Yue Wu
Wenpeng Zhang
Zilin Chen

Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Wuhan University, Ministry of Education, and Wuhan University School of Pharmaceutical Sciences, Wuhan, P. R. China

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Research Article

A poly (4-vinylpridine-co-ethylene glycol dimethacrylate) monolithic concentrator for in-line concentration-capillary electrophoresis analysis of phenols in water samples

A poly(4-vinylpridine-co-ethylene glycol dimethacrylate) monolith was synthesized in a capillary and constructed as a concentrator for the in-line polymeric monolith microextraction coupling with capillary electrophoresis. The integrated system was then used for the simultaneous determination of five trace phenols (2-nitrophenol, 3-nitrophenol, 4-nitrophenol, 2-chlorophenol, and 2,4-dichlorophenol) in water samples. The experimental parameters for in-line solid-phase extraction, such as composition and volume of the elution plug, pH of sample solution, and the time for sample loading were optimized. The sensitivity for the mixture of phenols (2-nitrophenol, 3-nitrophenol, 4-nitrophenol, 2-chlorophenol, and 2,4-dichlorophenol) enhanced to 615–2222 folds at the optimum condition was compared to the sensitivity for a normal hydrodynamic injection in capillary electrophoresis. Linearity ranged from concentration of 10–500 ng mL^{-1} ($R^2 > 0.999$) for all five phenols with the detection limits of 1.3–3.3 ng mL^{-1}. In tap, snow and Yangtze River water spiked with 20 ng mL^{-1} and 200 ng mL^{-1}, respectively, the recoveries of 84–105% were obtained. It has been demonstrated that this work has great potential for the analysis of phenols in genuine water samples.

Keywords:
Capillary electrophoresis / In-line concentration / Phenols / Polymeric monolith
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1 Introduction

Phenols are serious pollutants in the environment. Due to the high toxicity even at low concentration [1] and the unpleasant organoleptic property, some phenols are listed as major toxic pollutants by the Environmental Protection Agency (EPA) of the USA and other countries [2]. Nowadays, the worldwide production of phenols is continuously growing. They are widely used in the manufacture of dyes, wood, rubber, chemicals, explosives, and pesticides all over the world [3, 4]. Nitrophenols could even be synthesized by photochemical atmospheric reactions in the environment due to nitrogen oxides emission of factories and automobiles. On the other hand, the strong chemical stability and resistance to microbial degradation also lead to the accumulation of phenols in the environment. Therefore, the determination and monitoring of trace phenols in the environment are very important for the protection of water resources and food supplies for humans.

Various methods have been reported for the determination of phenolic compounds, such as enzyme-linked immunosorbent assay [5], spectrophotometry [6, 7], electrochemical methods [8–12], GC [13, 14], LC [15–21], GC-MS [22–25], LC-MS [26], and CE [27–31]. Some important works have already been reviewed in the literatures [32, 33]. Due to many inherent merits such as low sample and solvent consumption, high separation efficiency, short analytical time, and versatility of separation modes, CE can be used as a powerful technique for the analysis of phenols. However, the determination of phenols in environmental samples by CE is always limited because of the low content of analyte in the real sample and low detection sensitivity of CE, which results from both short optical path of the capillary used as detection cell and small sample volume (usually a few nanoliters) injected in CE [27, 34]. Therefore, the extraction techniques are needed in the analysis of phenols in real samples by CE, especially when the low sensitive UV detection is employed.

Correspondence: Professor Zilin Chen, Luojia Chair Professor, Vice Dean and Institute Director, School of Pharmaceutical Sciences, Wuhan University, Wuhan, P. R. China
E-mail: chenzl@whu.edu.cn
Fax: 86-27-68759850

Abbreviations: **2,4-CP**, 2,4-dichlorophenol; **2-CP**, 2-chlorophenol; **2-NP**, 2-nitrophenol; **3-NP**, 3-nitrophenol; **4-NP**, 4-nitrophenol; **4-VP**, 4-vinylpridine; **β-CD**, beta-cyclodextrin; **BMA**, butyl methacrylate; **EGDMA**, ethylene glycol dimethacrylate; **EPA**, Environmental Protection Agency; **LPME**, liquid-phase microextraction; **PME**, solid-phase microextraction; **SBSE**, stir bar sorptive extraction; **SPME**, solid-phase microextraction

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Conventional liquid–liquid extraction and solid-phase extraction are the most commonly used techniques for the preconcentration of phenols in real samples [13, 15–17, 20–22, 26, 32, 33]. However, the multiple step extraction procedures are laborious and time consuming. The large consumption of organic solvent is environmentally unfriendly and harmful to the health of operators. Some miniaturized preconcentration techniques with easy operation and less consumption have been reported for the extraction of phenols, such as solid-phase microextraction (SPME) [14, 23, 24], stir bar sorptive extraction (SBSE) [18, 35, 36], and liquid-phase microextraction (LPME) [25]. The fiber-based SPME is a solvent-free extraction method, which is quite suitable for the concentration of volatile phenols. But the fiber used as concentrator is fragile and has limited lifetime, and the carryover of sample is always a problem in this method. SBSE is another valuable technique for the extraction of phenols. SBSE has higher recovery and sample capacity than SPME, but the volume of sample solution (usually 50 mL) is relatively large, and the manual transfer of the stir bar is required. LPME has been successfully applied to the extraction of phenols from aqueous matrices. However, there are problems such as relatively low precision and sensitivity, caused when the volume of solvent used in the extraction is too small [25]. An ionic liquid-based single-drop microextraction with improved precision has been reported for detection of phenols. The detection limits less than 0.05 $\mu g\ mL^{-1}$ were obtained for three phenols [37].

As an alternative to SPME, polymeric SPE, a newly developed technique, has been demonstrated to be very effective on the preconcentration of trace analytes in real samples. In polymeric SPE, a capillary monolithic column with larger surface area and highly porous microstructure is used as the concentrator. As compared to the conventional SPME, not only is the fabrication of concentrator simplified in polymeric SPE, the extraction efficiency is also greatly improved due to the convective mass transfer and low back pressure drop of the porous monolith. Polymeric SPE has been proved to have good compatibility to the CE analysis with multiple coupling modes of off-line [38–40], on-line [41], and in-line [42–53]. With in-line concentration, the monolithic concentrator is constructed directly into the inlet end of the electrophoresis capillary. Thus, the concentration and determination of analytes can be carried out in the same capillary without the further transfer of the eluting solution. In this way, not only is the automation of the extraction procedure on the commercial CE instruments greatly facilitated, the sample volume required for analysis and the consumption of the organic solvents for the elution can also be minimized. In addition, due to the use of small volume of eluent (typically dozens of nano liters) and the total introduction of preconcentrated analytes into the separation-detection unit, higher enrichment efficiency could be obtained with in-line polymeric SPE, as compared to the off-line methods in some cases [54]. To date, the in-line polymeric SPE coupled CE analysis has been widely applied for the determination of neurotransmitters [42], amino acids [43], drugs [44, 49, 52], inorganic anions [46], carbamate pesticides [53], peptides, and proteins [47, 48, 50, 51]. A poly-(methacrylic acid-co-ethylene glycol dimethacrylate) (MAA-co-EGDMA)-based off-line polymeric SPE has been coupled to CE for the concentration and determination of phenols (catechol, resorcinol, 2,6-dimethylphenol, and 2,4,6-trinitrophenol) in water samples with the LOD of 6–159 $ng\ mL^{-1}$ [55].

In this work, an in-line polymeric SPE coupled CE analysis was proposed for the preconcentration and determination of trace phenols (2-nitrophenol, 3-nitrophenol, 4-nitrophenol, 2-chlorophenol, and 2,4-dichlorophenol) in water samples. A poly(4-vinylpridine-co-EGDAMA) (4-VP-co-EGDMA) monolith was prepared and employed as the concentrator. It has been demonstrated that the concentrator is very effective for the concentration of phenols. With the integration of in-line polymeric SPE and CE analysis, the detection sensitivity was greatly improved and the tedious pretreatment of real samples was avoided. The systematic optimization of experimental parameters, such as composition and volume of the elution plug, pH of sample solution, and the time for sample loading, were carried out carefully. A detailed evaluation of the method was also performed to demonstrate that this method could be applied for the determination of trace phenols in real water samples.

2 Materials and methods

2.1 Chemicals

All chemicals and solvents were of analytical reagent grade. 4-Vinylpridine (4-VP) and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma-Aldrich (USA) and Alfa Aesar (UAS), respectively. 2-Nitrophenol (2-NP), 3-nitrophenol (3-NP), 4-nitrophenol (4-NP), 2,2′-azobis (2-methylpropionitrile) (AIBN), dodecanol, toluene, β-cyclodextrin (β-CD), methanol (MeOH), and ACN were purchased from Shanghai Chemical (Shanghai, China). 2-Chlorophenol (2-CP) and 2,4-dichlorophenol (2,4-CP) were purchased from Aladdin (Shanghai, China). Double-distilled water was used in the experiments. A stock standard solution of 5 $mg\ mL^{-1}$ for each analyte was prepared in ACN. The composite standard containing 20 $\mu g\ mL^{-1}$ of each analyte was prepared by diluting the stock solution with double-distilled water.

2.2 Instrumentation

The CE analysis was performed on a CAPEL 105 CE system (LUMEX, Russia) equipped with a UV-Vis detector. Fused-silica capillaries with 50 μm id and 100 μm id were purchased from Yongnian Fiber Plant (Hebei, China). Data collection and processing were carried out on Chrom & Spec software for chromatography. Teflon tube for construction of in-line monolithic concentrator was obtained from Innosep

(part No. JR-T4011, 0.25 mm id and 1.59 mm od, Henan, China).

2.3 Pretreatment of capillaries

A capillary of 50 μm id and 60 cm in length with effective length of 50.5 cm was used for CE analysis. Before use, the capillary was activated with 0.1 M NaOH for 30 min, washed with water for 10 min, and conditioned with the running buffer for 20 min. At the beginning of each day, the capillary was conditioned with 0.1 M NaOH for 5 min, with water for 3 min, and with running buffer for 5 min.

2.4 Procedure of normal CE

The UV-Vis detection was carried out at 280 nm. An aqueous solution of 35 mM sodium dihydrogen phosphate and 10 mM of β-CD was used as running buffer, and the pH was adjusted to 8.5 with 1 M NaOH. The applied voltage was 19 kV and the temperature of the capillary was kept at 22°C. Hydrodynamic injection was carried out by applying a pressure of 30 mbar for 5 s.

2.5 Procedure of in-line polymeric SPE-CE

A capillary of 64 cm in length and 50 μm id was used for in-line polymeric SPE-CE analysis. The distance from the inlet end to the polymeric concentrator is 3.0 cm; the length of the concentrator is 5 mm; distance from the concentrator to the detection window is 50.5 cm; and distance from the detection window to the outlet end is 9.5 cm. The analytical procedure includes the following steps: (i) Sample loading and concentration: sample solution was introduced into the capillary with concentrator by the pressure of 1000 mbar for 30 min. The analytes were concentrated on the monolithic concentrator. (ii) Sample washing: the analytes unconcentrated and the sample solution remained in the capillary were washed out with 35 mM phosphate buffer (pH 7.5) by applying a pressure of 1000 mbar for 48 s. (iii) Eluent injection: a plug of ACN was injected by a pressure of 100 mbar for 15 s. (iv) Eluting: both ends of the capillary were put into the running buffer, and the pressure of 100 mbar was applied on the inlet end for 4 min to push the ACN plug through the concentrator for the desorption of the concentrated analytes. (v) CE separation: the experimental conditions are the same as in normal CE described in Section 2.4 except that the pH of the running buffer was adjusted to 10.5 with 1 M NaOH. After each injection, the capillary was conditioned with double distilled water for 2 min and ACN for another 1 min to avoid sample carryover between consecutive injections. Before each sample loading, the concentrator was preconditioned with an aqueous solution of pH 7.0 adjusted by NaOH solution.

2.6 Preparation of poly(4-VP-co-EGDMA) monolith capillary column

The poly(4-VP-co-EGDMA) monolith column was synthesized by in situ polymerization inside a fused silica capillary with 100 μm id. The capillary was activated with 1M NaOH for 4 h at 40°C and washed with distilled water and 0.1 M HCl. After the pretreatment, the capillary was dried under N_2 for 24 h and filled with 50% (v:v) 3-(triethoxysilyl)propyl methacrylate methanol solution. After being kept at 40°C for 12 h with both ends sealed, the capillary was washed with methanol and dried by N_2 again before further use.

The prepolymerization mixture comprised of monomer 4-VP 0.84 mmol, cross-linker EGDMA 4.19 mmol, initiator AIBN 4.4 mg was dissolved in 160 μL toluene and 1.5 mL dodecanol. After bubbling with N_2 for 20 min, the mixture solution was introduced into the methacryloyl-modified capillary, which was then sealed immediately with silicon rubber at both ends. The capillary was kept at 45°C for the polymerization for 16 h and washed with methanol to remove the residual components and porogenic solvent.

2.7 Construction of in-line monolithic concentrator

For the construction of in-line concentrator, a 5 mm of poly(4-VP-co-EGDMA) monolithic column was cut off under the microscope to guarantee a perfect flat cutting was made. The concentrator was then introduced carefully into the middle of a piece of Teflon tube with a length of 1 cm. After that, 3 cm of bare fused-silica capillary (50 μm id × 360 μm od) was butted to one side of the concentrator and separation capillary (50 μm id × 360 μm od) to another side of the concentrator.

2.8 Treatment of the real sample

Tap water, snow water and Yangtze River water were collected and kept in refrigerator before use. The sediments in the real samples were removed by centrifugation at 14 000 rpm for 10 min.

3 Results and discussion

3.1 Construction of the in-line concentrator for CE

For the fabrication of monolithic in-line concentrator, the in situ polymerization is a common method. However, when the very short concentrator is used to obtain high separation efficiency and when a strong alkali running buffer is used in CE separation, the concentrator often falls apart. In addition, because the concentrator is synthesized in the separation capillary in situ, the extraction capacity is always limited by the diameter of the capillary used for the separation. In this work, an easy and effective method was used to construct the

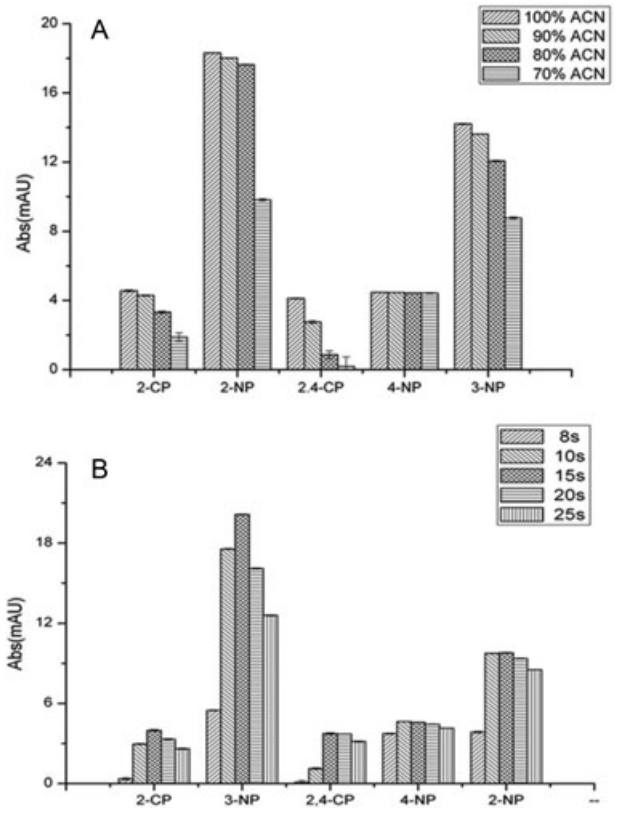

Figure 1. Effects of ACN in water (A) and desorption volume (B) on the desorption of nitrophenols for the in-line concentrator. Standard solutions of 200 ng mL^{-1} at pH 7.5 were used. Extraction and CE conditions are described in Section 2.5.

in-line concentrator. The polymeric monolith with a bigger diameter was connected to the separation capillary by a Teflon tube. With this design, not only was the disintegration of the concentrator avoided effectively; higher extraction capacity was also obtained compared to the concentrator prepared by the in situ polymerization. Although a Teflon tube was used to connect the monolithic concentrator and separation capillary, no trouble of bubble formation and the decrease in separation efficiency were observed in this work.

For the construction of in-line concentrator, the length of the monolithic polymer was studied. Monoliths with different lengths were prepared and coupled in-line with CE. The extraction efficiency and CE analysis of phenols were then investigated. Although higher extraction capacity could be obtained with longer concentrator (longer than 1 cm), the eluent plug required for the entire elution was consequently enlarged and lead to the band broadening and the decline of sensitivity. In addition, a longer concentrator causes higher fluidic resistance in the capillary that results in a significant decrease of resolution and even the overlap of the peaks of phenols. Although no separation problems were observed when the monolith with the length less than 5 mm was used, the extraction capacity was not satisfactory for the sensitive detection of the phenols in the real samples. Thus, based on the consideration of both sensitivity required for the analysis of trace phenols in real samples and the CE separation after the in-line concentration, 5 mm of the concentrator in length was finally chosen in this work.

3.2 Optimization of the electrophoretic separation with in-line concentrator

At first, the CE separation of 2-CP, 2-NP, 3-NP, 4-NP, and 2, 4-CP was studied before the connection of the monolithic concentrator. Although capillary zone electrophoresis showed good compatibility with the retention and elution on the polymeric monolith, the separation of five phenols was hardly achieved in CZE mode due to their slight differences in electrophoretic mobility. After adding 10 mM β-CD to the running buffer of 35 mM phosphate at pH 8.5, the base line separation was obtained with the host-guest inclusion interaction between phenols and β-CD. But when the same conditions were applied for the CE separation with in-line concentrator, the overlaps of the peaks were observed, possibly due to the band broadening caused by both larger sample volume (changed from 100 mbar × 5 s to 100 mbar × 15 s) and the increase of fluidic resistance benefited from monolithic concentrator. Taking advantage of the enhancement of EOF at higher pH values, running buffer of pH 10.5 was finally adopted in CE analysis with in-line concentrator.

It was observed that running buffer containing β-CD could elute out the phenols adsorbed on the monolithic concentrator due to the inclusion interaction between the phenols and β-CD. Thus, in order to avoid the eluting effects of the β-CD, the sample solution remained in the capillary was washed out from the capillary by 35 mM phosphate buffer without β-CD at pH 7.5 after sample loading. When a separation voltage was applied on both running buffers containing β-CD, neutral β-CD moved with EOF toward cathode and negatively charged phenols moved toward anode, but electrophoretic velocity of phenols was less than that of EOF. The counter movement and interaction between β-CD and phenol finally resulted in the baseline separation of phenols.

3.3 Optimization of the variables involved in the in-line concentration

In order to achieve the highest performance of in-line concentration, several parameters were carefully optimized in this work, including composition and volume of the eluent plug, the pH of sample solution, and the time of injection.

At first, for the complete elution of the concentrated phenols, the composition and volume of the elution plug were optimized. Various proportions of ACN in water were examined as elution solutions. It was found that recoveries were increased with the increase of ACN content (Fig. 1A), and the highest recovery was achieved with pure ACN. In order to get higher recovery, a little acetic acid (0.2–0.5, v:v) was added into the ACN since acetic acid can hinder potential hydrogen bonding between 4-VP and

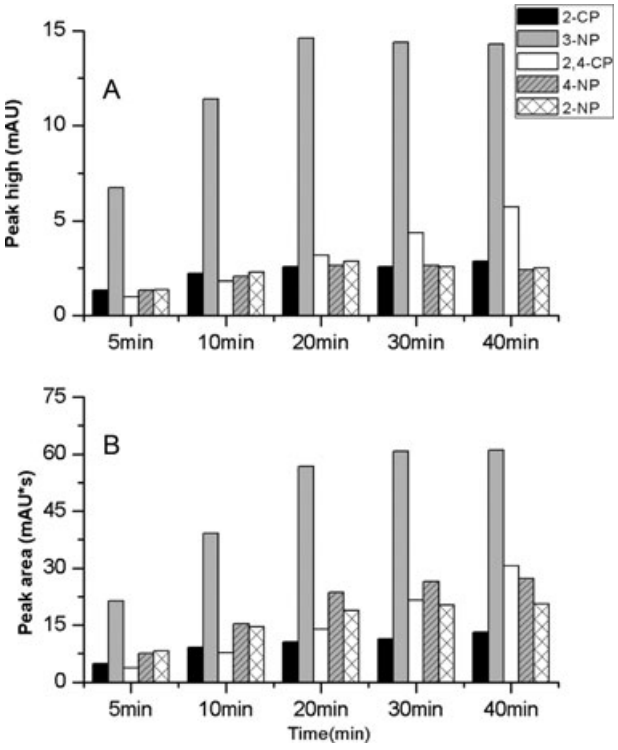

Figure 2. Effects of sample loading time on individual peek heights (A) and individual peek areas (B) of five phenols with in-line concentration. Other conditions are the same as in Fig. 1.

-OH group of phenols, which can possibly contribute to the elution. However, no significant improvement in recovery was found by the further addition of acetic acid. Therefore, ACN was finally chosen as the desorption solution for the in-line concentration.

The volume of the eluent plug was also optimized for the complete elution and the minimization of band broadening. The volumes of the elution plugs were controlled by adjusting the injection times to 8, 10, 15, 20, and 25 s under the injection pressure of 100 mbar. As shown in Fig. 1B, the highest sensitivity was obtained with injection time of 15 s where the highest peak height was observed. Significant decrease in peak height was observed as injection time increased, indicating the occurrence of the band broadening with large volumes of the eluent plugs.

The pH value of the sample solution was reported to have great influence on the retention of analytes in polymer-based solid phase extraction [38, 39]. In this work, the effect of sample pH values was evaluated in the range of pH 5.0 to 10.0. The extraction efficiencies of five phenols were higher in weak acidic, neutral, and weak alkaline solutions than those in strong alkaline solutions. It suggested that the hydrogen-bonding interaction might be involved in the extraction procedure. On the term of the chemical structures, the hydrogen bond might form between the phenolic group of phenols and the nitrogen atoms in the pyridine residues of the monolithic polymer. In strong alkaline solutions such as pH 10.0, the ionization of phenolic group occurred. The hydrogen bonding between phenols and polymer was greatly interrupted, which resulted in the decrease of recoveries. This phenomenon indicated that potential ionic interactions between the phenolate forms of the phenols and pyridine residue of polymer might contribute little to the concentration of phenols on 4-VP-based polymeric concentrator. For the most sensitive detection with in-line concentrator, pH 7.5 was finally chosen in the work because the highest recovery was obtained.

In this work, the volumes of the sample loading were studied by adjusting the injection time under press of

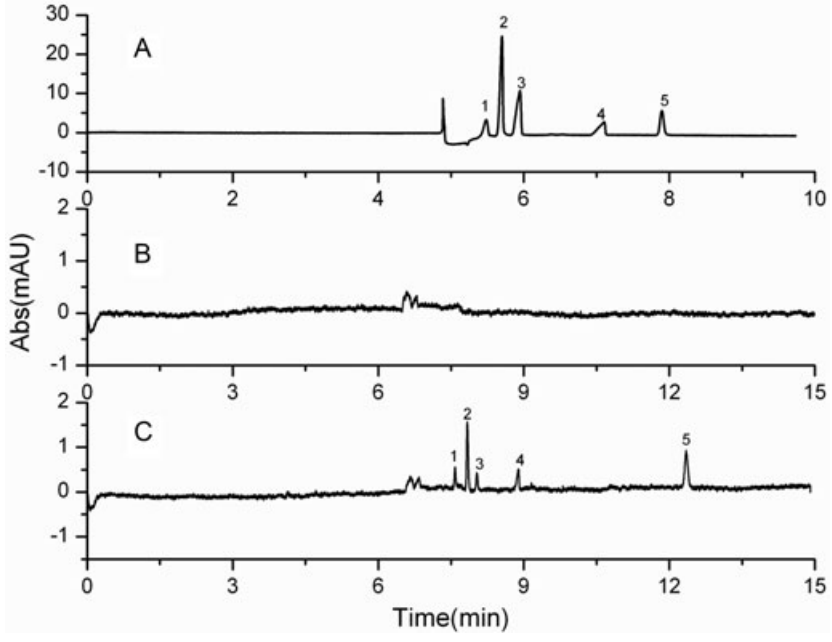

Figure 3. Electropherograms of separating phenols (A) by polymeric SPE-CE at sample concentration of 500 ng mL^{-1} (B) by CE at sample concentration of 500 ng mL^{-1} (C) by CE at sample concentration of 20 μg mL^{-1}. Peak identification: (1) 2-CP; (2) 3-NP; (3) 2,4-CP; (4) 4-NP; (5) 2-NP. Other conditions are the same as in Fig. 1.

Table 1. Calibration curves for phenols with in-line concentration

Compound	Linear range (ng/mL)	Calibration curves			LOD (ng/mL)	LOQ (ng/mL)
		Slope (mAU·s/(ng/mL))	Intercept (mAU·s)	*r*		
2-Chlorophenol	10–500	0.073	0.9331	0.9994	3.3	5.6
3-Nitrophenol	10–500	0.342	4.3060	0.9999	1.3	1.5
2,4-Dichlorophenol	10–500	0.205	1.0313	0.9992	1.8	5.0
4-Nitrophenol	10–500	0.140	1.8116	0.9994	1.3	4.0
2-Nitrophenol	10–500	0.146	1.7188	0.9991	1.5	3.0

1000 mbar. The injection time of 5, 10, 20, 30, and 40 min were tested with standard sample solution of 200 ng mL^{-1}. The changes of peek heights and areas for five phenols are illustrated in Fig. 2A and B, respectively. Although the peak heights of two analytes (3-NP and 2-NP) started to decrease slightly when loading time was over 20 min (Fig. 2A), the obvious increases of peak areas for all five phenols were observed with the increase of the injection time up to 30 min (Fig. 2B). From 30 to 40 min, the growths of the peak areas were found to slow down. In addition, the losses of resolution caused by the increase of peak widths were also raised. Thus, based on the consideration of the resolution required and the acceptable time for a whole analysis, a sample injection of 30 min was selected for subsequent analysis with satisfactory sensitivity.

The analysis of phenols by in-line polymeric SPE-CE and direct CE under the optimized conditions were carried out, as shown in Fig. 3. The concentration of five phenols was 500 ng mL^{-1}, and the injection was 1000 mbar for 30 min and 100 mbar for 5 s for in-line SPE-CE and direct CE, respectively. As compared with the detection in direct CE, sensitivity was dramatically improved with in-line concentration. It indicates that the developed method has great potential for the determination of the trace substances. As shown in the electropherograms, a little decrease in resolution was also found with in-line SPE-CE compared to direct CE analysis. It was possibly due to the larger injection volume used in eluting step in SPE process.

3.4 Validation

Under the optimized conditions as described in Section 2.5, the analytical characteristics including linearity, reproducibility, and limits of detection and quantification were evaluated. In the concentration range of 10–500 ng mL^{-1}, calibration curves of each phenol were constructed by plotting peak areas as a function of the concentrations. The LOD and LOQ were considered as the minimum analyte concentration yielding an S/N ratio equal to 3 and 10, respectively. As listed in Table 1, good linearity was obtained with the regression coefficients (*r*) more than 0.999. It has been demonstrated that phenols at the level of several nanograms could be detected with UV detection by in-line polymeric SPE-CE. As compared with the detection limits in direct CE, the enhancements in sensitivity for the mixture of phenols (2-NP, 3-NP, 4-NP,

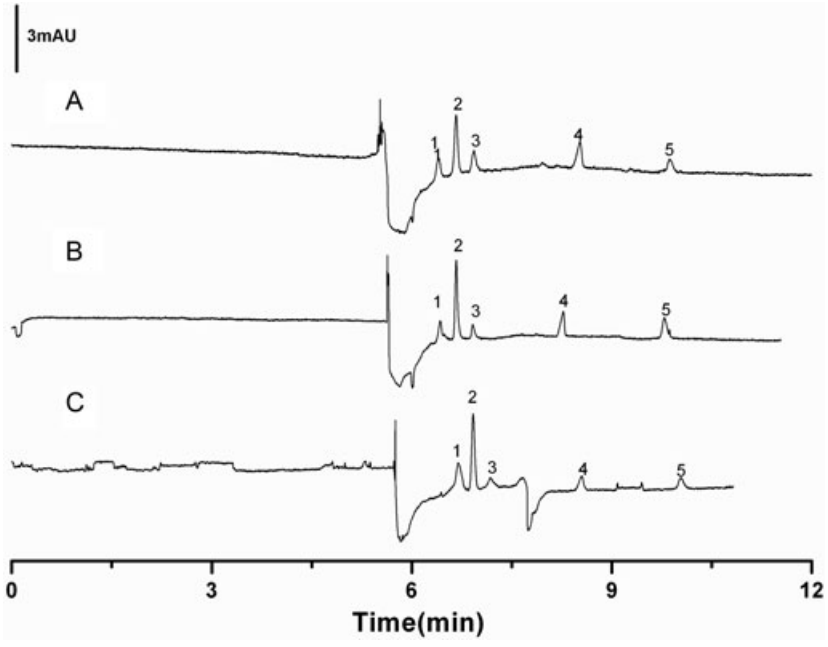

Figure 4. Electropherograms of separating phenols by in-line polymeric SPE-CE for real water samples (A) snow water (B) tap water (C) Yangtze River water spiked with 20 ng mL^{-1} of each phenol. Peak identification: (1) 2-CP; (2) 3-NP; (3) 2,4-CP; (4) 4-NP; (5) 2-NP. Other conditions are the same as in Fig. 1.

Table 2. Intraday and interday precisions of migration time and peak area at two different concentrations for phenol in-line concentration

Compound	Concentration (ng/mL)	Precision (RSD,%)			
		Intraday ($n = 3$)		Interday ($n = 3$)	
		MT	Area	MT	Area
2-Chlorophenol	200	1.0	1.4	0.3	4.8
	10	1.9	5.5	0.8	4.6
3-Nitrophenol	200	1.0	1.0	0.4	4.6
	10	1.8	4.3	0.7	5.8
2,4-Dichlorophenol	200	1.0	2.9	0.8	3.4
	10	1.7	3.4	0.7	3.9
4-Nitrophenol	200	1.5	3.2	2.8	1.3
	10	1.8	3.1	0.6	5.2
2-Nitrophenol	200	1.4	1.9	1.8	4.1
	10	1.7	3.5	1.3	4.0

2-CP, and 2,4-CP) were 970, 615, 2222, 1846, and 1333 folds, respectively.

The reproducibilities were also investigated by interday and intraday precision. Standard sample solutions of 10 ng mL^{-1} and 200 ng mL^{-1} were tested in three consecutive days with the optimized procedure. The RSD were calculated and presented in Table 2. For the intraday precision, the RSDs of the migration time were less than 1.9% and peak areas were less than 5.5%. For interday precision, the RSDs of the migration time and peak area were less than 2.8 and 5.8%, respectively.

The robustness and stability of the polymeric concentrator were examined. After over hundreds of runs for 4 months, no obvious changes in permeability, extraction efficiency, or recovery were observed. It suggests that the concentrator has satisfied robustness and stability.

3.5 Application of the in-line concentrator in analysis of real samples

The developed method was applied for the determination of five phenols (2-NP, 3-NP, 4-NP, 2-CP, and 2,4-CP) in real samples. Tap water, snow water, and Yangtze River water spiked with 20 ng mL^{-1} of each phenol were analyzed. As shown in Fig. 4, trace amount of phenols in the real water samples could be well determined. In natural water, the concentrations of nitrophenols and chlorophenols were less than 0.1 ng/mL. EPA has regulated the level of 2-nitrophenol, 4-nitrophenol, and 2-chlorophenol in the water, which are set at 2700, 240, and 2000 ng/mL, respectively [56, 57]. It is difficult to determine the low level of nitrophenols and chlorophenols in natural waters, but the methods can be used to determine nitrophenols and chlorophenols in the polluted water samples.

The recoveries of the in-line polymeric SPE-CE were calculated by the ratio of determined to spiked standard phenols. As listed in Table 3, satisfactory recoveries of 84–105% and acceptable RSD less than 7% ($n = 3$) were obtained. It suggests that the 4-VP-based in-line monolithic concentrator has great potential for the analysis of phenols in real water samples.

4 Concluding remarks

In this work, a poly(4-VP-co-EGDMA) monolithic in-line polymeric SPE coupling with CE was constructed and evaluated for the determination of trace phenols in environmental

Table 3. Extraction recoveries (%) and RSDs ($n = 3$) obtained for the in-line polymeric SPE-CE of tap, snow, and Yangtze River water spiked with phenols at 20 ng mL^{-1} and 200 ng mL^{-1}

Compound	Real sample	Spiked concentrations			
		20 ng/mL		200 ng/mL	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
2-Chlorophenol	Tap water	89	5.0	99	0.8
	Snow water	91	2.4	104	1.4
	Yangtze River water	102	1.7	101	2.4
3-Nitrophenol	Tap water	105	5.0	102	1.4
	Snow water	93	0.6	98	3.5
	Yangtze River water	97	3.4	105	1.7
2,4-Dichlorophenol	Tap water	84	3.2	91	4.6
	Snow water	104	0.3	95	6.2
	Yangtze River water	97	1.8	88	1.7
4-Nitrophenol	Tap water	87	4.6	89	1.4
	Snow water	97	2.4	104	4.6
	Yangtze River water	93	6.6	101	1.4
2-Nitrophenol	Tap water	101	4.2	86	7.0
	Snow water	86	0.3	99	1.0
	Yangtze River water	90	1.7	87	6.4

samples. It has been demonstrated that this monolithic concentrator is robust after hundreds of runs and exhibits high extraction efficiency as well as good regeneration. With in-line polymeric SPE, the sample volume required for the analysis was reduced to several microliters, and real samples could be injected directly into the capillary without the tedious pretreatment. In addition to precision and reproducibility, detection sensitivity was improved. Satisfactory recoveries and low RSD values were obtained when 4-VP-based in-line polymeric SPE-CE was applied in the analysis of real water samples.

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5 References

[1] Herterich, R., *J. Chromatogr. A* 1991, *549*, 313–324.

[2] Ahmaruzzaman, M., Gayatri, S. L., *J. Chem. Eng. Data* 2010, *55*, 4614–4623.

[3] Christophersen, M. J., Cardwell, T. J., *Anal. Chim. Acta*. 1996, *323*, 39–46.

[4] Wise, H. E., Fahrenthold, P. D., *Environ. Sci. Technol.* 1981, *15*, 1292–1304.

[5] Oubiña, A., Ballesteros, B., Galve, R., Barcelo, D., Marco, M. P., *Anal. Chim. Acta.* 1999, *387*, 255–266.

[6] Lupetti, K. O., Rocha, F. R. P., Fatibello-Filho, O., *Talanta* 2004, *62*, 463–467.

[7] Norwitz, G., Nataro, N., Keliher, P. N., *Anal. Chem.* 1986, *58*, 639–641.

[8] Kochana, J., Gala, A., Parczewski, A., Adamski, J., *Anal. Bioanal. Chem.* 2008, *391*, 1275–1281.

[9] Santos, A. S., Pereira, A. C., Sotomayor, M. D. P. T., Tarley, C. R. T., Durán, N., Kubota, L. T., *Electroanalysis* 2007, *19*, 549–554.

[10] Sun, J. G., Wang, G. F., Jiao, S. F., Wei, Y., Fang, B., *Chinese J. Anal. Chem.* 2007, *35*, 335–339.

[11] Kim, M. A., Lee, W.-Y., *Anal. Chim. Acta* 2003, *479*, 143–150.

[12] García, C. D., Ortiz, P. I., *Anal. Sci.* 1999, *15*, 461–465.

[13] Barták, P., Frnková, P. n., Čáp, L. r., *J. Chromatogr. A* 2000, *867*, 281–287.

[14] van Doorn, H., Grabanski, C. B., Miller, D. J., Hawthorne, S. B., *J. Chromatogr. A* 1998, *829*, 223–233.

[15] Masqué, N., Galià, M., Marcé, R. M., Borrull, F., *J. Chromatogr. A* 1997, *771*, 55–61.

[16] Puig, D., Barcelo´, D., *J. Chromatogr. A* 1997, *778*, 313–319.

[17] Puig, D., Barceló, D., *J. Chromatogr. A* 1996, *733*, 371–381.

[18] Huang, X., Qiu, N., Yuan, D., Huang, B., *Talanta* 2009, *78*, 101–106.

[19] Busto, O., Olucha, J. C., Borrull, F., *Chromatographia* 1991, *32*, 566–572.

[20] Pocurull, E., Calull, M., Marcé, R. M., Borrull, F., *J. Chromatogr. A* 1996, *719*, 105–112.

[21] Pocurull, E., Marce, R. M., Borrull, F., *Chromatographia* 1995, *41*, 521–526.

[22] Kovács, Á., Kende, A., Mörtl, M., Volk, G., Rikker, T., Torkos, K., *J. Chromatogr. A* 2008, *1194*, 139–142.

[23] Llompart, M. a., Lourido, M., Landı'n, P., Garcı'a-Jares, C., Cela, R., *J. Chromatogr. A* 2002, *963*, 137–148.

[24] Pino, V., Ayala, J. H., González, V., Afonso, A. M., *Talanta* 2007, *73*, 505–513.

[25] Chung, L.-W., Lee, M.-R., *Talanta* 2008, *76*, 154–160.

[26] Nistor, C., Rose, A., Farré, M., Stoica, L., Wollenberger, U., Ruzgas, T., Pfeiffer, D., Barceló, D., Gorton, L., Emnéus, J., *Anal. Chim. Acta* 2002, *456*, 3–17.

[27] Porras, S. P., Kuldvee, R., Palonen, S., Riekkola, M.-L., *J. Chromatogr. A* 2003, *990*, 35–44.

[28] Ebber, A., *Chromatographia* 2001, *53*, S307–S311.

[29] Gray, A. L., Hsu, J. T., *J. Chromatogr. A* 1998, *824*, 119–124.

[30] Maichel, B., Potocek, B., Gas, B., Chiari, M., Kenndler, E., *Electrophoresis* 1998, *19*, 2124–2128.

[31] Kaniansky, D., Krčmová, E., Madajová, V., Masár, M., Marák, J., Onuska, F. I., *J. Chromatogr. A* 1997, *772*, 327–337.

[32] Rodrı'guez, I., Llompart, M. P., Cela, R., *J. Chromatogr. A* 2000, *885*, 291–304.

[33] Puig, D., Barceló, D., *TrAC Trends Anal. Chem.* 1996, *15*, 362–375.

[34] Xie, T., Liu, Q., Shi, Y., Liu, Q., *J. Chromatogr. A* 2006, *1109*, 317–321.

[35] Quintana, J. B., Rodil, R., Muniategui-Lorenzo, S., Lopez-Mahia, P., Prada-Rodriguez, D., *J. Chromatogr. A* 2007, *1174*, 27–39.

[36] Huang, X. J., Qiu, N. N., Yuan, D. X., *J. Sep. Sci.* 2009, *32*, 1407–1414.

[37] Wang, Q., Qiu, H., Li, J., Liu, X., Jiang, S., *J. Chromatogr. A* 2010, *1217*, 5434–5439.

[38] Zhang, M., Wei, F., Zhang, Y.-F., Nie, J., Feng, Y.-Q., *J. Chromatogr. A* 2006, *1102*, 294–301.

[39] Wei, F., Zhang, M., Feng, Y. Q., *Electrophoresis* 2006, *27*, 1939–1948.

[40] Li, T., Shi, Z.-G., Zheng, M.-M., Feng, Y.-Q., *J. Chromatogr. A* 2008, *1205*, 163–170.

[41] Rodríguez-Gonzalo, E., Ruano-Miguel, L., Carabias-Martínez, R., *Electrophoresis* 2009, *30*, 1913–1922.

[42] Thabano, J. R. E., Breadmore, M. C., Hutchinson, J. P., Johns, C., Haddad, P. R., *J. Chromatogr. A* 2009, *1216*, 4933–4940.

[43] Breadmore, M. C., *Anal. Chim. Acta* 2006, *556*, 121–126.

[44] Schaller, D., Hilder, E. F., Haddad, P. R., *Anal. Chim. Acta* 2006, *556*, 104–111.

[45] Thabano, J. R. E., Breadmore, M. C., Hutchinson, J. P., Johns, C., Haddad, P. R., *Analyst* 2008, *133*, 1380–1387.

[46] Hutchinson, J. P., Zakaria, P., Bowie, A. R., Macka, M., Avdalovic, N., Haddad, P. R., *Anal. Chem.* 2005, *77*, 407–416.

[47] Armenta, J. M., Gu, B., Humble, P. H., Thulin, C. D., Lee, M. L., *J. Chromatogr. A* 2005, *1097*, 171–178.

[48] Armenta, J. M., Gu, B., Thulin, C. D., Lee, M. L., *J. Chromatogr. A* 2007, *1148*, 115–122.

[49] Faure, K., Delaunay, N., Alloncle, G., Cotte, S., Rocca, J.-L., *J. Chromatogr. A* 2007, *1149*, 145–150.

[50] Zhang, L., Zhang, W., Zhang, Y., *Electrophoresis* 2005, *26*, 2172–2178.

[51] Vizioli, N. M., Rusell, M. L., Carbajal, M. L., Carducci, C. N., Grasselli, M., *Electrophoresis* 2005, *26*, 2942–2948.

[52] Baryla, N. E., Toltl, N. P., *Analyst* 2003, *128*, 1009–1012.

[53] Rodríguez-Gonzalo, E., Domínguez-Álvarez, J., Ruano-Miguel, L., Carabias-Martínez, R., *Electrophoresis* 2008, *29*, 4066–4077.

[54] Almeda, S., Arce, L., Benavente, F., Sanz-Nebot, V., Barbosa, J., Valcarcel, M., *Anal. Bioanal. Chem.* 2009, *394*, 609–615.

[55] Li, T., Jia, Q., Song, L., Su, R., Lei, Y., Zhou, W., Li, H., *Talanta* 2009, *78*, 1497–1502.

[56] *Nitrophenols, Ambient Water Quality Criteria*, U.S. Environmental Protection Agency, Washington, DC 1980.

[57] *Ambient Water Quality Criteria for 2-chlorophenol*, U.S. Environmental Protection Agency, Washington, DC 1980.

