Direct determination of chlorophenols in environmental water samples by hollow fiber supported ionic liquid membrane extraction coupled with high-performance liquid chromatography

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Abstract

The 1-octyl-3-methylimidazolium hexafluorophosphate ([C8MIM][PF6]) ionic liquid was immobilized in the pores of a polypropylene hollow fiber for hollow fiber-protected liquid-phase microextraction. Analytes including 4-chlorophenol (4-CP), 3-chorophenol (3-CP), 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP) were extracted into this ionic liquid membrane, and back extracted into 10 M sodium hydroxide acceptor solution in the lumen of the hollow fiber. Then, the acceptor solution was withdrawn into the high-performance liquid chromatography (HPLC) microsyringe connected to the hollow fiber, and directly injected into the HPLC system for analysis. Some parameters that might affect the extraction efficiency were optimized, and low detection limits (0.5 μg L⁻¹ for 4-CP, 3-CP, DCP and 1.0 μg L⁻¹ for TCP) were obtained. Good repeatability was achieved because of the stability of the hollow fiber-supported ionic liquid membrane. The proposed procedure was applied for direct determination of the four chlorophenols in some real water samples including groundwater, river water, wastewater and tap water. All of the four chlorophenols in these water samples were under the limits of determination, and the recoveries were in the range of 70.0–95.7% at 5 μg L⁻¹ spiked level.
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Keywords: Supported liquid membrane; Ionic liquids; Chlorophenols; High-performance liquid chromatography

1. Introduction

Chlorophenols (CPs) are important pollutants that extensively exist in environmental waters and soils [1]. The main sources of CPs are effluent discharges of industries such as paper and pesticide industries [2], but the chlorination process of non-chlorinated phenols in water can also generate some chlorophenols. Because of their high toxicity and potential carcinogenicity, most of them are on the US Environmental Protection Agency priority pollutant list [3]. In 1982, the European Economic Community [now European Union (EU)] issued another pollutant list [4] that included many polychlorophenols and established their maximum allowable concentration in drinking waters (0.5 μg L⁻¹). Therefore, developing reliable, sensitive and easily operating detection methods are of great interest for the determination of these compounds in environmental samples.

Over the past decades, high-performance liquid chromatography (HPLC) was one of the mainly used methods for the separation and determination of chlorophenols by many researchers [5–7]. Various detectors such as ultraviolet (UV) [8,9], fluorescence [10], electrochemical [11], and mass spectroscopy [12] were in conjunction with HPLC for the determination of CPs.

Because of the low concentration of CPs and the complexity of the environmental samples, an enrichment step is usually needed prior to the instrumental analysis. Liquid–liquid extraction (LLE) [13] and solid-phase extraction (SPE) [14] are the most commonly used techniques for separation or preconcentration of CPs in environmental samples. However, for extraction or elution, these steps often require an appreciable amount of toxic solvent, which are hazardous to the operators and result in threat to the environment. Therefore, a variety of microextraction techniques that use no or small amounts of solvent were developed in recent years. Among them, solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) are the two pre-
dominant extraction techniques for analysis of chlorophenols. SPME has some drawbacks such as limited lifetime, fragility of fibers and possibility of sample carry-over [15]. In addition, it is very difficult for SPME to extract some highly polar compounds like chlorophenols without derivatization. Therefore, LPME technique, especially hollow fiber-protected liquid-phase microextraction (HF-LPME) [16], was developed. Usually, two extraction modes including two-phase extraction and three-phase extraction (extraction/back extraction) are used for HF-LPME. For both of the two extraction modes, the selection of an appropriate extraction solvent is of major importance to achieve efficient extraction. Selection of a solvent is based on the proper immobilization of pores of fiber, immiscibility of the solvent with water, low volatility, and good extraction efficiency of analytes. Toluene, undecane, 1-octanol and dihexyl ether were often used as the extraction solvent in HF-LPME. Based on the basic principle of extraction of ‘like dissolves like’, polar solvents should more efficiently extract polar compounds such as chlorophenols. However, most of the conventional polar solvents such as dichloromethane are volatile, so it is very strict with the operators to set up the HF-LPME device. During the extraction, the hollow fiber impregnated with the volatile solvent must be immersed in the sample solutions very quickly to avoid the loss of the extraction solvent in the air, which may cause big deviations between extractions. Therefore, finding novel solvents that are polar and nonvolatile for HF-LPME is of great interest.

It is well known that ionic liquids are polar and nonvolatile. Previous studies also demonstrated that ionic liquid in the pores of supported membrane could not be displaced and the supported ionic liquid membrane was very stable at mild stirring conditions [17,18]. Furthermore, ionic liquids have high affinity to polar compounds [19], and the ionic liquid membrane can transport some organic compounds selectively [20–22]. These specific characteristics make ionic liquids suitable to be used as the extraction solvent for separation and preconcentration of chlorophenols in environmental water samples. In the last three years, several hydrophobic ionic liquids consisted of alkylimidazolium hexafluorophosphate were used for drop-based two phases LPME of some analytes from different environmental samples in our laboratory [23–26]. To the best of our knowledge, ionic liquids-based supported liquid membrane technique was not applied in sample pretreatment in analytical chemistry. Therefore, it is of great interest to test the applicability of ionic liquids as membrane liquids in three-phase LPME for the purpose of development of sample pretreatment method with high selectivity and enrichment factor.

In the present work, the polar and nonvolatile ionic liquid 1-octyl-3-methylimidazolium hexafluorophosphate ([C₈mIM][PF₆]) was employed as the extraction solvent for HF-LPME by using CPs as model analytes. After investigating the relative parameters that affected extraction efficiency, the optimum preconcentration conditions were established for HPLC determination of CPs with a UV detector. Because of the nonvolatilibility of the ionic liquid and the stability of the hollow fiber supported ionic liquid membrane, the proposed method was environmentally benign, easily operating and reliable, with good repeatability and spiked recoveries.

2. Experimental

2.1. Materials and chemicals

The Q 3/2 Accurel polypropylene hollow fiber membrane (600 μm I.D., 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrane, Wuppertal, Germany. A 25-μL microsyringe (Agilent, Palo Alto, CA, USA) with a needle of 0.5 mm-outer diameter was used to introduce the acceptor into the lumen of the hollow fiber for extraction and inject the acceptor into HPLC system after extraction.

HPLC-grade acetonitrile was purchased from J.T. Baker (Phillipsburg, NJ, USA). 3-Chlorophenol (3-CP) and 4-chlorophenol (4-CP) standards (1.00 mg mL⁻¹) were purchased from the Institute for Reference Materials of SEPA (Beijing, China). 2,4-Dichlorophenol (DCP) and 2,4,6-trichlorophenol (TCP) standards (1.00 mg mL⁻¹) were obtained from the National Research Center for Reference Material (Beijing, China). Standard stock solution was prepared by dissolving 1 mL each standard in 10 mL of methanol and stored at 4 °C. Working solutions were obtained daily by appropriately diluting the stock solutions with water. Humic acid was purchased from Acros Organics (Morris Plains, NJ, USA. Technical: 50–60% as humic acid). All other chemicals were purchased from Beijing Chemicals Corporation (Beijing, China) and were of analytical grade or better. A Barnstead (Dubuque, IA, USA) EasyPure LF System which can supply Type I reagent water with resistivity up to 18.2 MΩ cm was used to produce ultrapure water throughout the experiments. The ionic liquid of [C₈mIM][PF₆] (≥98%) was purchased from Solvent-Innovation (Cologne, Germany).

Sodium hydroxide (pH 13.0 and 14.0) solutions and 50 mM phosphate buffers (pH 9.0, 11.0 and 12.0) were used as acceptor solutions, while hydrochloric acid solutions (pH 1.0 and 2.0) and 50 mM phosphate buffer (pH 3.0, 5.0 and 7.0) were used to prepare the sample solutions. All the used reagents were purchased from Beijing Chemicals (Beijing, China). A pH 211 microprocessor pH meter (Hanna Instruments, Sarmeola di Rubano, Padova, Italy) was used to adjust the sample and acceptor buffer pH.

Groundwater samples were collected from a deep well in west suburb of Beijing, river water samples from the Haihe River in Tianjin, China, wastewater at a sewage outfall of a wastewater treatment factory in Beijing and tap water samples from our laboratory after flowing for about 5 min. These samples were all stored at the temperature of 4 °C.

2.2. Extraction procedure

The polypropylene hollow fiber that was cut manually and carefully into 5.0 cm length was immersed in 20 mL [C₈mIM][PF₆] ionic liquid for 10 min to immobilize the ionic liquid in the pores of the hollow fiber. This hollow fiber was taken out and its outside and inside were washed five times with water. Then it was mounted onto the needle tip of the microsyringe holding ~10 μL acceptor solution. Thereafter, the plunger of the microsyringe was depressed to flush out ~10 μL acceptor solution to wash and fill the lumen of the hollow fiber with-
out any air bubbles. Afterwards, the other end was sealed with heated tweezers. By this preparation, the obtained hollow fiber-supported ionic liquid extraction device had an effective fiber length of ∼4.5 cm, with an acceptor phase volume of ∼10 μL. This hollow fiber with the microsyringe was fixed with the clamer of the experimental apparatus. The experimental schematic diagram was similar to that described by Lee et al. [27]. The extraction was carried out by adding 15 mL sample solution to a 20-mL vial, and the prepared hollow fiber with the microsyringe was immersed into the sample solution. And the magnetic stirrer was turned on to start the extraction. After stirring for a prescribed time, the hollow fiber was taken out from the aqueous solution. The sealed end was cut open and the acceptor solution was withdrawn into the microsyringe and injected into the HPLC system directly for determination. The used fiber was discarded and a fresh one was used for the next extraction.

2.3. HPLC determination

The HPLC equipment used was an Agilent 1100 LC system including a binary pump and a VWD detector set at 220 nm. A personal computer equipped with an Agilent ChemStation program for LC was used to process chromatographic data. A 7725 injector valve with a 20 μL loop (Rheodyne, USA) and a 250 mm × 4.6 mm I.D. C18 column (Inertsil ODS-P, GL Sciences, Japan, 5 μm particles) were used for injection and separation of the analytes.

The chromatographic separation was performed with a mobile phase consisting of 20 mmol L⁻¹ phosphate buffer adjusted by hydrochloric acid to pH 2.5 and acetonitrile, and delivered at a flow rate of 1.0 mL min⁻¹. The gradient program was as follows: keeping constant 40% acetonitrile during 0–7 min, then increasing to 60% in 7–12 min, increasing to 80% in 12–20 min and keeping constant until 22 min, thereafter restored to 40% in 3 min followed by a 3 min equilibration time.

3. Results and discussion

3.1. Selection of extraction solvents

According to the basic principle of ‘like dissolves like’, in addition to the [C₈MIM][PF₆] ionic liquid, some other polar solvents such as dichloromethane and chloroform were also tried as liquid membrane for the extraction of chlorophenols. Experiments show that these solvents are very easy to volatilize in the air when setting up the LPME device and the extraction efficiencies deviate greatly between two extractions. Furthermore, according to Seddon’s research [28], [C₈MIM][PF₆] has higher polarity than dichloromethane and chloroform. Therefore, the [C₈MIM][PF₆] ionic liquid was adopted in the following study.

3.2. Immobilization of ionic liquid

The SEM photographs of the inner surface of the hollow fiber before and after impregnation with ionic liquid are shown in Fig. 1. The Fig. 1a shows that the structure of the hollow fiber wall was combined with many micropores. Fig. 1b shows that the micropores were full of ionic liquid, and the ionic liquid membrane formed. Comparing the two photographs, we can see that the ionic liquid was immobilized in the pores effectively and the ionic liquid membrane for extraction formed.

3.3. Effect of the alkalinity of acceptor solution and the acidity of sample solution

Because the extraction in this experiment is a three-phase extraction process, according to the basic principle of extraction/back extraction, adjusting the acceptor solution to a suitable alkalinity can keep the chlorophenols ionized, which ensures that the ionized analyte molecules are irreversibly trapped and thereby concentrated. Fig. 2 shows that for the four analytes, the extraction efficiency increased with the acceptor solution pH up to 13, followed by decrease with further increase of acceptor solution pH. The possible reason for the lower peak area at pH 14 is that the buffer capacity of 20 mM phosphate in the mobile phase is not high enough, and part of the CPs enriched in the 1 M NaOH acceptor solution were still ionized after injecting into the HPLC system. Therefore, acceptor solution at pH 13 was chosen in the following studies.
The sample solution acidity can also influence the extraction efficiency of weak organic base or acid. The sample solutions were often adjusted to appropriate acidity to de-ionize analytes for obtaining higher extraction efficiency. In our experiment, the effect of sample acidity was studied by adding varied amount of 6 M HCl or 1 M NaOH into the sample solution, and no significant change of extraction efficiency was obtained in the studied range of pH 1–7. This result is reasonable as the pKₐ values of the four chlorophenols are 9.41 for 4-CP, 9.12 for 3-CP, 7.89 for DCP and 6.23 for TCP [29], and most of the CPs are existed in the molecule form in the range of pH 1–7. Theoretically, pH 3.0 is enough to keep all the studied CPs in the molecule form as the lowest pKₐ value is 6.23. This corresponded to spike about 3 L of 6 M HCl into the 15 mL standard sample solution. For real environmental samples with different buffer capacity, however, this amount of HCl probably cannot adjust the sample solution to the expected acidity (pH 3.0). As a sample solution of pH 1.0 (0.1 M HCl) can give the same extraction efficiency as that of pH 3.0, 0.1 M HCl in sample solution (spiking 250 µL of 6 M HCl into the 15 mL sample) was adopted to ensure all the studied CPs be deionized in all samples.

### 3.4. Effect of salt addition

Salt was often added into the sample solution for improving the extraction efficiency in most traditional extraction processes. Sodium (Na⁺) and chloride (Cl⁻) were reported to exhibit low affinity towards the ionic liquids used [30], thus their exchange with the cations or anions of ionic liquids should be minor. Therefore, sodium chloride (NaCl) was chosen to study the salt effect in this present study. Varied amounts of NaCl were added into the sample solution to investigate the effect of the salt concentration on the extraction. Fig. 3 shows that for the four analytes, the extraction efficiency increased with the NaCl concentration up to 20%, followed by a descent with further increasing of NaCl concentration. The results can be explained by the two simultaneously occurring processes: the salting out effect and the electrostatic interactions between polar molecules and salt ions in sample solution. At the beginning, the former process played the predominant role. But the salt molecules began to interact with analyte molecules when salt concentration increased further, which directly lead to the decrease of the response. Furthermore, ionic liquid immobilized in the hollow fiber pores became more easily dissolved in the aqueous phase at higher salt concentration, and thus affected the stability of the ionic liquid membrane greatly and decreased the extraction efficiency. Therefore, 20% NaCl was adopted as the final addition in the following studies.

### 3.5. Effect of stirring rate and extraction time

Higher stirring rate could accelerate diffusion of the analytes and abbreviate the extraction dynamic equilibrium time in HF-LPME. In the experiments, the stirring rate was divided into three different classes: low, medium and high. The results show that at the extraction time of 30 min, the medium stirring rate was appropriate to obtain the highest extraction efficiency. High stirring rate caused many bubbles to attach on the surface of the hollow fiber, which impeded the transfer of the analytes. Therefore, the medium stirring rate was chosen for the following investigation.

In addition, by a common rule, increasing extraction time gives increased extraction efficiency until equilibrium. Thus, longer extraction time will achieve higher extraction efficiency. High stirring rate caused many bubbles to attach on the surface of the hollow fiber, which impeded the transfer of the analytes. Therefore, the medium stirring rate was chosen for the following investigation.

In addition, by a common rule, increasing extraction time gives increased extraction efficiency until equilibrium. Thus, longer extraction time will achieve higher extraction efficiency. However, this is not a comprehensive rule for all the experiments. Experiment results shown in Fig. 4 indicate that for all of the four analytes, the peak areas reached the highest at the extraction time of 60 min and then decreased rapidly with further increasing of extraction time. This may attribute to the loss of the ionic liquid membrane when it was exposed to the salt concentration.
solution for a long time. According to this investigation, 60 min was chosen in the following studies.

3.6. Effect of humic acid

For most of environmental water samples analysis, the effect of humic acid on the extraction is an important parameter. In this present study, humic acid concentration covering the range in most environment surface water (0–50 mg L$^{-1}$) [31] was investigated. From Fig. 5, we can see that the addition of humic acid did not significantly affect the extraction efficiency when the humic acid concentration was less than 25 mg L$^{-1}$. If much more humic acid was added, the chlorophenols probably associated with the humic acid in sample solution, which made the extraction more difficult and decreased the extraction efficiency greatly. Increasing the alkalinity of acceptor solution can improve the extraction efficiency of analytes in the acceptor solution. Thus, the equilibrium between the associated humic acid and the freely dissolved analytes will be destroyed and more associated analytes will be released and trapped into the acceptor solution. Higher alkaline acceptor solution was not compatible with the direct injection into the HPLC determination system, and it was not adopted in this proposed method.

3.7. Analytical performance and application

Some characters of the proposed method such as linear range, correlation coefficients, limits of detection (LODs) and repeatability were all investigated by enriching 15 mL of CPs standard solutions and the results were shown in Table 1. Each analyte exhibited good linearity with correlation coefficient $r^2 > 0.99$ in the studied range. The limits of detection, calculated on the basis of signal-to-noise ratio of 3 (S/N = 3), were in the range of 0.5–1.0 μg L$^{-1}$. The repeatability of the analytical performance was studied for five replicate experiments at 5 μg L$^{-1}$ CPs standard solution with relative standard deviations (RSDs) lower than 6.0%. The detection limits and RSDs of this proposed method are comparable with that of a relevant method reported in literature [5], which were 0.5–2.5 μg L$^{-1}$ and 5.6–11.5%, respectively.

In order to validate the proposed method, four real environmental water samples including groundwater, river water, wastewater and tap water were analyzed and the recoveries were determined by spiking the samples with 5 μg L$^{-1}$ CPs. Results show that the contents of CPs in the four samples were all under the detection limits. Table 2 showed that the spiking recoveries were all in the range of 70.0–95.7%. Fig. 6 shows the chromatograms obtained after enriching 15 mL of river water without and with spiking 5 μg L$^{-1}$ CPs, respectively, by the proposed method.

<table>
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<tr>
<th>Table 1</th>
<th>Some analytical performance data of the proposed method</th>
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<tr>
<td>Analytes</td>
<td>Linearity range (μg L$^{-1}$)</td>
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<tr>
<td>---------</td>
<td>--------------------------------</td>
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<tr>
<td>4-CP</td>
<td>5–400</td>
</tr>
<tr>
<td>3-CP</td>
<td>5–400</td>
</tr>
<tr>
<td>DCP</td>
<td>5–400</td>
</tr>
<tr>
<td>TCP</td>
<td>10–200</td>
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<tr>
<td>&lt;sup&gt;a&lt;/sup&gt; Determined at a concentration of 5 μg L$^{-1}$ for each analyte.</td>
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<tr>
<th>Table 2</th>
<th>The recoveries of 4-CP, 3-CP, DCP and TCP in real environmental samples at 5 μg L$^{-1}$ spiking level (mean ± s, n = 3)</th>
</tr>
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<tbody>
<tr>
<td>Samples</td>
<td>4-CP</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>Groundwater</td>
<td>91.2 ± 4.3</td>
</tr>
<tr>
<td>River water</td>
<td>95.7 ± 3.2</td>
</tr>
<tr>
<td>Wastewater</td>
<td>77.5 ± 5.7</td>
</tr>
<tr>
<td>Tap water</td>
<td>88.9 ± 4.6</td>
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</table>

Fig. 4. Effect of extraction time on the peak area of CPs. Sample solutions, prepared by spiking 400 μg L$^{-1}$ of CPs and 3.0 g NaCl into 15 mL of 0.1 M HCl, were extracted with pH 13.0 acceptor buffer for different time at the medium stirring rate.

Fig. 5. Effect of humic acid on the peak area of CPs. Sample solutions, prepared by spiking 400 μg L$^{-1}$ of CPs, 3.0 g NaCl and various amount of humic acid into 15 mL of 0.1 M HCl, were extracted with pH 13.0 acceptor buffer for 60 min at the medium stirring rate.
4. Conclusions

A new analytical method based on hollow fiber supported ionic liquid membrane extraction coupled with HPLC determination for chlorophenols at low μg L\(^{-1}\) level in water samples was proposed. The proposed method has good reproducibility with RSDs lower than 6% and reliable analytical results with spiked recoveries in the range of 70.0–95.7% at 5 μg L\(^{-1}\). The present work also demonstrated that ionic liquid is a promising solvent for supported liquid membrane extraction. It is expected that, by using more hydrophobic or tailored ionic liquid as membrane liquid, extraction procedures with higher extraction efficiency and selectivity can be developed for applications in drug analysis, incitant determination and other fields.

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References


