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Ionic liquid based three-phase liquid–liquid–liquid solvent bar microextraction for the determination of phenols in seawater samples

Liang Guo, Hian Kee Lee*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

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ABSTRACT

For the first time, an ionic liquid based three-phase liquid–liquid–liquid solvent bar microextraction (IL-LLL-SBME) was developed for the analysis of phenols in seawater samples. The ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), was used as the intermediary solvent for LLL-SBME, enhancing the extraction efficiency for polar analytes. In the procedure, the analytes were extracted from the aqueous sample into the ionic liquid intermediary and finally, back-extracted into an aqueous acceptor solution in the lumen of the hollow fiber. The porous polypropylene membrane acted as a filter to prevent potential interfering materials from being extracted, and no additional cleanup was required. After extraction, the acceptor solution could be directly injected into a high-performance liquid chromatographic system for analysis. Six phenols, 2-nitrophenol, 4-chlorophenol, 2,3-dichlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol were selected here as model compounds for developing and evaluating the method. The most influential extraction parameters were evaluated, including the ionic liquid, the composition of donor solution and acceptor solution, the extraction time and the extraction temperature, the effect of ionic strength, and the agitation speed. Under the most favorable extraction parameters, the method showed good linearity (from 0.05–50 to 0.5–50 μg/L, depending on the analytes) and repeatability of extractions (RSD below 8.3%, $n = 5$). The proposed method was compared to conventional three-phase LLL-SBME and ionic liquid supported hollow fiber protected three-phase liquid–liquid–liquid microextraction, and showed higher extraction efficiency. The proposed method was demonstrated to be a simple, fast, and efficient method for the analysis of phenols from environmental water samples.

1. Introduction

In spite of the tremendous development of analytical techniques in the past several decades, sample preparation, which is an unavoidable step for complex matrices to isolate and preconcentrate the target analytes rendering them suitable for the detection system, remains a bottleneck in modern analytical methodology.

To date, much effort has been devoted to establish simple, rapid, minimized as well as environment-friendly sample preparation methods to provide good and effective extraction. Solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) are the two widely developed extraction techniques in the past 15–20 years.

SPME, a solvent free and efficient extraction process, combining extraction and pre-concentration in a single step, has been developed and applied for the extraction of various types of organic compounds. However, highly polar compounds like chlorophenols need to be derivatized prior to SPME [1–3]. In addition, SPME has some shortcomings, such as analyte carry over, and the limited lifetime and fragility of the most commonly used fibers.

More recently, liquid-phase microextraction (LPME), an alternative solvent-miniaturized extraction method, has been developed to overcome the problems associated with SPME, and demonstrated to be a simple, fast and cost-effective sample preparation technique. In addition, LPME uses only a few microliters of solvent and reduces exposure to the operator, and discharge into the environment. The simplest form of LPME, single-drop microextraction (SDME) is not a robust system due to the instability of the microdrop held on a Teflon rod or at the tip of needle of a microsyringe.

Hollow fiber protected LPME (HF-LPME) was developed to address this aforementioned drawback of SDME. In this method, the solvent is held and protected by an HF fixed to a microsyringe during the extraction, and after extraction, the extract can be easily withdrawn into the microsyringe and directly analyzed. Due to the protection afforded by the HF, the agitation speed can be increased, thus enhancing the extraction. A variation of HF-LPME that involves a free-moving solvent-filled HF, solvent bar

* Corresponding author. Tel.: +65 6516 2995; fax: +65 6779 1691.
E-mail address: chmleehk@nus.edu.sg (H.K. Lee).

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Table 1
Physical properties of target phenols.[a]

Analyte	pK_a	CAS number
4-CP	8.81	106-48-9
2-NP	7.23	88-75-5
2,3-DCP	7.70	576-24-9
2,4-DCP	7.89	120-83-2
2,4,6-TCP	5.99	88-06-2
PCP	4.70	87-86-5

[a] Values taken from Ref. [22].

microextraction (SBME), developed by Jiang and Lee [4], demonstrated higher extraction efficiency. The free movement of the solvent bar in the aqueous sample solution greatly increases the transfer of analytes from the aqueous sample to the extraction solvent.

In SBME, the selection of an appropriate solvent is essential to extraction efficiency. There are some considerations in choosing a solvent, high extraction capability of analytes, immiscibility with water, low volatility, compatibility with HF, and less interference with the chromatographic analysis of the target analytes [1,5–7]. Toluene and 1-octanol are widely used extraction solvents [8,9]. Moreover, based on the "like dissolves like" principle, polar solvents should have higher extraction efficiency for polar analytes such as phenols. For these polar analytes, three-phase SBME, whereby analytes in aqueous donor solution are first extracted into an intermediary organic solvent and subsequently back-extracted into an aqueous acceptor solution, is more suitable [10].

Ionic liquids (ILs) are salts that are usually composed of large asymmetric organic cations and either an organic or an inorganic anion [5]. They are polar, of low volatility, and are able to dissolve a lot of organic compounds [11]. Due to their negligible volatility, ILs are considered green solvents to both operator and environment. Furthermore, ionic liquids have been used in hollow fiber membrane extraction applications and have high affinity for polar analytes [1,12–15]. These significant features make ILs as good alternatives to conventional organic solvents used for extraction or preconcentration. Since their introduction in LPME by Liu et al. [16], ILs have been widely used in extracting a variety of organic compounds [16–21].

In this study, the hollow fiber-supported ionic liquid based three-phase liquid–liquid–liquid solvent bar microextraction (IL-LLL-SBME) was developed and applied for the determination of trace phenols in seawater samples followed by analysis with HPLC–UV. This was the first time an ionic liquid was used as the intermediary solvent in a three-phase LLL-SBME procedure in which the IL was impregnated in the HF wall pores, with aqueous solution as acceptor in the lumen of the HF. Since protection was afforded by the hollow fiber, no extra cleanup procedure was needed. The method combined analyte extraction and concentration in a single step. Since the final extract was aqueous, it could be directly analyzed by reverse phase HPLC. The extraction parameters were optimized and the proposed method was applied to analyze genuine seawater samples.

2. Experimental

2.1. Chemicals and materials

The compounds, 2-nitrophenol (2-NP), 2,3-dichlorophenol (2,3-DCP), and 2,4-dichlorophenol (2,4-DCP) were supplied by Sigma-Aldrich (Milwaukee, WI, USA), while 4-chlorophenol (4-CP), 2,4,6-trichlorophenol (2,4,6-TCP), and pentachlorophenol (PCP) were bought from Fluka (Buchs, Switzerland). Their physical properties are shown in Table 1.

HPLC-grade methanol and acetonitrile were purchased from Tedia Company (Fairfield, OH, USA). Phosphoric acid and 1-octanol were bought from Merck (Darmstadt, Germany). Sodium hydroxide (NaOH) was from Chemicon (Temecula, CA, USA) and sodium chloride (NaCl) was acquired from Goodrich Chemical Enterprise (Singapore). Ultrapure water was produced on a Nanopure water purification system (Barnstead, Dubuque, IA, USA). A magnetic stirrer plate was purchased from Heidolph (Kelheim, Germany).

Six room temperature ionic liquids (>98% purity); 1-butyl-3-methylimidazolium methylsulfate ([BMIM][$MeSO_4$]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF4]), were purchased from Merck (Darmstadt, Germany), while N-butyl-3-methylpyridinium bis(trifluoromethylsulfonyl)imide (BMPIm), 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (EMIIm), 1-butyl-3-methylimidazolium phosphate ([BMIM][PO_4]), were bought from Strem Chemicals (Newburyport, MA, USA).

2.2. Apparatus and instrumentation

Separation and analysis of analytes were carried out on a Shimadzu (Kyoto, Japan) HPLC system. The chromatographic system consists of a LC-20AD binary pump, an SPD-20A ultraviolet–visible (UV–vis) detector, a DGU-20A degasser, a SIL-20A auto sampler, and a dynamic mixing chamber.

An Agilent Technologies (Palo Alto, CA, USA) Eclipse C_{18} column (4.6 mm × 250 mm I.D., 5 μm) was used for separation. The mobile phase used for separations was a binary solvent of acetonitrile:water (pH = 3.0, adjusted by phosphoric acid). Gradient elution with a flow-rate of 1.0 mL/min was applied: initial 50% acetonitrile for 1 min, then a linear ramp to 65% in 8 min, held at 65% for 1 min and then, followed by a linear ramp to 50% in 15 min. The detection wavelength was set at 220 nm and the analysis was carried out at ambient temperature. All the experiments were performed in triplicate.

The Q 3/2 Accurel polypropylene hollow fiber was purchased from Membrana (Wuppertal, Germany). The inner diameter of the hollow fiber was 600 μm, the thickness of the wall was 200 μm, and the wall pore size was 0.2 μm. The hollow fiber was ultrasonically cleaned in HPLC-grade acetone and dried before use. It was cut into 2.80 cm segments for subsequent experiments.

A microsyringe (10 μL) with a cone needle tip (SGE, Sydney, Australia) was used for filling the hollow fiber membrane with acceptor solution. Another microsyringe (10-μL) with a flat-cut needle tip (Hamilton, Reno, NV, USA) was used for drawing out analyte-enriched acceptor solution from the hollow fiber membrane after extraction.

2.3. Sample preparation

A stock solution containing 1000 mg/L of each analyte was prepared with methanol and was stored in the refrigerator at 4 °C until analysis. Water samples were prepared by spiking deionized water with analytes at known concentrations (20 μg/L) to study extraction performance and optimize the extraction conditions as indicated in the individual experiments. Quantification of the analytes was done by external calibration, where a series of standard solutions was prepared by diluting the stock solution and analyzing with HPLC–UV to obtain linear calibration plots for each analyte based on the chromatographic peak areas.

Genuine seawater samples were collected from the west coast of Singapore using pre-cleaned glass bottles. The bottles were covered with aluminum foil to prevent any photochemical degradation. All collected seawater samples were transported to the laboratory immediately, and stored in the refrigerator at 4 °C. The genuine

seawater samples were extracted and analyzed without any prior treatment or filtration to avoid the loss of target phenols.

2.4. IL-LLL-SBME process

To prepare the solvent bar, the hollow fiber was manually and carefully cut into 2.8-cm segments. One end of the hollow fiber was heat-sealed. A suitable volume of acceptor solution was withdrawn into a 10-μL microsyringe with the cone needle tip. The needle tip was carefully inserted into the open end of the hollow fiber, and the acceptor solution was introduced into the lumen of the fiber. Then the fiber was carefully removed from the needle, and its open end was heat-sealed. The fiber formed a solvent bar with two ends heat-sealed. No leakage was observed when heat-sealing the fiber. According to our experiments, the effective length of the solvent bar was ~2.2 cm, and the acceptor phase volume inside the fiber lumen was ~4.8 μL.

The solvent bar was immersed in the ionic liquid for 5 s to impregnate the wall pores of the hollow fiber. The ionic liquid-impregnated solvent bar was then placed in the sample solution for extraction. The volume of the aqueous solution was 10 mL in a 15-mL vial. The aqueous solution was kept under stirring at 700 rpm during extraction procedure. After 20 min of extraction, the solvent bar was taken out. One end of the solvent bar was trimmed off with a sharp blade, and the analyte-enriched acceptor solution was carefully withdrawn into a microsyringe with a flat-cut needle tip. Finally, a 2-μL aliquot of the extract was directly injected into the HPLC–UV system for analysis. The used fiber was discarded, and a fresh one was used for the next extraction.

2.5. Conventional LLL-SBME (non-IL-LLL-SBME)

To carry out conventional LLL-SBME, a 10-mL sample solution in a 15-mL vial was used. The preparation of solvent bar was the same as that of IL-LLL-SBME, except that no ionic liquid was used. Before the extraction, the solvent bar was placed into 1-octanol for 5 s to impregnate the pores of the wall of the fiber and subsequently, was immediately placed in the sample solution that was maintained at 60 °C and under magnetic stirring (700 rpm). After 40 min of extraction, the solvent bar was taken out. One end of the hollow fiber was trimmed off with a sharp blade, and acceptor solution was carefully withdrawn into the microsyringe with a flat-cut needle tip. Finally, the extractant was injected into the HPLC–UV system for analysis. The used fiber was discarded, and a fresh one was used for the next extraction.

2.6. Ionic liquid supported HF-LLLME (IL-HF-LLLME)

Prior to extraction, the hollow fiber was cut into 2.80 cm segments and ultrasonically cleaned in HPLC-grade acetone and dried and then, one end was heat-sealed. Using a 10-μL microsyringe with a cone tip needle, a 6-μL aliquot of acceptor solution was introduced into the lumen of the hollow fiber. The fiber was immersed in the ionic liquid for 5 s to impregnate the pores of the wall of the hollow fiber. The ionic liquid impregnated fiber with the microsyringe was placed in a 10 mL of sample solution (in a 15-mL vial) for extraction for 40 min. The sample solution was maintained at 60 °C and under magnetic stirring (700 rpm) during the extraction procedure. After extraction, the hollow fiber with microsyringe was removed from the sample solution. The acceptor solution was carefully withdrawn into the syringe and subsequently, was directly injected into a HPLC–UV instrument for analysis. The used hollow fiber was discarded and a fresh one was used for the next experiment.

3. Results and discussion

3.1. Basic principle of IL-LLL-SBME

The basic principle of IL-LLL-SBME is similar to that of conventional LLL-SBME [1,4,5,10,23]. Briefly, the three-phase system consists of the aqueous sample solution (serving as donor phase), the ionic liquid phase impregnated in the wall pores of the hollow fiber (intermediary organic solvent), and the aqueous solution in the lumen of the hollow fiber (serving as acceptor phase). The ionic liquid, which is immiscible with aqueous solution, prevented the mixture of donor phase and acceptor phase, and served as a carrier of analytes. The analytes were extracted from donor phase, through the ionic liquid immobilized in the pores of hollow fiber and finally, into the acceptor phase in the lumen of the hollow fiber. The analytes were ionized and trapped in the acceptor phase, preventing them from being re-extracted into ionic liquid.

3.2. Enrichment factor

Generally, the IL-LLL-SBME procedure may be illustrated by the following equation:

$$i_d \leftrightarrow i_i \leftrightarrow i_a$$

where d, i, and a represent the donor phase, the ionic liquid phase, and the acceptor phase, respectively. The enrichment factor (EF), defined as the ratio $C_{a,eq}/C_{d,initial}$ [4,5], where $C_{a,eq}$ and $C_{d,initial}$ are the final concentration of analytes in the acceptor phase and the initial concentration of analytes in the donor phase, can be given as follows [24–26]

$$EF = \frac{1}{1/K_1K_2 + V_{IL}/K_2V_d + V_a/V_d}$$

where V_{IL}, V_a and V_d are the volume of the ionic liquid in the pores of the hollow fiber, acceptor phase and the donor phase, respectively. K_1 and K_2 are the distributions ratios for the analytes from the donor phase into the ionic liquid phase, and from the ionic liquid phase into the acceptor phase, respectively.

$$K_1 = \frac{C_{IL}}{C_d}$$

and

$$K_2 = \frac{C_a}{C_{IL}}$$

where C_{IL}, C_a and C_d represent the equilibrium concentration of analytes in the ionic liquid phase, the acceptor phase, and the donor phase, respectively.

Since the volume of ionic liquid in the pores of hollow fiber is very small, Since

$$V_{IL} \ll K_2V_d$$

EF can be calculated simply as

$$EF = \frac{1}{1/K + V_a/V_d}$$

where K is the distribution coefficient of the three-phase equilibrium, and

$$K = K_1K_2 = \frac{C_a}{C_d}$$

From the equation above, it is obvious that high EF can be achieved by decreasing the ratio of the acceptor phase volume and the donor phase volume.

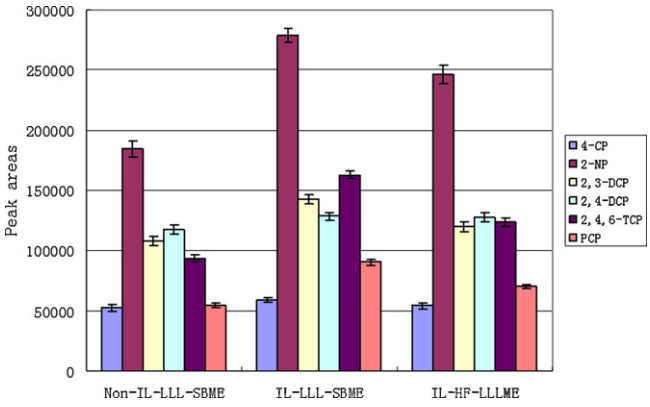

Fig. 1. Comparison in LLL-SBME, IL-LLL-SBME, and IL-HF-LLLME.

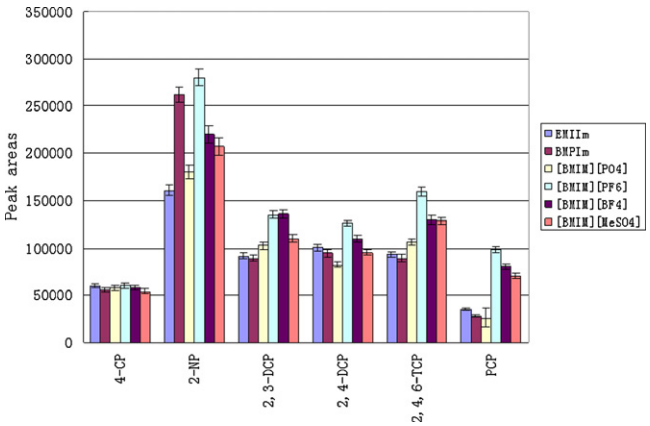

Fig. 2. Comparison of performance of different ionic liquids in IL-LLL-SMBE.

3.3. Comparative studies

The performance of IL-LLL-SBME was compared with that of conventional LLL-SBME and IL-HF-LLLME. Spiked ultrapure water samples (20 μg/L of each phenol) were used for the comparative extraction.

As seen from Fig. 1, the highest extraction efficiency was obtained by IL-LLL-SBME, followed by IL-HF-LLLME, and then, conventional LLL-SBME. The [BMIM][PF_6] was demonstrated to have significantly better extraction of the target analytes due to its higher affinity to polar analytes, insolubility in the aqueous sample solution, and higher stability in the wall pores of hollow fiber. The extraction efficiency of the proposed method was conceivably enhanced due to the free movement and random tumbling of the solvent bar in the aqueous sample solution during the extraction procedure. This facilitates the contact of the solvent bar with sample thereby accelerating the analyte transfer into the ionic liquid phase. Furthermore, the IL-LLL-SBME was reasonably fast and, only 20 min was required for the extraction procedure.

It is notable to mention that the extraction time for the proposed method was only 20 min, much less than that of LLL-SBME (40 min) and ionic liquid supported HF-LLLME (40 min), reducing the possible loss of ionic liquid during long extraction duration, which also conceivably contributed to the higher extraction efficiency.

3.4. Optimization

In order to obtain the most favorable extraction conditions, the IL-LLL-SBME extraction parameters that affect the extraction efficiency, including the type of ionic liquid, pH of sample solution and acceptor solution, extraction temperature and extraction time, agitation speed, and the effect of ionic strength, were investigated. The optimization was based on the extraction efficiency in terms of the peak areas of analytes. All experiments were performed in triplicate.

3.4.1. The selection of ionic liquid

The ionic liquid was selected based on the following considerations: (1) it should be compatible with the polypropylene hollow fiber and then, be easily and securely immobilized in the pores of the hollow fiber; (2) it should be immiscible with water since it served as a barrier between the donor phase and the acceptor phase; (3) it should permit the following situation to prevail during the extraction procedure: analytes should have higher solubility in the ionic liquid than in the donor phase and have less solubility in ionic liquid than in the acceptor phase; (4) it should also be relatively low volatile or non-volatile to prevent loss during extraction. The following six ionic liquids, [BMIM][PF_6], [BMIM][BF_4], [BMIM][$MeSO_4$], [EMIIm], [BMPIm], and [BMIM][PO_4], were studied. All of them could be easily immobilized in the pores of polypropylene hollow fiber using the procedure designed above. The extraction efficiencies with different ionic liquid are shown in Fig. 2.

[EMIIm], [BMPIm], and [BMIM][PO_4] gave comparable extraction efficiencies for most analytes, except for PCP. The highest extraction efficiency for all analytes was obtained by [BMIM][PF_6], followed by [BMIM][BF_4], and then, [BMIM][$MeSO_4$]. Compared to other ionic liquids studied here, the more hydrophobic of [BMIM][PF_6], as well as its insolubility in the aqueous solution, should contribute to its better extraction efficiency [5], or borne out by its better performance.

3.4.2. Composition of donor phase and acceptor phase

The compositions of the sample solution and the acceptor solution play critical roles in three-phase SBME. In order to obtain efficient extraction of target phenols, which are weekly acidic, the sample solution should be adjusted to a suitable acidity to de-ionize analytes and maintain their neutrality thereby increasing their distribution into the ionic liquid phase.

A series of concentrations of HCl in the sample solution were investigated to optimize the pH of the sample solution. There was no significant change in the extraction efficiency of analytes when the concentration of HCl was varied from 0.001 M to 1.0 M, as expected, since the pK_a of all analytes is higher than 4.70, and the optimum extraction efficiency for all analytes was obtained at 0.1 M HCl in general.

The acceptor solution can also affect the extraction efficiency. It is necessary to adjust the acceptor solution to an appropriate alkalinity to maintain the ionic status of the weakly acidic analytes, preventing them from being re-extracted into the ionic liquid phase, ensuring the analytes were trapped and concentrated in the acceptor solution. In the study, varying concentrations of NaOH from 0.001 M to 1 M were used as acceptor solution. As shown in Fig. 3, the extraction efficiency increased with the increase of concentration of NaOH from 0.001 M to 0.1 M and then flattened out. This result showed that 0.1 M NaOH was efficient to ionize all the analytes (whose pK_as range from 4.70 to 8.81) to give efficient extraction. Higher concentration of NaOH as the acceptor solution had no significant improvement on extraction efficiency.

Based on the above discussion, a combination of 0.1 M HCl and 0.1 M NaOH under which the most favorable extraction efficiency was obtained was selected as the donor solution and the acceptor solution, respectively.

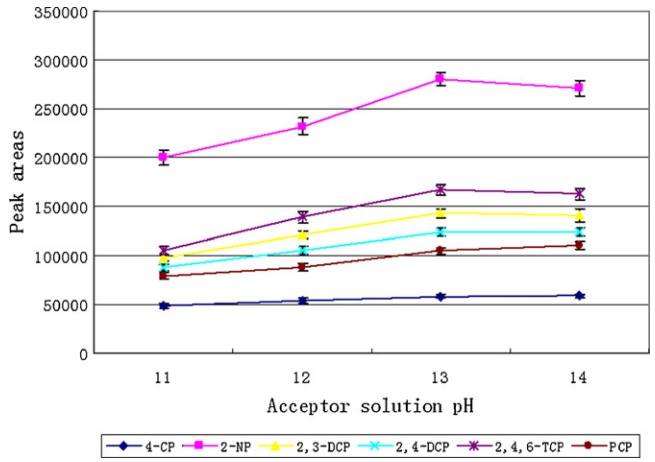

Fig. 3. Effect of acceptor solution pH on the extraction of phenols.

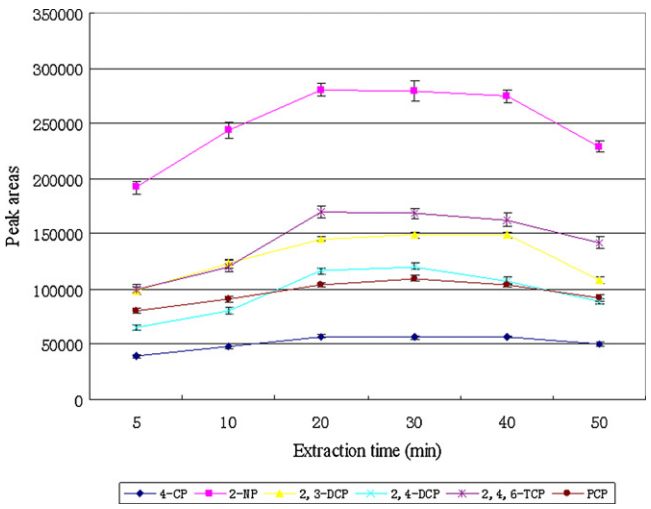

Fig. 5. Extraction time profiles of IL-LLL-SBME.

3.4.3. *The effect of extraction temperature*

The extraction temperature plays a significant role in LPME and SPME [27,28]. In order to investigate the effect of temperature on extraction efficiency, a series of experiments was carried out at 25 °C (ambient temperature), 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C. The effect of temperature on extraction efficiency is shown in Fig. 4. It was apparent that, for all the six analytes, the peak areas were enhanced with the increase of temperature, up to ca. 60 °C and subsequently, the peak areas started to decline.

Generally speaking, increasing sample solution temperature can increase the diffusion coefficients of analytes, thus facilitating the partitioning of analytes from the aqueous solution to the ionic liquid and thereby, leading to an equilibrium [29–31] state more rapidly. Furthermore, with the increase of temperature, the viscosity of the ionic liquid impregnated in the pores of hollow fibers can be decreased [32], speeding the transfer of analytes through the ionic liquid and finally partition into the acceptor solution. Nonetheless, too high a temperature may result in the increase of analytes distributed from aqueous solution into the headspace, thereby, reducing the availability of analytes for transfer to the acceptor solution. Thus, the temperature of 60 °C represented the most favorable extraction temperature.

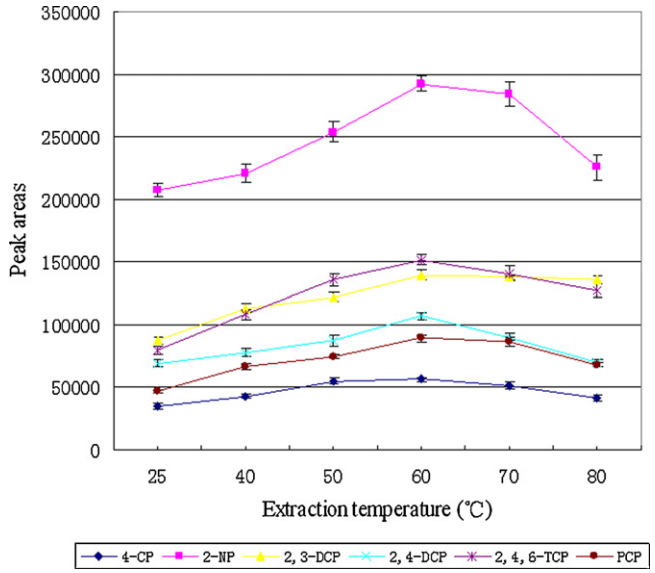

Fig. 4. Effect of temperature on the extraction of phenols.

3.4.4. *Extraction time profiles*

A series of extraction times was studied to evaluate the extraction efficiency. The sample solution was heated at 60 °C and under a constant magnetic stirring at 700 rpm. The extraction time investigated was 5, 10, 20, 30, 40, and 50 min, respectively. Fig. 5 shows the extraction time profiles. As can be seen, the peak areas of the analytes increased significantly when the extraction time increased from 5 to 20 min, and then, reached a plateau, indicating that the equilibrium had been reached. Above 40 min, the peak areas of most analytes decreased, depending on different analytes.

IL-LLL-SBME is an equilibrium extraction rather than an exhaustive extraction procedure. The extraction efficiency depends on the amount of analytes transferred from the sample solution to the acceptor solution. Since the mass transfer is a time-dependent process, a prolonged extraction time would achieve higher extraction efficiency. After a period of extraction time, the equilibrium of mass transfer was established and therefore, the highest extraction efficiency was obtained and thereon, increasing time would have no significant effect. Furthermore, too long an extraction time may conceivably lead to the loss of ionic liquid impregnated in the pores of hollow fiber since it was exposed to the salt solution, as was reported in a previous study [1], thus reducing the extraction efficiency. Twenty minutes was thus selected as the most favorable extraction time.

3.4.5. *Effect of ionic strength*

The salting-out effect is widely used in LLME and SPME to improve the extraction efficiency. In this study, the effect of addition of salt on extraction efficiency was investigated by adding various amounts of sodium chloride (NaCl) (ranging from 0 to 30%, w/v) into the sample solution. From Fig. 6, it was observed that the extraction efficiency of all analytes increased with the increase of the NaCl concentration, up to 15% (w/v) and then, reached a plateau. Above 20%, the extraction efficiency of all analytes decreased with the further increase of the NaCl concentration.

This observation can be explained as follows [33]. Firstly, the salting-out effect had a definite effect on the extraction efficiency when the salt concentration was relatively low. The presence of salt increased the ionic strength of the aqueous solution and therefore, decreased the solubility of analytes and further, enhanced their partitioning from this solution into the ionic liquid phase, resulting in the increase in extraction efficiency.

Secondly, with the increase of the NaCl concentration, the electrostatic interaction process between polar analytes and salt ions, occurring simultaneously with the salting-out effect, played

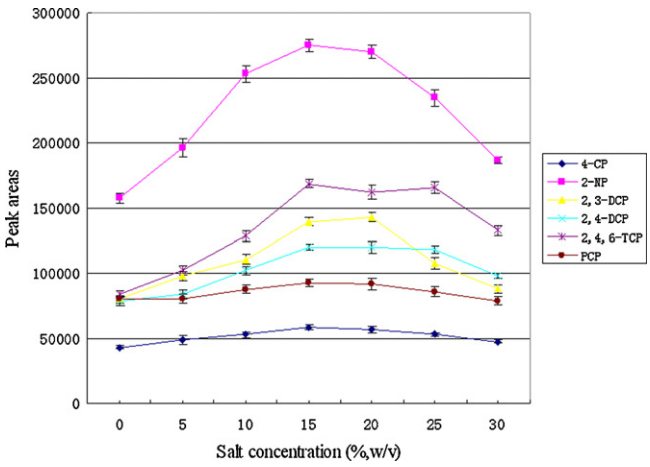

Fig. 6. Effect of ionic strength on the extraction of phenols.

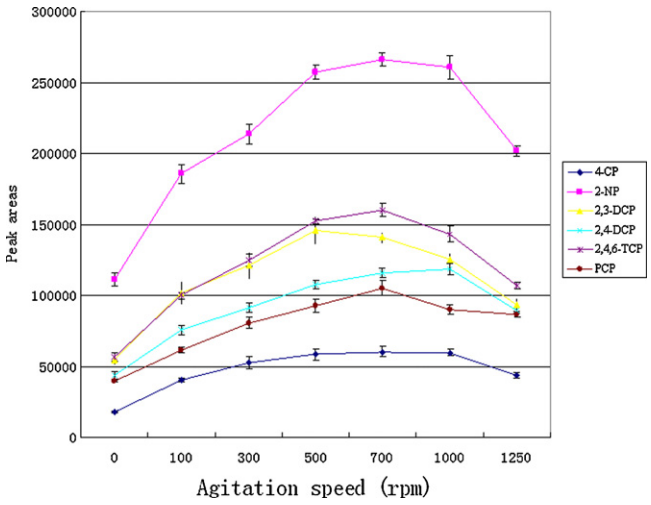

Fig. 7. Effect of agitation speed on extraction.

a more predominant effect on the extraction efficiency. This process obstructed the transfer of analytes into the ionic liquid phase thereby, reducing the extraction efficiency.

Thirdly, high NaCl concentration in the aqueous solution may lead to a loss of ionic liquid impregnated in the pores of hollow fiber due to the solubility of [BMIM]Cl in the aqueous solution, due to an ion exchange process between [BMIM][PF_6] and chloride [5,34]. This might decrease the extraction efficiency.

Based on the above considerations, salt addition was limited to 15% NaCl.

3.4.6. Agitation speed

The extraction efficiency of IL-LLL-SBME procedure depends on the partitioning rate of analytes from the aqueous solution, through the ionic liquid phase and finally, into the acceptor solution. As with SPME and LPME, agitation is an important factor that affects the extraction efficiency [35].

The effect of agitation speed on the extraction efficiency was investigated under different stirring speed from 300 to 1250 rpm. It can be clearly seen from Fig. 7, that initially, the peak areas increased rapidly, followed by a slower rate of increase and subsequently, the signals flattened out with the agitation speed of 700 rpm. However, the peak areas decreased when the stirring speed was higher than 1000 rpm.

Agitation can effectively enhance the partitioning of the analytes into the ionic liquid thereby, reducing the time needed to achieve partitioning equilibrium. Furthermore, under agitation the solvent bar moved and tumbled freely in the aqueous solution and, would be continuously exposed to fresh aqueous solution. On the other hand, when the agitation speed was above 1000 rpm, air bubbles were observed and adhered to the hollow fiber membrane surface, which conceivably obstructed the partitioning of analytes and inevitably, affected the extraction efficiency and precision [4,36]. Additionally, too high an agitation speed may conceivably lead to a loss of ionic liquid impregnated in the pores of hollow fiber or any extractant. Therefore, an agitation speed of 700 rpm was chosen as the most favorable agitation speed for the extraction.

Based on the discussion above, the most favorable IL-LLL-SBME conditions for phenols were using [BMIM][PF_6] as ionic liquid, 0.1 M HCl as donor solution and 0.1 M NaOH as acceptor solution, agitation speed 700 rpm, addition of 15% (w/v) NaCl, extraction time 20 min and extraction temperature 60 °C, and the injection volume for HPLC–UV analysis is 2 μL. All the following experiments were carried out under these conditions.

3.5. Method validation

Under the aforementioned extraction conditions, using spiked ultrapure water samples (20 μg/L of each analyte), the developed method was evaluated by determining the linear range, repeatability, limits of detection (LODs), and enrichment factors. These results are summarized in Table 2.

By plotting the mean peak areas against the concentrations of analytes in the sample solution, calibration curves were obtained (based on seven points). Good linearity of each analyte was obtained in the range of 0.05–50, 0.1–50, 0.2–50, and 0.5–50 μg/L, respectively, depending on analytes, with correlation coefficient (r) higher than 0.9869. The repeatability in peak areas was investigated for five replicate analyses at the same operation parameters. The relative standard deviations (RSD) were lower than 8.3% for all the six analytes, showing good repeatability of the method.

Based on a signal-to-noise ratio (S/N) of 3, the LODs were in the range of 0.01–0.1 μg/L. The LODs obtained were lower than those obtained by hollow fiber protected LPME-CE-DAD [37], cloud point preconcentration-HPLC-electrochemical detection [38], single drop coacervative microextraction-HPLC–UV [39], and at the same range with those obtained by continuous flow liquid membrane extraction-LC-DAD [40], dispersive liquid–liquid microextraction-derivatization-GC-ECD [41], immersed solvent microextraction-GC–MS [42], solid phase microextraction-GC–MS [43], solid-phase

Table 2
Linear range, limits of detection, enrichment factors, and precision of phenols of IL-LLL-SBME.

Analyte	Linear range (μg/L)	Correlation coefficient (r)	LOD (μg/L)	Enrichment factors	RSD (%, n = 5)
4-CP	0.5–50	0.9869	0.1	81	6.1
2-NP	0.05–50	0.9933	0.01	158	5.2
2,3-DCP	0.1–50	0.9946	0.05	132	8.3
2,4-DCP	0.2–50	0.9907	0.05	116	7.6
2,4,6-TCP	0.1–50	0.9912	0.02	143	6.8
PCP	0.2–50	0.9894	0.05	99	7.2

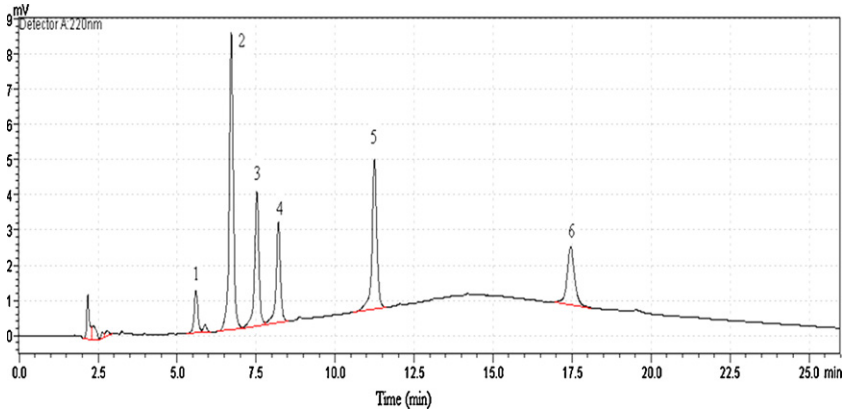

Fig. 8. Chromatography of extract of spiked genuine seawater sample (2.0 μg/L of each analyte) under the most favorable extraction conditions, as given in the text. (1) 4-Chlorophenol, (2) 2-nitrophenol, (3) 2,3-dichlorophenol, (4) 2,4-dichlorophenol, (5) 2,4,6-trichlorophenol, and (6) pentachlorophenol.

extraction-dispersive liquid–liquid microextraction-GC-ECD [44], solid-phase extraction-HPLC–UV [45], membrane assisted solvent extraction-GC–MS [46], but higher than these achieved by stir bar sorptive extraction-thermal desorption-GC–MS [47] and solid-phase microextraction-HPLC-electrochemical detection [48]. The advantage of the present procedure over the last two mentioned methods is the non-dependence on commercial microextraction devices.

3.6. Genuine water samples analysis

The applicability of the developed method was evaluated by determining phenols in genuine seawater samples collected from the west coast of the island of Singapore. The seawater samples were directly extracted using the developed method without any pretreatment. There were, however, no target analytes detected in the seawater samples after IL-LLL-SBME; it is likely they were not present, or they were below the LODs of the present procedure.

Nevertheless, in order to assess matrix effects, genuine seawater samples were spiked with phenols from a standard solution at a concentration 25 or 2.0 μg/L of each analyte (Table 3). Fig. 8 shows a chromatogram of extract of spiked seawater samples (2.0 μg/L of each analyte) after extraction by the developed method. The relative recoveries, defined as the ratios of the measured concentration of the analytes in real samples and the measured concentration of analytes in pure water samples spiked with the same amount of analytes, are summarized in Table 3. Relative recoveries of above 82.5% were obtained for all analytes, demonstrating that the matrices of the genuine seawater samples have little effect on the extraction efficiency of the developed method. The method is suitable for the determination of phenols at trace level concentrations in environmental water samples; the usual concentrations of phenols in contaminated seawater are usually in the parts per billion rage.

Table 3
Summary of results of analysis of phenols in spiked genuine seawater samples by IL-LLL-SBME.

Analyte	Spike seawater water (25 μg/L)		Spike seawater water (2.0 μg/L)	
	Relative recovery (%)	RSD (%)	Relative recovery (%)	RSD (%)
4-CP	83.3	9.2	88.6	8.9
2-NP	96.5	8.3	101.7	7.4
2,3-DCP	86.7	7.5	90.1	9.6
2,4-DCP	89.4	8.9	82.5	5.4
2,4,6-TCP	97.8	6.3	98.8	5.7
PCP	101.0	5.2	105.4	6.3

4. Conclusion

In the present work, a new organic solvent-free three-phase microextraction method, ionic liquid based liquid–liquid–liquid solvent bar microextraction was developed and applied for the determination of phenols in seawater samples with subsequent analysis by HPLC–UV.

Higher extraction efficiency for polar analytes was obtained by using the ionic liquid ([BMIM][PF_6]) as the intermediary solvent impregnated in the wall pores of the hollow fiber.

Since protection was afforded by the hollow fiber, no extra cleanup was needed. After extraction, the acceptor solution could be directly injected into a HPLC–UV system for analysis. In addition, any potential carry-over was avoided by using the hollow fiber only once.

With the proposed method, good LODs, linearity, and acceptable repeatability were achieved. Furthermore, seawater matrix effects were investigated and demonstrated to have little effect on the extraction. IL-LLL-SBME, in conjunction with HPLC–UV analysis, was shown to be a simple and efficient extraction method for phenols in environmental water samples.

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