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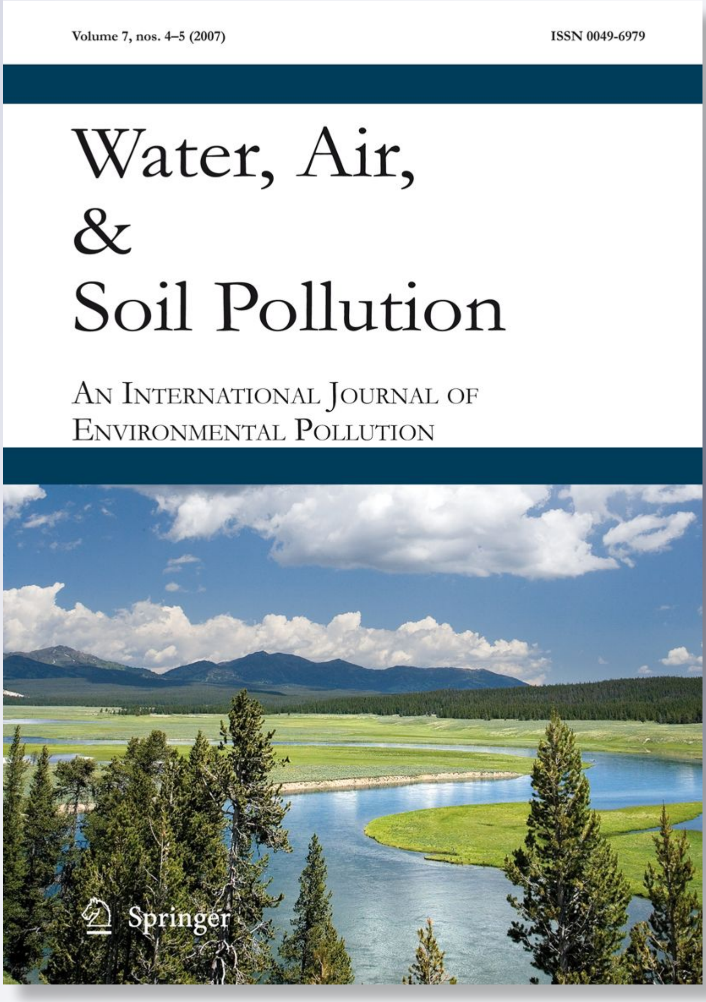

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Separation and Determination of Three Phenolic Xenoestrogens in Industrial Wastewater by Micellar Electrokinetic Chromatography on Polydimethylsiloxane Microchip

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Abstract The separation on microchip provides the advantages including high efficiency, increased throughput, reduced quantities of hazardous materials, cost saving, relatively facile instrumentation, improved portability, etc. A technique of micellar electrokinetic chromatography (MEKC) coupled with amperometric detection has been actualized on a polydimethylsiloxane microchip for the rapid separation and determination of three phenolic xenoestrogens as octylphenol (OP), 4-nonylphenol (4-NP), and bisphenol A (BPA). The baseline separation of these phenolic xenoestrogens is successfully obtained within 55 s under the optimized MEKC conditions with borate running buffer of pH 8.0 containing sodium dodecyl sulfate and β-cyclodextrin. The linear range for OP, 4-NP, and BPA are 20–1,000, 15–1,000, and 20–1,000 μg/L with the detection limit of 5.0, 4.0, and 3.0 μg/L, respectively. The present method is successfully applied for the determination of these phenolic xenoestrogens in some industrial wastewater samples from mainland of China with the recoveries ranged from 90.2 to 109.4%.

Keywords Polydimethylsiloxane microchip · Micellar electrokinetic chromatography · Amperometric detection · Phenolic environmental estrogen · Industrial wastewater

1 Introduction

Presently, a lot of phenolic compounds, usually be used as industrial plasticizer, have been reported to be the endocrine disrupters as the simulant estrogen. They might damage the health and procreation of animals and human (Wakida et al. 2006; Volkel et al. 2002; Park and Choi 2009). In last year, the Taiwan food scandal is a typical occurrence over the abuse of those plasticizers such as DEHP to simulate the palm oil in foods and drinks as a clouding agent. It has been linked to the problems of lost balance of hormones of children. The occurrence widely embroils the mainland, Hong Kong, and Macao of China and some other Asiatic countries. Therefore, it results severe economic damnums and the civil anxiety. Among those phenolic plasticizers, some most typical compounds, such as *p-tert*-octylphenol (OP), 4-nonylphenol (4-NP), bisphenol A (2, 2-bis-(4-hydroxyphenyl)-propane) (BPA), etc., deserved particular attention because of their strong endocrine disrupting effects, wide application, and

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ubiquity in environment (Jin et al. 2004; Li et al. 2004; Morrissey et al. 1987; White et al. 1994).

OP has been reported as an estrogen in fish, avian, and mammalian cells which mimicked the effects of 17β-estradiol (second predominant sex hormone) by binding to the estrogen receptor (White et al. 1994). In vitro studies, it has been demonstrated as the most potent alkylphenol to stimulate a number of biological responses, such as cell growth and specific gene transcription like 17β-estradiol (Harazono and Ema 2001; Jobling and Sumpter 1993). 4-NP has been reported to cause the estrogenic effects in fish and other aquatic organisms (Jobling et al. 1996; Gronen et al. 1999). And the exposure in BPA has been reported to cause a chromosomal abnormality in the oocytes of female mice, leading to the risk of galactophore developing abnormity (Timms et al. 2005) and urethral malformations (Markey et al. 2001). 4-NP and OP have been classified in priority hazardous substances in the European Union (EU 2000). And they are also included in the priority list of pollution control substances in Oslo–Paris Convention (OSPAR 2007). On March 1 of last year, the EU has banned the use of plastic infant feeding bottles containing BPA (EU 2011). Such products are also prohibited to be imported into the USA. Therefore, it is very important to develop simple, rapid, and efficient methods for monitoring these compounds.

Because of the polarity of hydroxyl groups, the regular analytical methods of OP, 4-NP, and BPA such as LC-FD (Cai et al. 2004a) or LC-MS (Xiao et al. 2011; Ferrer et al. 2011; Ma et al. 2010) basically focus on their derivatives. However, the derivate procedure is complex and time-consuming. Nowadays, the micellar electrokinetic chromatography (MEKC) method is proved to be a rapid, powerful, and efficient technique due to its major merit of high separation efficiency, extremely low solvent consumption, nano-liter sample requirement, facility of automation, and low running cost (Li et al. 2009). Several authors have reported the separation of OP, 4-NP, and/or BPA using MEKC in capillary (Cai et al. 2004b; Takeda et al. 2000) or glass microchip (Wakida et al. 2006). However, the glass microchip is somewhat expensive, fragile, and susceptible to plugging. In recent years, the polydimethylsiloxane (PDMS) polymer has become an important alternative material for chip fabrication due to its low price and facility for operation (McDonald and Whitesides 2002; Slentz et al. 2002; Garcia et al. 2005; Tokuyama et al. 2005; Roman et al. 2005). But the separation of those phenolic compounds on PDMS microchip integrated with electrochemical detection has not been reported yet.

In this paper, the optimization of conditions for the separation of OP, 4-NP, and BPA was actualized on a PDMS microchip using MEKC mode. The effect of buffer pH, surfactants, and additives in MEKC was investigated to get a baseline separation of those targets. The proposed method had been successfully applied to detect the targets in solid-phase extractions (SPE) (Tong et al. 2010; Oleschuk et al. 2000; Sun et al. 2009; Han et al. 2005; Koeber et al. 2001; Barcelo and Hennion 1995) of industrial wastewater samples from mainland of China with satisfied results.

2 Experiments

2.1 Instruments and Chemicals

A double-channel programmable power source (Smart-2, Nanjing Hengyue Electro-optical Technique Co., Nanjing, China) is used to provide the potential for chip electrophoresis and electro-driven sampling. The separation voltage is set as 800 V, and the sampling voltage is set as 600 V. A RST3100 electrochemical workstation (RST Instrument Co. Ltd., Suzhou, China) is applied for amperometric detection with three-electrode system. A Trisep^{TM}-2100 pressurized capillary electrochromatography system (Unimicro Technologies, USA) equipped with a UV detector serves for capillary electrophoresis. An uncoated fused-silica capillary with dimension of 50 μm i.d. was purchased from Yongnian Fiber Plant

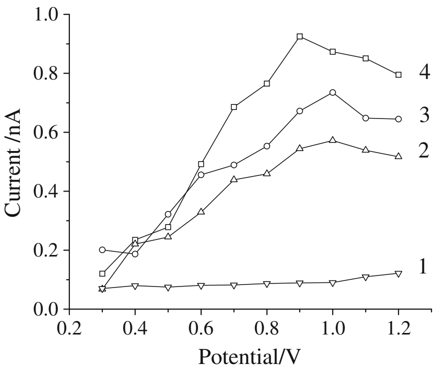


Fig. 1 The dependence of amperometric currents of *1* base line, *2* 200 μg/L OP, *3* 200 μg/L 4-NP, and *4* 200 μg/L BPA upon the applied potential. Conditions: Separation voltage, 800 V; sample injection, 600 V for 1 s; running buffer solution, 12.5 mM boric acid-borax (pH=8.0), 17 mM SDS, 5 mM β-CD

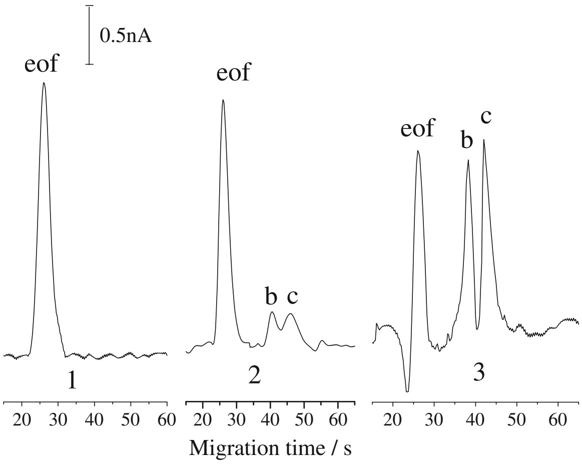


Fig. 2 Electropherograms of *b* 4-NP and *c* BPA with *1* 12.5 mM borax buffer solution (pH 8.0), *2* 12.5 mM borax buffer, 20 mM SDS (pH 8.0), and *3* 12.5 mM borax buffer, 20 mM SDS, 5 mM β-CD (pH 8.0). Other conditions are same as in Fig. 1

(Hebei, China). Sep-pak C18 SPE cartridges (500 mg/3 mL, Waters, USA) are used for sample enrichment. They were conditioned by being washed with 10 mL methanol and followed by 10 mL diluted HCl (pH within 2.0 to 3.0).

Referred to the paper (Wang et al. 2006), the PDMS sheet with cross-channel is cast with a silica mold, and the prospected PDMS microchip is achieved by sealing together with another PDMS sheet. The working electrode for amperometric detection is made of a sharpened graphite rod. The side surface of cylinder was insulated, and the polished front disk (100 μm of diameter) is served as the active area of electrode. It was held on a fine-tunable 3D regulator (Shanghai Lianyi Instrument Factory of Optical Fibers and Lasers, Shanghai, China) and exactly aimed opposite the mouth of microchannel under the CVM-100E video-microscope (Shanghai Changfang Optical Instrument Co., Shanghai, China). The gap size between electrode and microchannel mouth obviously influenced the detection that would be very remarkably noisy if the gap was too small otherwise the lower sensitivity and severe peak tailing. The optimized gap size is ca. 40 μm. Aside, there are Pt counter electrode and Ag/AgCl reference electrode inserted in the detection pool.

Octylphenol, bisphenol A, 4-nonylphenol, and β-cyclodextrin (β-CD) were purchased from Aladdin Reagent (Shanghai, China). Sodium dodecyl sulfate (SDS) was purchased from Di Yun Technology Co., Ltd (Hong Kong, China). Sylgard 184 (PDMS, including silicone elastomer and curing agent) was obtained from Dow Corning (Midland, MI, USA). $Na_2B_4O_7$, H_3BO_3, HCl, and NaOH were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All reagents are of analytical grade. All solutions were prepared with ultrapure water and passed through a 0.22-μm cellulose acetate filter (Shanghai Bandao Factory, Shanghai, China).

Running solution was prepared by dissolving adequate concentrated SDS and β-CD in borax (pH 8.0) buffer. The contents of those components were optimized for better separation efficiency. Standard stock solutions of OP, 4-NP, and BPA were prepared by dissolving 100 mg quantitative standard in 50 mL ethanol and kept in refrigerator at 4°C. They could be diluted to the desired concentration with the running buffer before use.

2.2 Sample Preparation

The real wastewater samples were collected from several factors in mainland of China. These samples were

Fig. 3 Effect of **a** pH and **b** β-CD concentration on resolution of 4-NP and BPA. Other conditions are same as in Fig. 1

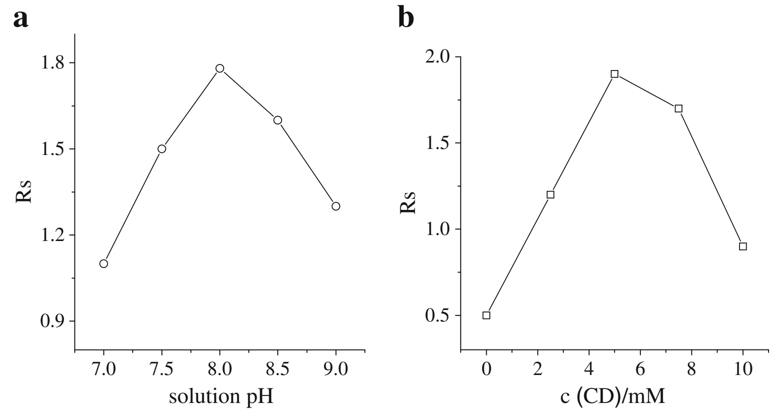
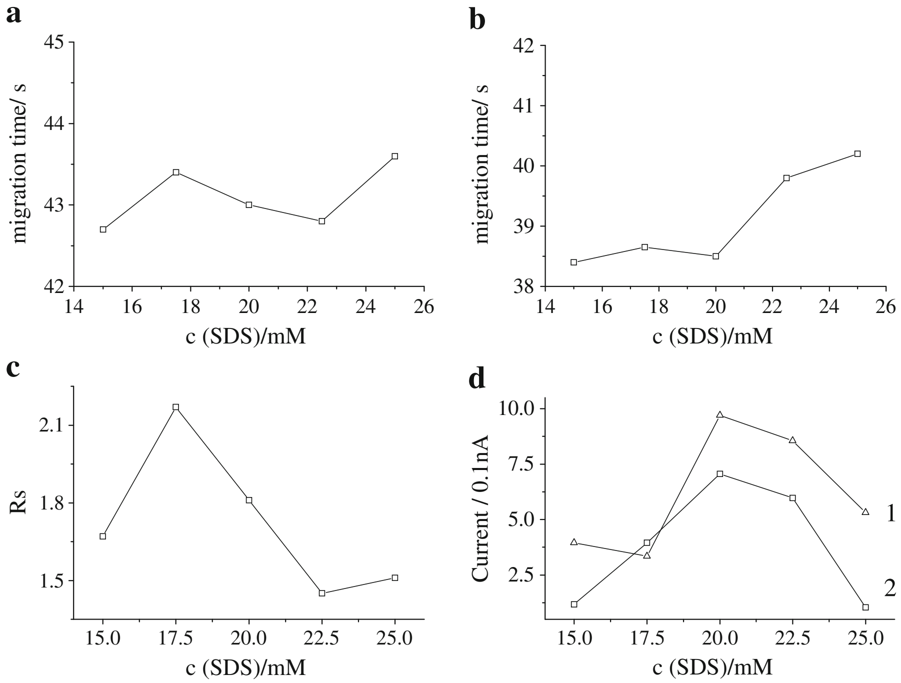


Fig. 4 Effect of SDS concentration on **a** migration time of BPA, **b** migration time of 4-NP, **c** the resolution of two analysts, and **d** the current response of *1* BPA and *2* 4-NP. Other conditions are same as in Fig. 1



stored in the amber glass bottles and kept at 4°C in refrigerator after biological treated in Lab of Environmental Engineering of Soochow University. After twice filtered with 0.22 μm syringe filter and adjusted their pH between 2.0 to 3.0 with 0.1 M HCl, 20 mL of every sample was flowed through SPE cartridge at the flow rate of 5 mL/min. After that, the solutes were washed off from cartridges with total 10 mL of ethanol for four times. These fractions were collected and blow-condensed with a gentle stream of nitrogen to 1.0 mL and then diluted to 2.0 mL with the running buffer for the final analysis. The C18 cartridge is in commonest use for SPE, and it has sufficient and reproducible recovery for organic compounds including phenolic compounds (Santana et al. 2009). A phase ratio of 10 for condensation was obtained.

3 Results and Discussion

3.1 Selection of Detection Potential for Phenolic Xenoestrogens

In amperometric detection mode, the detection potential should be optimized because the electrochemical response of the analytes greatly depended on it. The hydrodynamic voltammetric experiments were carried out

Table 1 Analytical performance of presented method

	Compound	Regression equation	*r*	Linear range (μg/L)	LOD (μg/L)
Condition 1[a]	4-NP	$Y=0.825+0.698X$	0.9941	10–1,000	4.0
	BPA	$Y=1.467+0.556X$	0.9959	20–1,000	2.0
Condition 2[b]	OP	$Y=0.118X-5.476$	0.9944	20–1,000	5.0
	4-NP	$Y=0.144X-1.112$	0.9901	15–1,000	4.0
	BPA	$Y=0.121X-1.412$	0.9952	20–1,000	3.0

[a] With 12.5 mM boric acid-borax (pH=8.0), 20 mM SDS, and 5 mM β-CD as running buffer; at 800 V of separation voltage and 600 V for sample injection for 1 s

[b] With 12.5 mM boric acid-borax (pH=8.0), 17 mM SDS, and 5 mM β-CD as running buffer; at 800 V of separation voltage and 600 V for sample injection for 1 s

Table 2 The detected results, recoveries, and RSD of three wastewater samples ($n=3$)

Compound		Sample 1[a]	Sample 2[b]	Sample 3[c]
BPA	Found (μg/L)	–	–	48.8(48.0)
	Added (μg/L)	100	100	100
	Detected (μg/L)	90.2	93.5	160.3
	Recovery (%)	90.2	93.5	107.7
	RSD (%)	5.5	4.5	3.1
	Plate number/m	– (–)	– (–)	75,439 (39,144)
4-NP	Found (μg/L)	51.3 (50.0)	25.9 (25.1)	17.1 (18.1)
	Added (μg/L)	60	60	50
	Detected (μg/L)	117.6	79.8	69.9
	Recovery (%)	105.7	92.9	104.2
	RSD (%)	6.4	4.9	2.9
	Plate number/m	99,650 (47,968)	128,243 (30,733)	89,571 (48,976)

[a] A coke factory in Shanxi

[b] A petrochemical company in Nanjing

[c] A petrochemical company in Hebei

to obtain an optimal detection potential. It was observed in Fig. 1 that the signals were both increased obviously with the increase of the potential until 0.9 to 1.0 V for three analytes. When the detection potential was higher than 1.0 V, the current of the analytes decreased meanwhile the background current increased (curves 1–4), so 1.0 V (vs. Ag/AgCl) was decided as the optimal detection potential for high response and signal/noise ratio. This factor is similar with what we had reported previously (Qian et al. 2010) as use of same working electrode.

3.2 The Separation Efficiency of MEKC on PDMS Microchannel

As a simplest model, the separation of BPA and 4-NP was investigated for evaluation of electrophoretic separation ability of PDMS microchannel. Unfortunately, only one peak of electroosmotic flow (EOF) but not any signal of analytes was observed with pure borax solution (pH=8.0) as running buffer (see curve 1 in Fig. 2). It might be assumed that BPA and 4-NP were co-migrated with EOF because they were kept to be electroneutrality in this running buffer.

The MEKC mode is more efficient for their separation. As curve 2 in Fig. 2, the peaks of BPA and 4-NP appeared when SDS was added into the borax buffer solution; here the micelles of SDS acted as pseudostationary phase which was expected to improve the selectivity for analytes, but still could not be baseline separated. As reported, the formation of β-CD inclusion complexes would result in the good separation (Bo et al. 2002), which could be attributed to the decreased mobility of analytes and the increased buffer viscosity (Tong et al. 2010) that changed along with the concentration of β-CD; the simultaneous existed β-CD in running buffer led to the baseline separation meanwhile greatly improving the detection response (see curve 3 in Fig. 2).

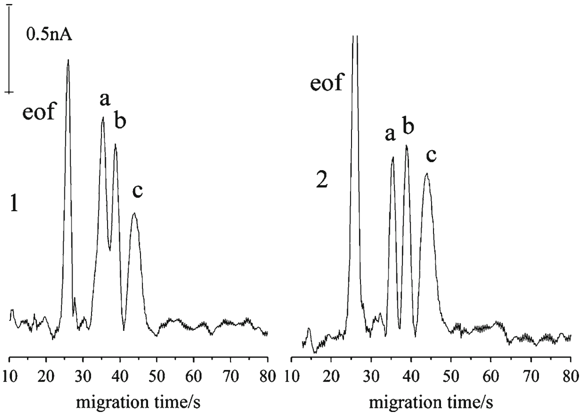


Fig. 5 Electropherograms of *a* OP, *b* 4-NP, and *c* BPA under the conditions of *1* 12.5 mM borate, 20.0 mM SDS, 5.0 mM β-CD (pH 8.0) and *2* 12.5 mM borate, 17.0 mM SDS, 5.0 mM β-CD (pH 8.0). Other conditions are same as in Fig. 1

The optimal conditions as pH of running buffer and the concentrations of SDS and β-CD for separation were screened. As shown in Fig. 3a, pH 8.0 was selected as the optimal pH value. The effects of β-CD concentration on the resolution of BPA and 4-NP are shown in Fig. 3b. As can be seen, the resolution increased with its concentration from 0 to 5 mM but declined under higher concentration.

The effect of SDS concentration on separation efficiency was also studied. When the concentration of

Table 3 Detected results of real industrial wastewater samples (*n*=3)

Sample		4[a]	5[b]	6[c]	7[d]	8[e]	9[f]	10[g]	11[h]
OP	Found (μg/L)	80.6	–	–	–	–	–	–	39.3
	Added (μg/L)	100	100	100	100	100	100	100	50
	Detected (μg/L)	190.5	93.2	91.9	95.4	107.9	95.6	96.9	85.1
	Recovery (%)	105.4	93.2	91.9	95.4	107.9	95.6	96.9	95.3
	RSD (%)	2.6	4.1	3.6	3.5	4.9	3.1	2.2	5.6
4-NP	Found (μg/L)	–	–	60.7	–	–	20.9	22.4	17.7
	Added (μg/L)	100	100	100	100	100	30	30	30
	Detected (μg/L)	107.6	104.1	172.5	109.3	106.3	52.1	56.2	52.2
	Recovery (%)	107.6	104.1	107.3	109.3	106.3	102.4	107.3	109.4
	RSD (%)	3.1	4.5	4.2	3.0	3.8	2.3	4.1	3.7
BPA	Found (μg/L)	–	–	–	41.5	24.6	20.5	–	–
	Added (μg/L)	100	100	100	50	50	50	100	100
	Detected (μg/L)	98.4	103.4	105.1	98.4	68.3	67.4	107.9	106.5
	Recovery (%)	98.4	103.4	105.1	107.5	91.6	95.6	107.9	106.5
	RSD (%)	4.4	6.5	3.2	4.9	4.0	5.2	3.6	4.9

[a] A plastic factory in Jiangsu

[b] A petrochemical company in Henan

[c] A coking factor in Anhui

[d] A food processing plant in Jiangsu

[e] A brewery in Jiangsu

[f] A food company in Jiangsu

[g] A refining and chemical company in Daqing

[h] A refinery in Ningxia

SDS was lower than 15 or over 25 mM, the electrophoretic peaks of BPA and 4-NP were found to overlap severely. In the concentration range from 15 to 25 mM, it was found that the migration time of the analytes was altered with SDS concentration (see Fig. 4a, b) and then induced different separation resolution (see curve in Fig. 4c). Also the detection responses of analytes are influenced by SDS concentration (see Fig. 4d). So SDS is not only a crucial factor for separation but also for detection. For balanced consideration between separation resolution and detection response, 20 mM SDS was selected as the optimum because there was sufficient resolution of 1.8 between two analytes in this case.

Under aforementioned conditions, BPA and 4-NP are well separated within 55 s (Fig. 2, curve 3). The related analytical properties are listed in Table 1 (condition 1).

Table 4 The repeatability (RSD) of standard solution of analytes (100 μg/L) in 6 days

Compound		1	2	3	4	5	6	RSD (%)
OP	*i* (nA)	0.61	0.64	0.68	0.75	0.65	0.72	7.8
	Migration time (s)	35.0	35.5	36.0	36.5	34.0	35.0	2.5
4-NP	*i* (nA)	1.33	1.43	1.49	1.40	1.35	1.31	4.9
	Migration time (s)	38.5	39.0	40.0	39.5	41.0	38.5	2.5
BPA	*i* (nA)	0.99	1.11	1.08	1.03	1.15	1.12	5.6
	Migration time (s)	44.5	43.5	45.0	46.0	46.5	45.0	2.4

3.3 Validation of Separation Capability of PDMS Microchannel with MEKC Mode

For validating the capability of this PDMS microchannel with MEKC method, the contents of 4-NP and BPA in three wastewater samples were detected, meanwhile the capillary electrophoretic analysis are synchronously carried out for comparison. The results are listed in Table 2; there the data in parentheses are obtained in capillary electrophoresis. These results indicate that the relative deviation of two methods was less than 5.6%. Meanwhile, the numbers of theoretical plates of two methods are also listed in Table 2. It is found that the plate numbers in PDMS microchannel were about doubled greater than in silica capillary. So it does, the faster separation (55 s) has achieved on this shorter microchannel than in silica capillary of longer than 10 min. The results significantly revealed the profits of chip electrophoresis.

3.4 Separation and Detection of the Targets in Wastewater Samples

The further application of MEKC for those phenolic xenoestrogens on this PDMS microchip was carried out. But it was found that the separation of three analytes was not sufficient (see curve 1 in Fig. 5) under above-mentioned conditions. Just need to change the concentration of SDS in the running buffer to 17 mM, the allowable baseline separation of three analytes had achieved incidental tiny loss of detection response. Under improved conditions, the analytical properties are also included in Table 1, and the detection results of eight real wastewater samples are listed in Table 3. The results of recovery from 90.2 to 109.4 %, the relative standard deviations (RSD) of below 6.5 %, and the relative deviation of less than 5.6% compared with capillary electrophoresis in Table 2 and 3 for real samples indicate a satisfactory detection with proposed approach. These results demonstrate that there were not only the adequate separation efficiency and detection ability of the MEKC on microchannel coupled with amperometric detection but also the reliable enrichment by SPE. Although there are not the evidence of the precise data about the condensed multiples of those targets because we had not the means to accurately determine them in uncondensed samples, it is clear that there must be the reproducible enriching effect from the SPE because there would not be the sufficient recoveries from variational enriching effect. Another important aspect to be considered is the interference of somewhat coexisted impurities in real samples. It was found that the peaks of some unknown impurities would appear after 1 min of migration time and they did not interfere the detection of those phenolic xenoestrogens.

The detections of standard solution with every 100 μg/L of three analytes in consecutive 6 days have carried out. The RSD of migration time are found to be below 3% for three analytes, and the RSD of quantitative response are below 8% (see Table 4). The results indicated the adequate reproducibility of proposed method for applications.

4 Conclusion

In this paper, a novel chip electrophoretic system was described for the separation of OP, 4-NP, and BPA by MEKC mode coupled with amperometric detection. The optimal conditions, such as buffer pH, concentrations of surfactant, and β-CD, were systematically studied. The proposed microchannel provides doubled greater theoretical plates number than silica capillary, which led to the higher separation efficiency and shorter separation time. And it was successfully actualized the high-speed separation of these three phenolic xenoestrogens within 55 s under optimized MEKC conditions. The present approach had been used for the determination of these phenolic xenoestrogens in industrial wastewater samples with satisfactory results.

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References

Barcelo, D., & Hennion, M. C. (1995). On-line sample handling strategies for the trace-level determination of pesticides and their degradation products in environmental waters. *Analytica Chimica Acta, 318*(1), 1–41.

Bo, T., Zhong, L., Li, M., Luo, Y. Q., Li, K. A., Liu, H. W., & Guo, D. A. (2002). Separation of isoquinoline alkaloids and saponins by microemulsion electrokinetic chromatography with anionic and cationic surfactants. *Chromatography, 56*(11–12), 709–716.

Cai, Y. Q., Jiang, G. B., & Zhou, Q. X. (2004a). Separation and determination of bisphenol A, 4-n-nonylphenol and 4-tert-octylphenol by micellar electrokinetic chromatography. *Chinese Journal of Analytical Chemistry, 32*(9), 1179–1181.

Cai, Y. Q., Jiang, G. B., Liu, J. F., Liang, X., Yao, Z. W., Liu, J. M., Liu, J. Y., & Zhou, Q. X. (2004b). Solid-phase microextraction coupled with high performance liquid chromatography-fluorimetric detection for the determination of bisphenol A, 4-n-nonylphenol, and 4-tert-octylphenol in environmental water samples. *Analytical Letters, 37*(4), 739–753.

EU (2000). EU Directive 2000/60/EC, establishing a framework for community action in the field of water policy.

EU (2011). EU Directive 2011/8/EU (Amending Directive 2002/72/EC) as regards the restriction of use of bisphenol A in plastic infant feeding bottles.

Ferrer, E., Santoni, E., Vittori, S., Font, G., Manes, J., & Sagratini, G. (2011). Simultaneous determination of bisphenol A, octylphenol, and nonylphenol by pressurised liquid extraction and liquid chromatography-tandem mass spectrometry in powdered milk and infant formulas. *Food Chemistry, 126*(1), 360–367.

Garcia, C. D., Dressen, B. M., Henderson, A., & Henry, C. S. (2005). Comparison of surfactants for dynamic surface modification of poly(dimethylsiloxane) microchips. *Electrophoresis, 26*(3), 703–709.

Gronen, S., Denslow, N., Manning, S., Barnes, S., Barnes, D., & Brouwer, M. (1999). Serum vitellogenin levels and reproductive impairment of male Japanese Medaka (*Oryzias latipes*) exposed to 4-tert-octylphenol. *Environmental Health Perspectives, 107*, 385–390.

Han, D. M., Fang, G. Z., & Yan, X. P. (2005). Preparation and evaluation of a molecularly imprinted sol–gel material for on-line solid-phase extraction coupled with high performance liquid chromatography for the determination of trace pentachlorophenol in water samples. *Journal of Chromatography. A, 1100*(2), 131–136.

Harazono, A., & Ema, M. (2001). Effects of 4-tert-octylphenol on initiation and maintenance of pregnancy following oral administration during early pregnancy in rats. *Toxicology Letters, 119*(1), 79–84.

Jin, X. L., Huang, G. L., Jiang, G. B., Zhou, Q. F., & Liu, J. F. (2004). Simultaneous determination of 4-tert-octylphenol, 4-nonylphenol and bisphenol A in Guanting Reservoir using gas chromatography-mass spectrometry with selected ion monitoring. *Journal of Environmental Sciences (China), 16*(5), 825–828.

Jobling, S., & Sumpter, J. P. (1993). Detergent components in sewage effluent are weakly oestrogenic to fish: an in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquatic Toxicology, 27*(3–4), 361–372.

Jobling, S., Sheahan, D., Osborne, J. A., Matthiessen, P., & Sumpter, J. P. (1996). Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology and Chemistry, 15*(2), 194–202.

Koeber, R., Fleischer, C., Lanza, F., Boos, K. S., Sellergren, B., & Barcelo, D. (2001). Evaluation of a multidimensional solid-phase extraction platform for highly selective on-line cleanup and high-throughput LC-MS analysis of triazines in river water samples using molecularly imprinted polymers. *Analytical Chemistry, 73*(11), 2437–2444.

Li, W., Seifert, M., Xu, Y., & Hock, B. (2004). Comparative study of estrogenic potencies of estradiol, tamoxifen, bisphenol-A and resveratrol with two in vitro bioassays. *Environment International, 30*(3), 329–335.

Li, T. T., Jia, Q., Song, L. H., Su, R. Y., Lei, Y., Zhou, W. H., & Li, H. F. (2009). Coupling poly-(methacrylic acid-co-ethylene glycol dimethacrylate) monolith microextraction to capillary electrophoresis for the determination of phenols in water samples. *Talanta, 78*(4–5), 1497–1502.

Ma, Q., Bai, H., Wang, C., Zhang, Q., Zhou, X., Dong, H., & Wang, B. L. (2010). Simultaneous determination of n'onylphenol, octylphenol and bisphenol A in textiles and food packaging materials by liquid chromatography tandem mass spectrometry. *Chinese Journal of Analytical Chemistry, 38*(2), 197–201.

Markey, C. M., Luque, E. H., De Toro, M. M., Sonnenschein, C., & Soto, A. M. (2001). In utero exposure to bisphenol a alters the development and tissue organization of the mouse mammary gland. *Biological Reproductivity, 65*(4), 1215–1223.

McDonald, J. C., & Whitesides, G. M. (2002). Poly(dimethylsiloxane) as a material for fabricating microfluidic devices. *Accounts of Chemical Research, 35*(7), 491–499.

Morrissey, R. E., George, J. D., Price, C. J., Tyl, R. W., Marr, M. C., & Kimmel, C. A. (1987). The developmental toxicity of bisphenol A in rats and mice. *Fundamental and Applied Toxicology, 8*(4), 571–582.

Oleschuk, R. D., Shultz-Lockyear, L. L., Ning, Y. B., & Harrison, D. J. (2000). Trapping of bead-based reagents within microfluidic systems: On-chip solid-phase extraction and electrochromatography. *Analytical Chemistry, 72*(3), 585–590.

OSPAR (2007). OSPAR convention for the protection of the marine environment of the North-East Atlantic. OSPAR list of chemicals for priority action.

Park, S. Y., & Choi, J. (2009). Genotoxic effects of nonylphenol and bisphenol A exposure in aquatic biomonitoring species: Freshwater crustacean, daphnia magna, and aquatic midge, *Chironomus riparius*. *Bulletin of Environmental Contamination and Toxicology, 83*(4), 463–468.

Qian, X. M., Zhang, Q. L., Zhang, Y., & Tu, Y. F. (2010). Separation/determination of flavonoids and ascorbic acid in rat serum and excrement by capillary electrophoresis with electrochemical detection. *Analytical Sciences, 26*(5), 557–560.

Roman, G. T., Hlaus, T., Bass, K. J., Seekhammer, T. G., & Culbertson, C. T. (2005). Sol–gel modified poly(dimethylsiloxane) microfluidic devices with high electroosmotic mobilities and hydrophilic channel wall characteristics. *Analytical Chemistry, 77*(5), 1414–1422.

Santana, C. M., Ferrera, Z. S., Padrón, M. E. T., & Rodríguez, J. J. S. (2009). Methodologies for the extraction of phenolic compounds from environmental samples: New approaches. *Molecules, 14*, 298–320.

Slentz, B. E., Penner, N. A., & Regnier, F. E. (2002). Capillary electrochromatography of peptides on microfabricated poly (dimethylsiloxane) chips modified by cerium(IV)-catalyzed polymerization. *Journal of Chromatography. A, 948*(1–2), 225–233.

Sun, L., Chen, L. G., Sun, X., Du, X. B., Yue, Y. S., He, D. Q., Xu, H. Y., Zeng, Q. L., Wang, H., & Ding, L. (2009). Analysis of sulfonamides in environmental water samples based on magnetic mixed hemimicelles solid-phase extraction coupled with HPLC-UV detection. *Chemosphere, 77* (10), 1306–1312.

Takeda, S., Lida, S., Chayama, K., Tsuji, H., Fukushi, K., & Wakida, S. (2000). Separation of bisphenol A and three alkylphenols by micellar electrokinetic chromatography. *Journal of Chromatography. A, 895*(1–2), 213–218.

Timms, B. G., Howdeshell, K. L., Barton, L., Bradley, S., Richter, C. A., & Vom Saal, F. S. (2005). Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. *Proceedings of the National Academy of Sciences of the United States of America, 102*(19), 7014–7019.

Tokuyama, T., Fujii, S., Sato, K., Abo, M., & Okubo, A. (2005). Microbioassay system for antiallergic drug screening using suspension cells retaining in a poly(dimethylsiloxane) microfluidic device. *Analytical Chemistry, 77*(10), 3309–3314.

Tong, C. L., Guo, Y., & Liu, W. P. (2010). Simultaneous determination of five nitroaniline and dinitroaniline isomers in wastewaters by solid-phase extraction and high-performance liquid chromatography with ultraviolet detection. *Chemosphere, 81*(3), 430–435.

Volkel, W., Colnot, T., Csanady, G. A., Filser, J. G., & Dekant, W. (2002). Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chemical Research in Toxicology, 15*(10), 1281–1287.

Wakida, S., Fujimoto, K., Nagai, H., Miyado, T., Shibutani, Y., & Takeda, S. (2006). On-chip micellar electrokinetic chromatographic separation of phenolic chemicals in waters. *Journal of Chromatography. A, 1109*(2), 179–182.

Wang, A. J., Xu, J. J., Zhang, Q., & Chen, H. Y. (2006). The use of poly(dimethylsiloxane) surface modification with gold nanoparticles for the microchip electrophoresis. *Talanta, 69*(1), 210–215.

White, R., Jobling, S., Hoare, S. A., Sumpter, J. P., & Parker, M. G. (1994). Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology, 135*(1), 175.

Xiao, J., Shao, B., Wu, X. Y., Sun, X. J., & Wu, Y. N. (2011). A study on bisphenol A, nonylphenol, and octylphenol in human urine amples detected by SPE-UPLC-MS. *Biomedical and Environmental Sciences, 24*(1), 40–46.