on-chip MEKC separation for the first time as far as we know. As there are serious issues regarding low concentration sensitivity, we have two major strategies for the on-site monitoring as follows, one is on- or/and off-chip concentration and the other one is to investigate highly sensitive detection techniques such as electrochemical detection, laser-induced fluorescent detection. The studies on improved on-chip MEKC are currently under investigation.

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