Magnetic ionic liquid aqueous two-phase system coupled with high performance liquid chromatography: A rapid approach for determination of chloramphenicol in water environment

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ABSTRACT

A novel organic magnetic ionic liquid based on guanidinium cation was synthesized and characterized. A new method of magnetic ionic liquid aqueous two-phase system (MILATPs) coupled with high-performance liquid chromatography (HPLC) was established to preconcentrate and determine trace amount of chloramphenicol (CAP) in water environment for the first time. In the absence of volatile organic solvents, MILATPs not only has the excellent properties of rapid extraction, but also exhibits a response to an external magnetic field which can be applied to assist phase separation. The phase behavior of MILATPs was investigated and phase equilibrium data were correlated by Merchuk equation. Various influencing factors on CAP recovery were systematically investigated and optimized. Under the optimal conditions, the preconcentration factor was 147.2 with the precision values (RSD%) of 2.42% and 4.45% for intra-day (n=6) and inter-day (n=6), respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.14 ng mL⁻¹ and 0.42 ng mL⁻¹, respectively. Fine linear range of 12.25 ng mL⁻¹~2200 ng mL⁻¹ was obtained. Finally, the validated method was successfully applied for the analysis of CAP in some environmental waters with the recoveries for the spiked samples in the acceptable range of 94.6%~99.72%. Hopefully, MILATPs is showing great potential

to promote new development in the field of extraction, separation and pretreatment of various biochemical samples.

Keywords: magnetic ionic liquid; aqueous two-phase; chloramphenicol; preconcentration; microextraction

1. Introduction

Pretreatment of various analytes is the most challenging and time-consuming step in an analytical procedure [1]. Currently, there are many available extraction procedures. For example, conventional solid-phase extraction (SPE) [2] and liquid-liquid extraction (LLE) [3] have been used for pre-concentration and clean-up in the

analysis process as efficient techniques to separate and concentrate various substrates. Besides the advantages shown in their successful applications, they also have some limitations, such as high cost, time-consuming operation, use of large amount of organic solvents which generally are toxic and hazardous to organisms and the environment [4]. As a result, new methods for sample preparation continue to be developed [5]. Among them, dispersive liquid-liquid microextraction (DLLME) was introduced by Assadi et al. as a simple extraction and rapid pre-concentration method [6]. This technique has been widely applied to analyze trace amount of various compounds in environment, food and drink samples for its good repeatability, less extraction time, high recovery as well as high enrichment factor [7-10]. However, volatile organic solvent was also used as disperser solvent which is not environmentally friendly when large scale of samples is pretreated. Shi et al. applied magnetic nanoparticles in the DLLME system to simplify the extraction and separation of target analytes by employing an external magnetic field [11]. Nevertheless, additional desorption procedure was required; it increased time and operational difficulty. Addition of desorption solvent might probably reduce the enrichment factor, and recovery could not be maintained consistently at the high level during the reuse of sorbents.

Aqueous two-phase system (ATPs) is a specific thermodynamic mixture which is usually formed by two different compositions mixed with water. As a promising alternative for traditional organic-water solvent extraction system, it was first proposed in the 20th century [12]. Compared with the above mentioned methods, ATPs is more environmentally friendly without any use of harmful volatile organic solvent in the whole process, and it has been widely used to separate and purify

proteins [13], antibiotics [14-15], metal ions [15] and dyes [16]. In recent years, some ATPs containing ionic liquids have been being developed and proved to have promising extraction capacity in many separation applications [17-19]. Rogers and his co-workers demonstrated the ability of ILs to form ATPSs in the presence of inorganic salts for the first time in 2003 [20]. IL-salt aqueous two-phase systems (ILATPs) have shown many advantages, such as low viscosity, little emulsion, rapid phase separation and environmentally compatibility [21]. It has been successfully applied as a green, non-toxic and sensitive pretreatment technique for various samples [22]. However, ILATPs still revealed a shortage of low isolation efficiency of two phases because of small density difference [23].

Recently, applications of magnetic room temperature ionic liquid (MRTIL) become a new field and a hotspot of research in sample extraction and concentration techniques [24-27]. Some ILs were immobilized on the surface of magnetic nanoparticles to form functional solid materials and used in magnetic solid-phase extraction [28-29]. These systems not only employ environmentally friendly IL as extractant, but also use magnetic separation technology to simplify separation. However, organic solvent is still introduced in desorption procedure and desorption is really extra tedious operation. Lately, a novel class of genuine magnetic ionic liquids with single-component was discovered. The first example is 1-butyl-3-methylimidazolium tetrachloroferrate ($[C_4mim][FeCl_4]$), which was firstly reported in 2004 [30]. These magnetic task-specific ILs exhibit a strong response to a magnetic field [31] and have already been successfully applied in many fields especially in fuel desulfurization and separation processes [32-34]. Recently, a few literatures have reported about the application of MILs on the pretreatment and concentration of target

analytes in single drop microextraction (SDME) [33] and dispersive liquid-liquid microextraction (DLLME) systems [35-36]. The introduction of MILs can enhance pretreatment efficiency by means of an external magnetic field. However, magnetic SDME is still time-consuming; the use of organic solvent in magnetic DLLME is not that eco-friendly. All the above MILs of great application prospect contain metal elements such as Fe, Co, Dy et al. Another kind of MIL is non-metal organic MIL based on a radical ion, and the first one was synthesized and proposed by Yoshida and coworkers in 2007 [37]. Afterwards, bimagnetic ionic liquid with an organic magnetic center was prepared and efficiently applied in catalysis [38]. Up to now there exist so few organic magnetic ionic liquids. More applications of them were never found in the literatures up to now.

Chloramphenicol (CAP) is an effective antibiotic against a wide range of Gram-positive and Gram-negative bacteria in both human and animals [39]. Because of its serious toxicity in humans, CAP is completely banned in food-producing animals within the EU [40]. CAP is easily released or excreted to the natural water [41]. The presence of its residues in aquatic environment has caused increasingly environmental and ecological problems [42-43] and it even shows direct toxicity to certain aquatic microorganisms [44]. Therefore, it is significantly necessary to monitor the residual chloramphenicol in water environment. There are various reported methods for the determination of CAP such as dispersive liquid-liquid microextraction [45], solid-phase extraction [46], aqueous two-phase extraction [47] et al. These methods obtained expected results in the quantitative task; but they also have their own shortcomings as described above. More novel, rapid and accurate pretreatment methods are urgently required for the analysis of CAP.

Based on the above, a novel organic magnetic ionic liquid based on guanidinium cation was synthesized in this study, and then a new method of magnetic ionic liquid aqueous two-phase system (MILATPs) coupled with high-performance liquid chromatography (HPLC) was established to separate effectively and determine trace amount of CAP in water environment. As the result, magnetic separation technique, organic solvent free system and rapid extraction were successfully combined together in this analytical method for the first time. In this work, extraction equilibrium was almost immediately achieved when aqueous two-phase phenomenon occurred and a simple external magnetic field was applied to assist phase separation. Experimental investigations were conducted to optimize different variables in this novel pretreatment process and satisfactory recovery was obtained under optimal conditions. Finally, the method was successfully applied for the determination of CAP in environmental water samples. Hopefully, this novel method can be referenced to monitor trace amount of other antibiotics in water environment.

2. Experimental

2.1 Chemicals and materials

All reagents used were of analytical grade or above. 4-Hydroxy-2,2,6,6-tetramethyl piperidine 1-oxyl free radical (4-OH-tempo) and 1,1,3,3-tetramethyl-guanidine were purchased from Best Reagent Co., Ltd. (Chengdu, China). Anhydrous potassium phosphate was supplied by Guangfu Superfine Chemical Industry Institute (Tianjin, China). Moreover, chlorosulfonic acid was obtained from Aike Chemical Reagent Co. Ltd. (Chengdu, China). All the other used chemicals and reagents were supplied by Kelong Chemical Co. Ltd. (Chengdu, China). Stock solution I of CAP with

concentration of 300 $\mu\text{g mL}^{-1}$ was prepared by dissolving proper amount of CAP into volumetric flask and then diluting to 50 mL with deionized water, and the latter was obtained from ultra-pure water purification system (0.4 mm filter) manufactured by Millipore Co., Ltd. (Bedford, MA, USA). Stock solution II of CAP was prepared by diluting stock solution I in a 50 mL volumetric flask to obtain a concentration of 10 $\mu\text{g mL}^{-1}$. Then they were sealed and stored in a refrigerator until use. Working solutions were prepared weekly by appropriate dilution of stock solution II.

2.2 Apparatus

A P1201 high performance liquid chromatography system (Elite, Dalian, China) consisting of UV1201 UV-Vis detector and EC2006 chromatography workstation was used for the analysis and quantitation of CAP. A Welchrom C18 column (4.6×250 mm, 5 μm) was used as the HPLC analytical column. The pH measurement was carried out with a PHS-25 pH-meter (Leici Instrument Factory, Shanghai, China). FT-IR spectra of MILs were recorded on a Spectrum Two L1600300 infrared spectrometer (Perkin Elmer, Fremont, USA). Electrospray ionization mass spectra (ESI-MS) were collected on Amazon SL mass spectrometer (Bruker Daltonics, Bremen, Germany) and data were processed by Bruker Compass Data Analysis 4.0. Thermogravimetric (TG) analysis was performed on a HTG-2 Microcomputer Differential Thermal Balance (Hengjiu Scientific Instrument Factory, Beijing, China). The magnetic properties of MIL were investigated by a vibration sample magnetometer (VSM) using MPMS SQUID system (Quantum Design, San Diego, USA). Mass value was determined by a FA/JA type electronic analytical balance (uncertainty of ± 0.0001 g). A CY20A water thermostat (Boxun Industry & Commerce Co. Ltd., Shanghai, China) with an uncertainty of ± 0.05 K was applied for keeping

constant temperature of all the tested system.

2.3 Synthesis of organic magnetic ionic liquid

2.3.1 Synthesis of $H[TEMPO-OSO_3]$

$H[TEMPO-OSO_3]$ was synthesized according to the procedure of previous literature [48]. Certain amount of 4-hydroxyl-tempo was dissolved in dichloromethane at 0~5°C, equal molar of chlorosulfonic acid was added dropwisely. The mixture was continuously stirred for 12 h at room temperature. After reaction, the product was washed with dichloromethane for 3 times, dried by a DZF-6050 drying oven (Yingyuyuhua Instrument Factory, Gongyi, China) under vacuum at 45°C and then dissolved in ethanol, successively.

2.3.2 Synthesis of $[TMG][TEMPO-OSO_3]$

$[TMG][TEMPO-OSO_3]$ was prepared by simple neutralization of $H[TEMPO-OSO_3]$ with equimolar of 1,1,3,3-tetramethylguanidine in ethanol or acetone. The product was washed with toluene for 3 times and dried under vacuum at 45°C. The final product was dark-reddish viscous liquid with yield of 91.8%. The MIL was characterized by FT-IR spectra and ESI-MS, its structural formula was shown in Fig. 1.

2.4 Preparation and measurement of ATPs

The binodal curves were determined by the cloud point method [49] in a jacketed glass vessel containing a magnetic stirrer at constant temperature and atmospheric pressure. The vessel was immersed in a jacketed glass vessel using water thermostat to keep constant temperature in extrinsic cycle pattern. An IL solution of known mass fraction was taken into the vessel with vigorous stirring for 15 min. Then a salt solution of known mass fraction was added dropwise to the vessel with an interval of

5 min between every two drops. When the mixture turned cloudy, the stirring was stopped and the two phases began to appear clearly. At the end of ATPs formation, the composition of the mixture for each point on the binodal curve was weighed, noted and calculated by mass. Afterwards, a certain amount of water was added dropwisely to produce a clear one-phase system. The system was then kept stirring at constant temperature for another 15 min. Repetitive operation was kept on to obtain the phase equilibrium line. Finally, the equilibrium curves data were correlated by the Merchuk equation [50] as eq.(1):

$$w_1 = a \exp(bw_2^{0.5} - cw_2^3) \quad (1)$$

Where w_1 and w_2 are the IL and salt weight percentages, respectively; a, b and c are three constants obtained by regression of the experimental data.

For the determination of each tie-line (TL), a ternary mixture composed of salt+water+IL at the biphasic region was gravimetrically prepared. The system was stirred and left to equilibrate, and then a complete separation of two phases was obtained. The phases were separately weighed and noted. Each TL was then calculated according to eq.(2-5) proposed by Merchuk [50] et al.

$$w_1^t = a \exp(b(w_2^t)^{0.5} - c(w_2^t)^3) \quad (2)$$

$$w_1^b = a \exp(b(w_2^b)^{0.5} - c(w_2^b)^3) \quad (3)$$

$$w_1^t = w_1^m / \alpha - w_1^b (1 - \alpha) / \alpha \quad (4)$$

$$w_2^t = w_2^m / \alpha - w_2^b (1 - \alpha) / \alpha \quad (5)$$

Where the superscripts of t , b and m designate the top phase, the bottom phase and the mixture, respectively; α is the ratio of the mass of the top phase and the total mass of the mixture. The tie-line length (TLL) corresponds to the distance between the points of the bottom phase composition to that of the top phase composition in the binodal curve.

2.5 Extraction procedure

Working solution was prepared by appropriate dilution of stock solution 2 and adjusted to a certain pH value by phosphoric acid or ammonium hydroxide. A schematic representation of extraction procedure is shown in Fig.2. A certain amount of IL and working solution were successively weighed in a 10 mL centrifugal tube with the mass accuracy guaranteed by a syringe. Afterwards, complete dissolution of IL was achieved. On the other hand, anhydrous potassium phosphate was placed in thermostatic water bath beforehand to reach the certain required temperature, which could avoid the change of ATPs temperature during salt addition. Then appropriate amount of anhydrous potassium phosphate was added and dissolved in the system at the certain temperature. A turbid or cloudy solution was easily formed without any additional treatment. During this stage, CAP was immediately extracted into the IL-rich droplet. Then a strong NdFeB magnet was used to separate the extraction phase. The magnetic ionic liquid droplet could be attracted by the magnet, and moved gently towards the magnet; then two phases were separated more easily. Because the new MIL has strong magnetism, the complete collection of its dispersing droplets can be achieved within 1 min. Afterwards, the upper IL phase volume was accurately measured by a syringe with an uncertainty of $\pm 0.5 \mu\text{L}$. The mass of the upper IL phase was accurately weighed by an analytical balance with an uncertainty of $\pm 0.0001 \text{ g}$.

Then 20 μL of ionic liquid phase was directly injected into HPLC for quantitative analysis.

2.6 HPLC analysis and determination of the partition parameters of CAP

The optimal HPLC condition on the C18 column was determined as the mobile phase of water-acetonitrile (82:18, V/V) at a flow rate of 2.0 mL min^{-1} with 20 μL injection volume. The column effluent was monitored at 278 nm, which was the maximum UV absorption wavelength of CAP. Extraction recovery (ER) of CAP was calculated according to eq. (6).

$$\text{ER}(\%) = v_{IL} \times c_{IL} / m_s \quad (6)$$

Where v_{IL} (mL) and C_{IL} (g mL^{-1}) are the volume of IL-rich phase and concentration of CAP in IL-rich phase, respectively; m_s (g) is the amount of CAP added.

All of CAP preconcentration experiments were performed 3 times repeatedly and their standard deviations (SD) were calculated as eq. (7).

$$SD = \left(\sum_{i=1}^N (w_i - w_{ave})^2 / N \right)^{0.5} \quad (7)$$

Where w_i and w_{ave} are experimental value each time and the average value, respectively. N represents the repeated experimental times ($N=3$).

Mass ratio (R) was defined as the mass ratio of the bottom phase (m_b) to top phase (m_t). The preconcentration factor (PF) was defined as the ratio of concentration of CAP in IL-rich phase to initial concentration of CAP in the sample solution.

3. Results and discussion

3.1. Characterization of MIL

The structure of new MIL has been identified via FT-IR spectra and ESI-MS as shown in Fig.3a and Fig.3c-3d. In FT-IR spectra, 3355 cm^{-1} is the N-H stretching vibration; 2976 cm^{-1} and 2934 cm^{-1} attribute to the methyl and methylene saturated C-H stretching vibration in the anion; 1607 cm^{-1} is the C=N stretching vibration; 1568 cm^{-1} is the N-H in-plane bending vibration; 1364 cm^{-1} , 1411 cm^{-1} and 1464 cm^{-1} are the methyl and methylene C-H in-plane bending vibration; 1215 cm^{-1} is the C-N stretching vibration; 1062 cm^{-1} is the N-O stretching vibration; 793 cm^{-1} is the N-H out-of-plane bending vibration. In ESI-MS spectrum, there existed fragment ions with m/e 116 ($\text{C}_5\text{H}_{14}\text{N}_3^+$) in positive ion mode and m/e 251 ($\text{C}_9\text{H}_{17}\text{NO}_5\text{S}^-$) in negative ion mode. Above data can prove the structure of new MIL after comparison with the recent study [23]. Thermogravimetric (TG) curve of novel [TMG][TEMPO-OSO₃] is shown in Fig.3b. It can be concluded that this MIL was very stable when temperature was lower than 140°C. So this novel MIL is suitable for the following analytical method.

3.2. Study of the magnetization and susceptibility of MIL

The magnetic property of MIL was investigated by a vibration sample magnetometer (VSM) using MPMS SQUID system (Quantum Design, America). As shown in Fig.4, the magnetic susceptibility at temperature of 298 K and 5 K revealed linear growth with the increasing intensity of applied magnetic field. In addition, static susceptibility measurement against the temperature under an applied field of 2 T was also investigated. It was found [TMG][TEMPO-OSO₃] exhibited a weak

paramagnetic property when temperature was above 150 K. Furthermore, when temperature was lowered, its magnetization rose sharply to a very strong magnetic susceptibility value as high as 855 emu mol^{-1} , which is about 80 times of the value at room temperature (298 K). This phenomenon reveals great potential for its magnetic application under low temperature. Additionally, the magnetic response of ionic liquid droplets to a neodymium magnet in an aqueous two phase system was demonstrated in Fig.5. It can be found that the droplets could be attracted by the magnet strongly and move with it. As a result, it is beneficial for enhancing the efficiency of phase separation.

3.3 Phase behavior of magnetic ILATPs

3.3.1 Effect of the property of salts on phase separation

The type of salts can play a very key role in the previous reports of ATPs, so a series of inorganic salts were firstly tested for the formation of the new MILATPs with [TMG][TEMPO-OSO₃]. Results demonstrate that MILATPs can form by adding appropriate amount of some specific salts, such as K₃PO₄, K₂CO₃, Na₂CO₃, Na₂SO₄ or (NH₄)₂SO₄. When other salts or some bases were selected, such as NaCl, KCl, K₂HPO₄, KOH or NaOH, they cannot drive MIL aqueous solution to separate into two phases. Fig.6 shows the phase diagrams of MIL aqueous two-phase systems with different inorganic salts at 298.15 K. It can be seen that experimental data of IL-(NH₄)₂SO₄ system do not fit very well with the calculated values. This result attributes to the relatively poor phase-separating ability of (NH₄)₂SO₄. As for those systems containing Na₂CO₃ and Na₂SO₄, the two salts have relatively low solubility in water and low salting-out ability. Consequently, MIL cannot be easily salted out if its amount is small. Considering the essence of microextraction, IL-K₃PO₄ and IL-

K_2CO_3 are much more suitable for preconcentration of CAP than other salts. Further comparison between these two systems was carried out by the experimental tie-lines. In IL- K_3PO_4 system, tie-line data reveal that when top phase is composed of nearly 80% of IL, the bottom phase only has 0.2% of IL dissolved. However, in IL- K_2CO_3 system, there still exists 2.8% of IL dissolved in the bottom phase when top phase is almost 100% composed of IL which is a saturation condition. Although both of them are fine salting-out agent, K_3PO_4 demonstrates more ideal performance and is more suitable in the microextraction application.

3.3.2 Effect of temperature

According to the result of the above section, K_3PO_4 was selected in the MILATPs to further investigate the effect of temperature on the phase separation ability. The thermal influences are illustrated in Fig.7. It is obvious that, when the mass fraction of anhydrous potassium phosphate is less than 19%, the phase-forming ability of magnetic ionic liquid will decrease with temperature rising. But there seems to be not much difference of phase behavior when K_3PO_4 content in the system becomes higher. It can be concluded that interaction of ionic liquid with water will increase when temperature is higher and their mutual solubility is enhanced. Although all temperatures could satisfy the conditions of subsequent study of CAP pretreatment, room temperature (298.15 K) was chosen for operation simplicity and energy saving.

3.4 Separation and concentration behaviors of CAP in MILATPs

3.4.1. Effect of the concentration of K_3PO_4

Effect of the concentration of K_3PO_4 on extraction recovery and preconcentration factor of CAP as well as mass ratio of the microextraction system was discussed in detail. The concentration of working solution of CAP was 1000 ng mL⁻¹. As shown in

Fig.8, with the increasing of K_3PO_4 concentration, the recovery of CAP can reach up to 99.82%. When K_3PO_4 is more than 4.08 g (36.92%), extraction recovery keeps relatively steady. It is regarded as a strong salting-out salt for its relatively higher hydrophilicity, and the MIL tends to separate from the system to form another phase as the amount of salt is increased. Moreover, CAP has a much better affinity with [TMG][TEMPO-OSO₃] than water probably due to the strong hydrogen bonds between CAP and IL. As a result, the salt will promote CAP to transfer into the IL-rich phase. The more salt was added, the more IL was separated out to form a new phase and the more amount of CAP was extracted to IL-rich phase. But when the salt is sufficient enough for the complete extraction of CAP, more addition of salt is not necessary. So K_3PO_4 with the amount of 4.08 g is selected for further investigation.

3.4.2. Effect of initial mass of [TMG][TEMPO-OSO₃]

In this section, the effect of initial mass of magnetic ionic liquid in the new MILATPs for pretreatment of CAP was discussed. As demonstrated in Fig.9, with the increase of the amount of IL, the extraction efficiency of CAP can be improved significantly while the preconcentration factor rises slightly and then drops significantly. Extraction efficiency is obviously enhanced with initial increase of MIL mass and then reaches equilibrium with further addition of MIL. Generally, both high extraction recovery and ideal pretreatment factor are crucial for the determination of trace amount of target component. Considering these two aspects, 0.05 g of IL was appropriate with extraction recovery of 99.82% and preconcentration factor of 146 for CAP.

3.4.3. Effect of pH of sample solution

Experiments were performed by changing the pH value of sample solution from 1

to 13 by adding appropriate amounts of phosphoric acid and ammonium hydroxide. Related effects of pH on the recovery of CAP and preconcentration factor as well as mass ratio are shown in Fig.10. According to the experimental result, it was found extraction recovery of CAP decreased sharply when $\text{pH} > 7$. The reason was that CAP could be gradually decomposed if it was preserved in alkaline condition for a certain time. On the contrary, when $\text{pH} \leq 7$, the level of CAP recovery keeps relatively steady and reaches the highest value at $\text{pH} = 3$. In the extraction process applied in our study, although the salt-rich phase was alkaline after dissolution of K_3PO_4 , CAP was almost immediately extracted into the fine droplets of MIL. In such a short time, K_3PO_4 and CAP could hardly coexist in the same phase. Meanwhile, the decomposition of CAP usually needs more time and enough alkaline. Such short exposure to alkaline cannot change its molecular structure, which should attribute to the rapid phase separation. To avoid possible decomposition, pH value of original sample solution can be determined to be 3 as the optimal one.

3.5 Method validation and comparison

A new analytical method of MILATPs coupled with HPLC was developed for quantitative determination of trace amount of CAP in environmental water. Under the optimum conditions, the calibration curve was performed by applying standard CAP solution at nine different concentrations with three replications for each one in the MILATPs in the range of 10~2500 ng mL^{-1} . The obtained linear regression equation was $y = 1.5332x + 1.6386$ ($R^2 = 0.9995$), where y is peak area of CAP and x represents the concentration (ng mL^{-1}) of CAP. Good linear range was achieved from 12.25 ng mL^{-1} to 2200 ng mL^{-1} .

The limit of detection (LOD) and the limit of quantification (LOQ) based on 3 S_b/m

and $10 S_b/m$ (where S_b is standard deviation of the blank signals and m is the slope of calibration curve after pretreatment) were determined as 0.14 ng mL^{-1} and 0.46 ng mL^{-1} , respectively. This reveals a good sensitivity for the presented method.

Finally, defined as the relative standard deviation (RSD%), the intra-day and inter-day precision were determined with several replications at the concentration level of 220 ng mL^{-1} of each analyte. They were analyzed by independent pretreatment of samples using the proposed method six times in a day or in different six days with achieved RSD% as 2.42% for intra-day precision and 4.45% for inter-day precision, respectively. Both of them were acceptable. Recoveries were all satisfactorily in the range of 90%~110% for this method. The preconcentration factor (PF) of this novel method was calculated as 147.2, which was much higher than those in reported ATPs literatures [22, 47]. As for other newer methods of extraction, the extractant droplet in single drop microextraction (SDME) is easily disturbed by water phase under high-speed stirring, which will result in the lower repeatability and limit its use [33], then dispersive liquid-liquid microextraction (DLLME) was developed as a simple and rapid pre-concentration method for the analysis of some organic compounds in water. But tedious centrifugation operation [6] and the use of organic solvent (e.g. ethyl acetate, *n*-hexane and so on) cannot be avoided [35-36]. New methods with magnetic nanoparticles applied in the DLLME system [11] can realize rapid separation, but the self-made nanoparticles cannot be easily obtained and inevitable desorption procedure is time-consuming. The earlier ATPs extraction method has avoided the use of volatile organic solvent successfully, such as in the analysis for human urine [51]. However, entire phase separation was difficult and the researchers had to employ extra post-processing before analysis. In this study, novel MILATPs method is fast and easy to

operate without centrifugation or desorption procedure, which enhances sample treatment efficiency greatly under greener condition. Compared with above methods, this is the first time to combine the advantages of organic solvent-free extraction, rapid preconcentration and magnetic separation simultaneously.

3.6 Analysis of real samples

In order to investigate the enrichment performance for real samples, the proposed method was applied for the pretreatment and determination of CAP in Jiangan river, Mingyuan lake and tap water of our laboratory, all located in Jiangan campus of Sichuan University, Shuangliu county, Chengdu city, China. All of the selected samples were centrifuged at 4000 rpm for 5 min and after that the supernatant was collected, then pH was adjusted for analysis. Spiked recovery experiments were performed by adding a very small amount of CAP working solution into the collected sample solution to obtain the required spiked concentration followed by pH adjustment. All the above environmental samples were filtered through microporous membrane with a pore size of 0.22 μm before HPLC analysis. The CAP analysis was carried out three times for each sample following the procedure described above. The results are listed in [Table 1](#). The accuracy of the proposed method was evaluated by analysis of spiked real samples with the extraction recovery (ER, %) acquired from [eq. \(8\)](#):

$$ER(\%) = (C_{\text{found}} - C_{\text{real}}) / C_{\text{added}} \times 100\% \quad (8)$$

Where C_{found} , C_{real} and C_{added} are the total detected concentration of CAP after addition of known amount of standard analyte into the real sample, the concentration of analyte in real environmental sample and the concentration of the known added concentration in real sample, respectively. As shown in [Table 1](#), good recovery of

analyte was obtained in the range of 94.6%~99.72% with RSD of 1.97%~4.35%. Additionally, it reveals that the present novel method has a good reliability and satisfactory reproducibility for the determination of trace amount of CAP in environmental waters. HPLC chromatograms with spiked concentration of 50 ng mL⁻¹ are shown in Fig.11, which exhibits a good base separation of the peak of CAP with others in such a complex analyte. In summary, the proposed method in this work can be satisfactorily applied for determination of trace amount of CAP in the environmental samples.

4. Conclusion

A unique aqueous two-phase system based on new magnetic ionic liquid with special property was developed, which was used to establish a novel analytical method with high performance liquid chromatography to separate effectively and determine trace amount of chloramphenicol in environmental waters. The advantages of magnetic separation, no use of organic solvent and rapid extraction were combined together in this MILATPs-HPLC method for the first time. An external magnetic field was applied for phase separation and it successfully enhanced the pretreatment efficiency. Extraction procedure could be almost immediately completed without any further operation such as vortex shaking. Preconcentration factor of 147.2 was obtained, which shows a significant enrichment effect. Additionally, good linear range of 12.25 ng mL⁻¹~2200 ng mL⁻¹ and fine precision RSD% (2.42% for intra-day, 4.45% for inter-day) as well as low value of limit of detection and limit of quantitation (0.14 ng mL⁻¹ and 0.42 ng mL⁻¹, respectively) were acquired by this method. Good recovery of 90%~110% for chloramphenicol was also achieved in the analysis of real

environmental samples. In a word, the novel magnetic aqueous two-phase system is showing great potential and is expected to promote new development in the field of extraction, separation and pretreatment of various samples.

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Figure captions

Fig.1 The structure of new magnetic ionic liquid

Fig.2 Scheme of MILATPs extraction procedure

Fig.3 FT-IR (a), TG curve (b) and ESI-MS (positive mode: c and negative mode: d) of MIL

Fig.4 Magnetic properties of new MIL.

Fig.5 Magnetic response of MIL droplets to a neodymium magnet

Fig.6 Binodal curves of MIL+salt+H₂O at 298.15 K. The points are experimental data; the curve is composed of calculated data according to Eq.(1), and the straight line is tie-line.

Fig.7 Binodal curves of MIL+ K₃PO₄+H₂O at different temperature. The points are experimental data; the curve is composed of calculated data according to Eq.(1).

Fig.8 Effect of the concentration of K₃PO₄ on the recovery of CAP with conditions: IL: 0.05 g; working solution: 6.92 g; pH of sample solution: 7; extraction temperature: 278.15 K; extraction time: immediately after dissolving K₃PO₄ (Error bars show the standard deviation of the mean, n=3)

Fig.9 Effect of initial mass of MIL on the recovery of CAP with conditions: K₃PO₄: 4.08 g; working solution: 6.92 g; extraction temperature: 298.15 K; extraction time: immediately after dissolving K₃PO₄ (error bars show the standard deviation of the mean, n=3)

Fig.10 Effect of pH on the recovery of CAP with conditions: K₃PO₄: 4.08 g; IL: 0.05 g; working solution: 6.92 g; extraction temperature: 298.15 K; extraction time: immediately after dissolving K₃PO₄ (error bars show the standard deviation of the mean, n=3)

Fig.11 HPLC chromatograms of three sample solutions added with 50 ng mL⁻¹ CAP after MILATPs extraction, including a: Jiangan river water, b: Mingyuan lake water and c: tap water in laboratory.

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Magnetic collection

Magnet moving upward

Magnetic collection

Aqueous two-phase occurred

Process of magnet-assisted phase separation

Final state after magnet-assisted phase separation

Fig.6

Fig.7

Fig.8

Fig.9

Fig.10

Fig.11